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TO CARRY OUT PHARMACOLOGICAL EVALUATION OF ANTIDEPRESSANT POTENTIAL OF BOSWELLIA SERRATA IN ALLOXAN INDUCED DIABETIC RAT

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ABSTRACT

One of the major health issues facing the world today is diabetes mellitus, whose rising incidence is linked to problems like depression. When depression and diabetes coexist, the management and prognosis of both illnesses are considerably poorer. In this work, a rat model of diabetes-induced depression is used to examine the therapeutic potential of Boswellia serrata extract treatment. Biochemical and behavioral alterations were measured using alloxan to cause diabetes. Oxidative stress, neurotransmitter levels, and neurobehavioral outcomes like as immobility in forced swim and tail suspension tests were the main areas of attention. As a result of notably lowering blood glucose levels, immobility duration, and oxidative stress markers, the Boswellia serrata extract was found to be an efficient means of alleviating depression symptoms in diabetic rats. Based on these findings, Boswellia serrata appears to be a viable herbal treatment option for diabetes-related sadness.

KEYWORD: Diabetes mellitus, depression, Boswellia serrata, Fluoxetine, alloxan, oxidative stress, diabetes-induced depression.

1. INTRODUCTION

1.1.Diabetes Mellitus

Diabetes is widely recognised as one of the leading causes of death and disability worldwide. The prevalence of diabetes will rise from 6% to over 10% in the next decade. In 2000, the

World Health Organization (WHO) recorded a total of 171 million people for all age groups worldwide (2.8% of the global population) who have diabetes, and the numbers are expected to rise to 366 million (4.4% of the global population) by 2030 (Wild et al., 2004).

Diabetes mellitus is a disease/group of syndromes characterized by chronic hyperglycaemia as a result of either lack of insulin or resistance to its action; hence there is altered metabolism of lipids, carbohydrates and proteins and increased risk of complications from vascular disease (Davis and Granner, 2001).

Characteristic symptoms of diabetes: Excessive urine (polyuria), excessive thirst, excessive hunger (polyphagia), increased fluid intake (polydipsia), blurred vision, unexplained body weight loss (cachexia), lethargy.

1.2.Classification of Diabetes

According to World Health of Organization (WHO) there are three type of diabetes mellitus i.e, Insulin Dependent Diabetes Mellitus (type 1), Non-Insulin Dependent Diabetes Mellitus (type-2) and Gestational diabetes.

1.2.1. Type 1 diabetes (T1D)

a) Description

i. Formerly known as Insulin-Dependent Diabetes Mellitus (IDDM).
ii. Characterized by hyperglycemia due to an absolute deficiency of the insulin hormone produced by the pancreas.
iii. Patients require lifelong insulin injections for survival.
iv. Usually develops in children and adolescents (although can occur later in life).
v. May present with severe symptoms such as coma or ketoacidosis.
vi. Patients are usually not obese with this type of diabetes, but obesity is not incompatible with the diagnosis.
vii. Patients are at increased risk of developing microvascular and macrovascular complications.

b) Etiology

i. Usually (but not always) caused by autoimmune destruction of the beta cells of the pancreas, with the presence of certain antibodies in blood.

ii. A complex disease caused by mutations in more than one gene, as well as by environmental factors.

c) Symptoms

i. Increased urinary frequency, thirst, hunger, and unexplained weight loss.
ii. Numbness in extremities, pain in feet (disesthesias), fatigue, and blurred vision.
iii. Recurrent or severe infections.
iv. loss of consciousness or severe nausea/vomiting (ketoacidosis) or coma. Ketoacidosis more common in T1D than in T2D.

d) Diagnosis

i. Diagnosis is made by the presence of classic symptoms of hyperglycemia and an abnormal blood test.
ii. A plasma glucose concentration >=7 mmol/L (or 126 mg/dL) or >=11.1mmol/L (or 200mg/dL) 2 hours after a 75g glucose drink.
iii. In a patient without classic symptoms, diagnosis can also be made by two abnormal blood tests on separate days.
iv. In most settings (although not always available in resource-poor countries), another test called HbA1C is done to approximate metabolic control over previous 2-3 months and to guide treatment decisions.

e) Treatment

i. Overall aim of treatment is symptom relief and prevention or delay of complications by targeting normal blood glucose levels.
ii. Lifelong insulin injections in different combinations: short-acting/long-acting, intensive management with multiple injections prior to meals, once or twice daily injections, insulin pump
iii. Consistent supply of insulin essential (however, insulin is unavailable and unaffordable in many poor countries)
iv. Glucometers to self-monitor blood glucose
v. Early detection and treatment of complications (at intervals recommended by national and international guidelines): eye exam, urine test, foot care, and specialist referral as needed
vi. Patient education about self-monitoring for sign/symptoms of hypoglycaemia (such as hunger, palpitations, shakiness, sweating, drowsiness and dizziness) and hyperglycemia.
vii. Patient education about diet, exercise, and foot care

viii. Where possible, patient-led support groups and community involvement.

1.2.2. Type 2 diabetes（T2D）

a) Description

i. Formerly named non-insulin-dependent diabetes mellitus (NIDDM).
ii. Characterized by hyperglycemia due to a defect in insulin secretion usually with a contribution from insulin resistance.
iii. Patients usually do not require lifelong insulin but can control blood glucose with diet and exercise alone, or in combination with oral medications, or with the addition of insulin.
iv. Usually (but not always) develops in adulthood (and is on the rise in children and adolescents).
v. Is related to obesity, decreased physical activity and unhealthy diets.
vi. As in T1D, patients are at a higher risk of microvascular and macrovascular complications.

b) Etiology

i. Associated with obesity, decreased physical activity and unhealthy diets (and involves insulin resistance in nearly all cases).
ii. Occurs more frequently in individuals with hypertension, dyslipidaemia (abnormal cholesterol profile), and central obesity, and is a component of "metabolic syndrome".
iii. Often runs in families but is a complex disease caused by mutations in more than one gene, as well as by environmental factors.

c) Symptoms

i. Patients may have no symptoms at all or minimal symptoms for years before being diagnosed.
ii. May have increased urinary frequency, thirst, hunger, and unexplained weight loss.
iii. May also experience numbness in extremities, pain in feet (dysesthesias), and blurred vision.
iv. May have recurrent or severe infections.
v. Patients may present with loss of consciousness or coma but this is less common than in T1D.

d) Diagnosis

i. Diagnosis is made by the presence of classic symptoms of hyperglycemia and an abnormal blood test.
ii. A plasma glucose concentration >=7 mmol/L (or 126 mg/dL) or >=11.1mmol/L (or 200mg/dL) 2 hours after a 75g glucose drink.
iii. In a patient without classic symptoms, diagnosis can also be made by two abnormal blood tests on separate days.
iv. In most settings (although it may not be available in some resource-poor settings), another test called HbA1C is done to approximate metabolic control over previous 2-3 months and to guide treatment decisions. This test can also be used to diagnose type 2 diabetes.
v. Some asymptomatic patients are diagnosed through "opportunistic screening" of high risk groups (at a routine medical visit, the health care provider may identify the patient as being at higher risk of diabetes and recommend a screening test).
vi. For example, age >45 years of age, a BMI >25 kg/m2 may, being of certain ethnic group or being hypertensive may prompt a screening test.
vii. In some cases, the patient him/herself requests screening.

e) Treatment

i. Overall aim of treatment is symptom relief and prevention or delay of complications by targeting normal blood glucose levels.
ii. Patients treated with diet/exercise, or with addition of one or more categories of oral medications, with a combination of oral medications and insulin, or with insulin alone.
iii. Glucometers to self-monitor blood glucose (with less frequency than with T1D).
iv. Early detection and treatment of complications (at intervals recommended by national and international guidelines): eye exam, urine test, foot care, and specialist referral as needed.
v. Self-monitoring for signs/symptoms of hypoglycemia (such as hunger, palpitations, shakiness, sweating, drowsiness and dizziness) and hyperglycemia.
vi. Patient education about diet, exercise, and foot care.

1.2.3. Gestational diabetes (GDM)

a) Description

i. include congenital malformations, increased birth weight and an elevated risk of perinatal mortality.
ii. Increased risk to woman of developing diabetes (T2D) later in life.

b) Etiology

i. The mechanism is not completely well understood but hormones of pregnancy appear to interfere with insulin action.

c) Symptoms

i. Increased thirst and increased urination are more commonly noted (although other symptoms can be present).
ii. Because pregnancy itself causes increased urination, these symptoms are difficult to recognize as abnormal.
iii. A larger than normal baby during pregnancy (noted on routine prenatal exam) may prompt diabetic screening.

d) Diagnosis

i. Standard OGTT is done at 24-28 weeks after an overnight fast (fasting plasma glucose and a plasma glucose 2 hours after 75g glucose drink is done).
ii. A 2 hour level >=7.8 mmol/L (or 140 mg/dL) is diagnostic of gestational diabetes.
iii. If fasting and postprandial blood sugars are elevated in the first trimester, this may indicate pre-existing diabetes mellitus (which is considered a different condition, with different implications).

e)Treatment

i. Strict metabolic control of blood glucose to lower obstetrical risks.
ii. Patients treated with diet/exercise, with addition of oral medications, or with the addition of insulin.
iii. Glucometers to self-monitor blood glucose.
iv. Patient education about diet and exercise.
v. Patient education after delivery regarding weight loss/exercise to prevent future diabetes.
vi. Lifelong screening for T2D as patient will be in high risk category.

1.3.Pathophysiology

Insulin facilitates glycogen synthesis from glucose in liver, muscle by stimulating the enzyme, glycogen synthetase. Reduction in insulin release increases glucose level which facilitates reverse conversion of glycogen to glucose. This action is controlled by another hormone, glucagon which acts contrary to insulin. Under constraint, glucose (from glycogen), re enters the blood when the insulin level is low. When insulin level is high there is an increase in cell growth and duplication, protein synthesis and fat storage. Insulin primarily

converts bidirectional processes of metabolism (catabolic to anabolic direction and vice versa). Insulin insufficiency triggers ketosis (fat burning metabolic phase) making the cells poorly respond, leading to insulin insensitivity or Resistance.

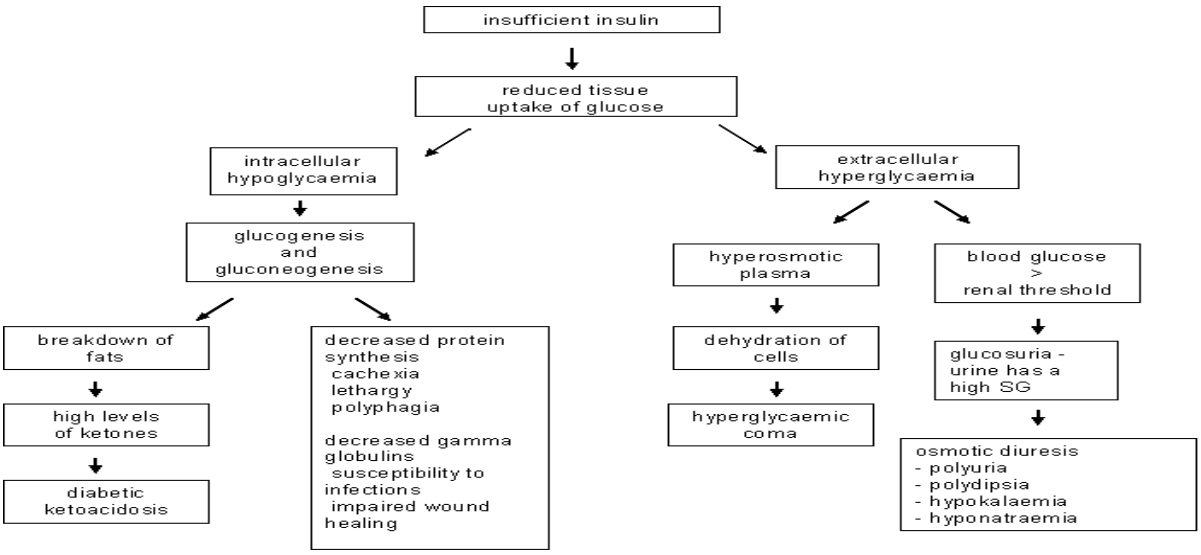


Fig. No. 1: Pathophysiology of diabetes mellitus.

(source:- www.cannininsulin.com/pathophysiology-algorithm.htm)

1.4. Chemically induced models

1.4.1. Streptozotocin-induced

Streptozotocin (STZ) has been selected by many investigators as an effective chemical for inducing experimental diabetic animal model STZ is an effective antibiotic whose diabetogenic action was firstly found in Upjohn Laboratories during testing of potential antibiotic from Streptomyces achromogenes. Actually, it is a broad spectrum antibiotic, but also destroys the pancreatic β-cells after a single injection. It is effective in different species at doses ranging from 50-200mg/kg in rat, dogs, mice, Chinese hamsters, monkeys, miniature pigs, and rabbits. Animals treated with STZ can usually survive without insulin treatment, although these animals are highly insulin-deficient. In previous report, a mild diabetic state can be induced in rats by a single low dose of approximately 35mg/kg (Yagihashi et al., 1992). STZ is unstable in solution even at acidic pH and should be injected quickly after dissolving in citrate buffer at pH 4.5. Its life span in vivo as a molecule is less than 15 minutes. Approximately 4 days after STZ administration to rats, the pancreatic β-cells appear degranulated, but not necrotic with evidence of limited proliferation (Yagihashi et al., 1992). For the mechanism of STZ diabetogenicity, it has been suggested that its nitrosourea moiety is responsible for pancreatic β-cells toxicity, while the deoxyglucose moiety facilitates its

transport across the cell membrane. There is a significantly lower mortality by injecting STZ to induce DM compared to alloxan. Furthermore, both type l and type 2 DM with various severities can be introduced through adjusting the dose and frequency of STZ administration. STZ-induced diabetic nephropathy and neuropathy is well-established and animal model is widely used to investigate the potential of bioactive moieties to treat diabetic nephropathy and peripheral neuropathies (Jakobsen and Lundbaek, 1976; Jakobsen, 1979; Visnagri et al., 2012; Gao et al., 2012).

1.4.2. Alloxan induced model

Alloxan is a chemical compound that selectively destroys insulin-producing beta cells in the pancreas, leading to hyperglycemia and symptoms similar to type 1 diabetes mellitus in experimental animals. Alloxan-induced diabetes is a form of insulin-dependent diabetes mellitus that occurs as a result of alloxan administration or injection to animals. It has been successfully induced in a variety of animal species; rabbits, mice, rats, monkeys, cats and dogs. Alloxan has been administered in single or multiple doses, through different routes (intraperitoneal, intravenous and subcutaneous); with single intraperitoneal administration apparently the most employed mode. The dosage of the drug also varies across studies, ranging between 90 and 200mg/kg of body weight (BW), with 150 mg/ kg BW being the most frequently used dosage. Animal species, route of administration and nutritional status have been considered to play a role in determining the dose of alloxan appropriate for induction of diabetes [Osasenaga Macdonald Ighodaroa et al., 2017].

1.4.3. Mechanism: Alloxan enters beta cells via the GLUT2 glucose transporter. Once inside the cell, it undergoes redox cycling, leading to the formation of reactive oxygen species (ROS) such as superoxide radicals and hydrogen peroxide. These ROS cause oxidative stress, DNA damage, and ultimately apoptosis (cell death) of beta cells [Lenzen S et al., 2008].

1.4.4. Characteristics:

i. **Diabetes Type**: This model typically results in insulin-dependent diabetes mellitus (Type 1 diabetes-like condition) due to the destruction of beta cells.
ii. **Onset**: Diabetes onset is rapid after alloxan administration, often within 24-72 hours.
iii. **Symptoms**: Animals exhibit hyperglycemia (high blood glucose levels), polyuria (excessive urination), polydipsia (excessive thirst), and weight loss [Rakieten N, Rakieten ML et al., 1963].

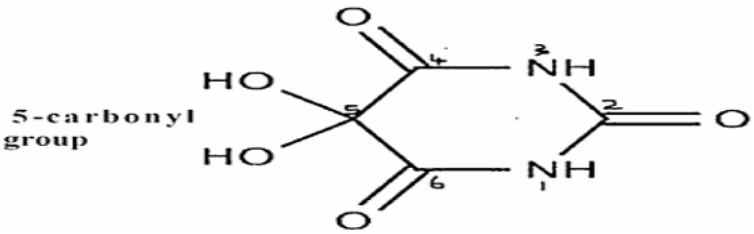


Fig.2 Chemical Structure of Alloxan

(source:- https://www.researchgate.net/figure/Chemical-structure-of-alloxan)

1.5. Diabetes induced depression

Major depressive disorder, or depression, is a common but dangerous mental illness that has an adverse effect on one's feelings, thoughts, behaviour, and perception of reality. Depression, also referred to as depressive disorder, is a prevalent mental illness. It is characterised by a protracted period of depression, loss of pleasure, or lack of interest in activities [Fu DJ et al,.2020].

Willis noted in the late 17th century that persons with diabetes were more likely to have had "significant life stress, sadness or long sorrow [Iosifescu DV et al,.2022]. Although similar clinical observations were documented in other nations, it wasn't until the latter half of the 1900s that a number of epidemiological studies revealed that diabetes increases the risk of depression in individuals with the condition, even if they are unaware of it. Apart from depression disorders, individuals with diabetes are also exhibiting noteworthy levels of diabetes-specific distress. This can be distinguished from depressive disorders but may also serve as a potential risk factor for depression. The United States of America (USA), the United Kingdom, and a few other high-income countries have all conducted epidemiological research on depression and diabetes and associated comorbidities. Although there are less reports about the situation in other parts of the world, those that do exist suggest that it is comparable in those other nations as well [Schatzberg AF et al,.2000].

The latest estimates of the global prevalence of depression and diabetes put the number of persons affected by these conditions at 350 million and 400 million, respectively.Six In general, 10% to 15% of people with diabetes have depressive disorders; this is roughly twice as common as depression in people without diabetes. The prognosis and mortality of both diseases are markedly worsened by comorbidity [Iosifescu DV et al,.2022; Harmer CJ et al ,. 2017;Santarelli L et al,.2003; Asnis GM et al,.2015].

Diabetics have a higher chance of depression, while those with depressive illnesses have a higher risk of diabetes[Hickie IB et al,.2011]. Numerous risk factors, such as low birth weight, traumatic childhood experiences, lifestyle choices, and obesity, have been linked to the occurrence of diabetes and depression [Iosifescu DV et al,.2022; Lambert O et al ,.2002]. and there is strong evidence that diabetes-related problems greatly raise the chance of developing depression [Fiedorowicz JG et al,.2004].

1.6. Type of Depression

There are several types of depressive disorders. Clinical depression, or major depressive disorder, is often just called "depression." It's the most severe type of depression.

Fig 3: Type of Depression.

(source:- https://www.verywellmind.com/common-types-of-depression-1067313).

1.7. Signs and symptoms

People usually experience more than one episode of depression, even if it may only happen once in their lifetime. Symptoms during these episodes can happen almost every day for the most of the day and can include.

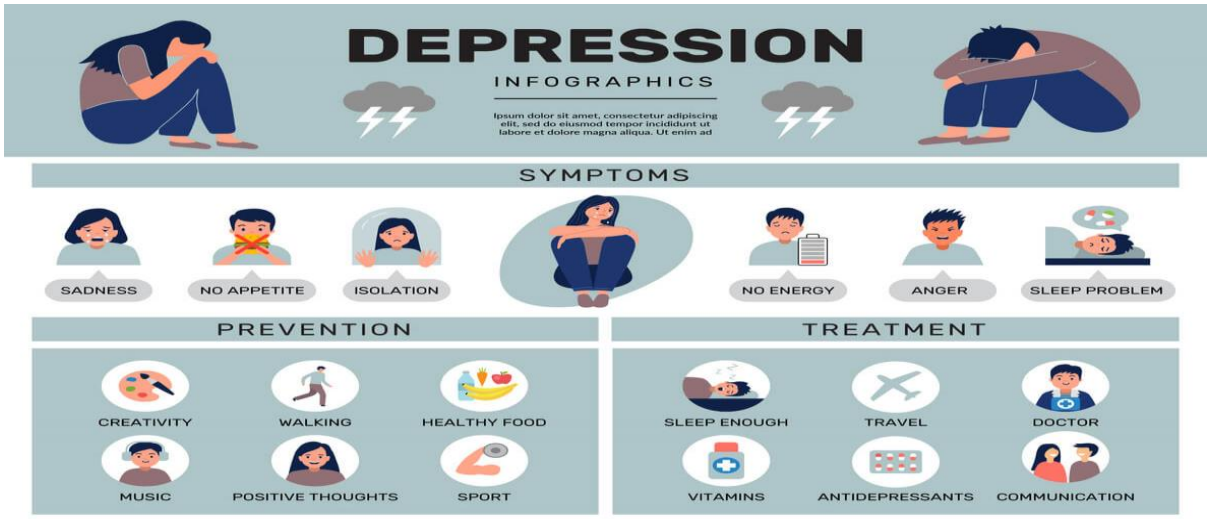


Fig.4 Symptoms of Depression.

(source:- https://www.27fchileanway.cl/what-is-a-major-depression/).

1.8. Pathology

The majority of the complicated etiology and pathophysiology of unipolar major depression is derived from neurobiological research conducted in clinical psychiatry. It is believed that a combination of environmental and hereditary variables leads to depression. About 30–40% of the pathophysiology of depression is due to genetic factors, with the remaining 60–70% being unique to each individual and ascribed to environmental circumstances [Ibrahim Abdullah et al,.2019; Kurt A et al,.2022].

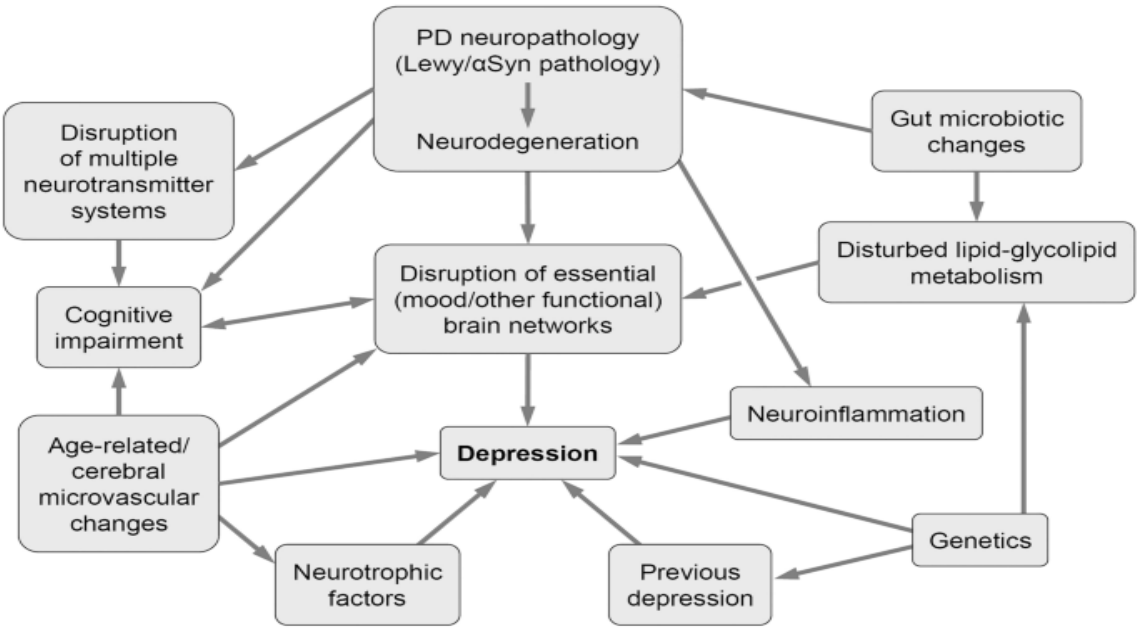


Figure 5 Pathophysiology of Depression.

(source:- https://link.springer.com/article/10.1007/s00702-022-02559-5).

1.9. Epidemiology

A depressive state is not the same as normal mood swings or sentiments related to daily living. It may have an impact on many facets of life, including ties to friends, family, and the community. It may originate from or contribute to issues at work and in the classroom. Anyone can experience depression. Depression is more likely to strike those who have experienced abuse, significant losses, or other stressful situations. Compared to males, women experience depression at a higher rate. [Woody CA et al., 2017]

1.10. Prevention and Treatment:

There are effective treatments for depression. These include psychological treatment and medications. Seek care if you have symptoms of depression. Psychological treatments are the first treatments for depression. They can be combined with antidepressant medications in moderate and severe depression.

1.10.1. Effective psychological treatment for depression include.

i. behavioural activation
ii. cognitive behavioural therapy
iii. interpersonal psychotherapy
iv. problem-solving therapy.

Selective serotonin reuptake inhibitors (SSRIs), like fluoxetine, are antidepressant drugs.

Self-care

In order to control depressive symptoms and enhance general wellbeing, self-care is crucial. Actions that you can take.

i. Make an effort to continue engaging in the things you used to like;
ii. Maintain relationships with friends and family;
iii. Exercise frequently, even if it's only a little stroll;
iv. Try your best to adhere to normal sleeping and eating schedules. Reduce or abstain from alcohol use, and abstain from illegal drug usage, as these behaviours can exacerbate depression.

Table 1: Classification of Antidepressant drugs.

Class	Therapeutic agents	Proposed benefits	Adverse effects and/or concerns
Selective serotonin reuptake inhibitors	Fluxetine, Paraxetine, Setraline, Citalopram, Dapoxetine	SSRIs treat depression by increasing levels of serotonin in the brain.	Side effect include sexual dysfunction, sleep disturbances, anxiety, dizziness, xerostomia, headache, QTc prolongation and gastrointestinal distress.
Serotonin-norepinephrine reuptake inhibitors (SNRIs)	Duloxetine, Venlafaxine, Desvenlafaxine, Levomilnacipran	Serotonin and norepinephrine reuptake inhibitors (SNRIs) block serotonin and norepinephrine reuptake in the synapse.	Hypertension Headache Diaphoresis Bone resorption
Tricyclic antidepressants (TCAs)	Imipramine, Amitriptyline Trimipramine	The increased levels of norepinephrine and serotonin in the synapse can contribute to the antidepressant effect.	Side effect include Dry mouth, Urinary Retention, Constipation, QRS prolongation, Seizures
Atypical Antidepressants	Trazodone Mitrazepine Bupropion	Bupropion, for example, works by inhibiting the reuptake of dopamine and norepinephrine at the presynaptic cleft.	Side effect include Sedation, Weight gain, Seizures
Reversible Inhibitors of MAO (RIMAs)	Moclobamide	MAOIs inhibit the monoamine oxidase enzyme responsible for catabolizing serotonin, norepinephrine, and dopamine.	Potential for serotonin syndrome, Sexual dysfunction

(source:- https://www.ncbi.nlm.nih.gov/books/NBK538182/)

2. Review of literature

Boswellia serrata is one of the ancient and most valued herbs in Ayurveda. “Gajabhakshya”, a Sanskrit name sometimes used for Boswellia, suggests that elephants enjoy this herb as a part of their diet[Siddiqui MZ et al,.2011]. Three renowned ancient texts form the pillars of classical Ayurvedic Science, which has its roots in India: Charaka's Charaka Samhita (c.B.C. 700), the first fundamental medical text; Susruta's Susruta Samhita (c.B.C. 600), which attempted to amass the entire medical knowledge, with special focus on surgery; and the two-volume tome comprising Astanga Samgraha and Astanga Hridaya (c.130-200 A.D.), written by Vagbhata the Elder and Vagbhata the Younger, which synthesized the works of Charaka and Susruta and summarized the eight parts of Ayurveda in prose and verse forms. The first two pillars of Ayurveda describe the antirheumatic (antiarthritis) activity of gugguls-the gum-resins of trees[Siddiqui MZ et al,.2011].

The phytochemistry of Boswellia serrata is quite complex. The oleo-resin-gum of the plant contains gum, acid resin and volatile oils. The acid resin of Boswellia serrata contains a high amount of boswellic acids 20. Acetyl 11-keto-β-boswellic acid (AKBA) obtained from the oleogum-resin of Boswellia serrata is a great source of anti-inflammatory drugs. Boswellia serrata phytochemicals are associated with wide range of therapeutic properties such as anti-arthritis, anti-oxidant, anti-diabetic, anticancer, hepatoprotective, nephroprotective, antibacterial, anti-plasmodium, diuretic, and analgesic [Siddiqui MZ et al,.2011].

Fig. 6: Boswellia Serrata image.

(Source:- https://www.botanichealthcare.net/boswellia-serrata-extract/)

Table No. 02: Taxonomical Hierarchy.

Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida
Order	Sapindales
Family	Burseraceae
Genus	Boswellia
Species	serrata.

(source:-https://www.researchgate.net/figure/Taxonomy-of-Boswellia-serrata 28_tbl2_354440359)

2.1. Morphological Description

Boswellia serrata is a deciduous tree that grows to a height of 10 to 15 metres; it has green branchlets and thin, greenish-grey, yellow or reddish bark that eventually turns to ash colour and peels off in exfoliating papery sheets or flakes. The plant has many long, tubular-structured resin canals that spread both vertically and horizontally throughout the tree; these canals are present throughout the tree, but the bark has the most resin canals compared to any other part of the tree. The young foliage of the plant is yellow or light brown, while the leaves are long (about 12-42 cm) with a greenish lower layer and 17–27 leaflet pairs. There is

pubescent veins[Isha Kumari et al,.2021]. Oblong-lanceolate leaflets make up the plant. The border might be completely absent, wavy, or crenate. Obtuse or subacute is the tip of the leaves. Secondary vein pairs number more than sixteen. A bright reddish colour can be seen in the mid-vein. Small, branching racemes of panicles measure 3–20 cm in length, which is shorter than leaves. Each pedicle is 2-4 mm in length [Isha Kumari et al,.2021].

2.2. Geographical Distribution of Boswellia serrata

Boswellia serrata is often found in the dry deciduous woods of the Middle East, North Africa, Pakistan, and India. Madhya Pradesh, Jharkhand, Andhra Pradesh, Bihar, Orissa, Gujarat, Punjab, Assam, Rajasthan, Uttar Pradesh, and Karnataka are the primary regions in India where it may be found. Gulf nations like Saudi Arabia and East Africa are major producers of BS[Isha Kumari et al,.2021].

2.3. Phytochemistry of Boswellia serrata

Secondary metabolites found in Boswellia serrata include quinones, alkaloids, fatty acids, tannins, saponins, coumarins, emodins, flavonoids, phenols, and reducing sugars. The plant is mostly recognised for its gum resin, which is made up of a complex blend of many phytochemical components. It has more terpenoids, carbs, and essential oils. Gum resin's primary essential oils include α-thujene, sabinene, terpinen-4-ol, cis-carveol, chavicol, linalool, terpinyl acetate, β-copaen-4-α-ol, and germacrene D, among others. The n-hexane essential oil fraction's GC-MS analysis showed the presence of alcohols, esters, monoterpenes, and diterpenes [A. Sharma et al,.2009; Isha Kumari et al,.2021].

Other essential oils identified are βeudesmene, γ-murolene, γ-cadinene, α-copaene, αmurolene, α-cubebene, α-cubebene, 3,5- dimethoxytoluene, 3,5-dimethoxytoluene, alloaromadendrene, allo-aromadendrene, o-methyl anisole, β-gurjunene, β-gurjunene, camphene, eucalyptol, valencene, S-cis-sabinol, α-phellandrene, methyl chavicol, α-terpinolene, bornyl acetate, α-terpineol, d-α-thujene, dlimonene, p-cymene, and 1,2,3,4,6,8a-hexahydro-1- isopropyl-4,7-dimethyl [Isha Kumari et al,.2021]. On the other hand, the plant's increased terpenoid content is mostly made up of diterpene alcohols, tetracyclic terpenoic acids, and pentacyclic triterpenes, such as boswellic acids. Boswellic acid (BA), a triterpenic acid, and its corresponding acetates, ABA and acetyl 11-keto-β-boswellic acid (AKBA), are the main forms of Boswellic acid [A. Sharma et al,.2009].

2.4. Pharmacological Activity of Boswellia Serrata

I. Antimicrobial activity

Boswellic acids: novel, specific, non-redox inhibitors of 5-lipoxygenase BA and derivatives concentration-dependently decreased the formation of leukotriene B4 from endogenous arachidonic acid in rat peritoneal neutrophils is [Nameeta Pilkhwal et al,.2019; Aman Upaganlawar et al,.2009].

II. Antidiarrhoeal

It has been discovered that Boswellia serrata extract (BSE) effectively treats diarrhoea in patients with inflammatory bowel syndrome without making them feel constipated. It was also discovered to be useful in preventing diarrhoea caused by acetylcholine and barium chloride by preventing the contraction of intestinal smooth muscles. [Nameeta Pilkhwal et al,.2019; Aman Upaganlawar et al,.2009].

III. Hypoglycemic

Action In a streptozocin-induced diabetic rat model, a herbal formulation containing B. serrata oleo-gum-resin as one of the ingredients has been shown to produce significant anti-diabetic activity, with a reduction in blood glucose levels comparable to that of phenformin [Nameeta Pilkhwal et al,.2019; Aman Upaganlawar et al,.2009].

IV. Hypolipidemic and Hepatoprotective activity

The water soluble portion of B. serrata extract demonstrated its hypolipidemic potential by reducing total cholesterol (38–48%) and raising HDL in rats given an atherogenic diet [Sudhanshu Mishra et al,.2020].

V. Anticancer Activity

When mice with ehrlic ascites carcinoma and S-180 tumours were given an alcoholic salai guggal (AESG) extract for anti-carcinogenicity, the results showed that the extract inhibited tumour growth by preventing cell proliferation and expansion because it interfered with the manufacture of proteins, DNA, and RNA [Sudhanshu Mishra et al,.2020].

VI. Immunomodulatory activity

The antianaphylactic and mast cell stabilising properties of an extract of B. serrata gum resin, which contains 60% acetyl 11-keto beta boswellic acid (AKBA) and other constituents like beta-boswellic acid, acetyl beta boswellic acid, and 11-keto beta-boswellic acid (KBA), have

been assessed using passive paw anaphylaxis and compound 48/80 induced degranulation of mast cell techniques[Sudhanshu Mishra et al,.2020].

VII. Anti inflammatory and Anti-arthritic activity's

When salai guggal extract was given orally (p.o.) in dose ranges of 50-200mg per kg -1 and intraperitoneally (i.p.) in dose ranges of 50-100mg per kg -1, respectively, it inhibited the carrageenan-induced rat hind paw oedema by 39.75% and 65-73%, as opposed to 47% inhibition observed with phenylbutazone (50mg/kg -1 p.o.). Salai guggal, at 50 and 100mg per kg -1 (p.o.) dosages, respectively, demonstrated a 49% and 34% suppression of paw swelling in the anti-arthritic investigation on the mycobacterium adjuvant-induced poly-arthritic in rats as compared to controls [Aman Upaganlawar et al,.2009].

VIII. Anti-asthmatic activity

Gupta et al. (1998) established the anti-asthmatic potential of alcohol extract of salai guggal (AESG) in a double blind placebo control clinical study with 300 mg thrice daily dose for 6 weeks. 70% of the patients with a prolonged history of asthma showed improvement in physical symptoms and signs of dyspnea, bronchi, number of attacks, and increase in stimulation of mitogen activated protein kinase MAPK and intracellular Ca2+ [Nameeta Pilkhwal et al,.2019].

IX. Anti-depressant

In an experimental animal model, the current study has demonstrated the significant antidepressant activity of Boswellia serrata. Thus, it may serve as a substitute for traditional antidepressant medications. To determine the safety and effectiveness profiles of Boswellia serrata, more research is necessary [Nameeta Pilkhwal et al,.2019; Aman Upaganlawar et al,.2009].

X. Anti-Alzheimer's Activity

An ongoing neurological illness is Alzheimer's disease (AD). Elevated oxidative stress has been shown to be a common and early characteristic of AD [Sudhanshu Mishra et al,.2020]. Antioxidant-producing medicinal plants are extensively employed in the treatment of several human illnesses [Isha Kumari et al,.2021]. Boswellia has the ability to cure AlCl3-induced Alzheimer's disease by raising Ach levels and lowering AchE activity in brain homogenates [Nameeta Pilkhwal et al,.2019; Aman Upaganlawar et al,.2009].

Literature Review

SrNo	Journal	Author, Year, & Pageno.	Title	Conclusion
1	THE PHARMA INNOVATION - JOURNAL .	Prabhakar Adake *, Chandrashekar R , S.N. Rao May 2013, Pages 1-6	Preclinical evaluation of antidepressant activity of Boswellia serrata by Tail Suspension Test	Present study has shown Boswellia serrata has significant antidepressant activity in experimental animal model. Hence can be an alternative to conventional antidepressant drugs. However, further studies are required to reveal both efficacy and safety profile of Boswellia serrata.
2	Ethnobotanical Leaflets	Aman Upaganlawar1* and Balu Ghule2 june2009; 1-9	Pharmacological Activities of Boswellia serrata Roxb	Present study has shown Boswellia serrata has significant antidepressant, Hypoglycemic Antimicribial, analgesic and antiinflamatory, anti cancer, anti asthamatic activity.
3	World Journal of Pharmaceutical Research	Amena Alam Shanta1*, K. M. Mazedul Adnan1, Md. Billal Hossain1, Tahani Jashim2, Rijve Ahamed1, Tashdid Binte Kamrull, Fatema-Tuz-Zohra1, Fahima Akhter Purni1, Farhan Rashid3 and Sanzida Khondoker1 2 August 2020 Volume 9 2021	In Vivo Evaluation Of Antidiabetic And Antidepressant Potential And Side Effect Study Of Aqueous Extract Of Leaves Of Centella Asiatica On Alloxan-Induced Diabetic Rat Model	From the mentioned outcomes, it can easily be said that the leaf extract of Centella asiatica impart alike metformin however the effect is somewhat lower than that of metformin. But this difference does not bear any statistical significance, also it improved the pathological state like SGPT, SGOT, and creatinine level as like as the hypoglycemic effect. Moreover, the data received from healthy rats those who were fed the extract, were similar to the negative control group. Hence we may infer that our plant can be applied in the control of diabetes mellitus type
4	Elsevier	Ahmed Al-Harrasi, Rene Csuk, Ajmal Khan, Javid Hussain et al 2019 May; Volume 161	Distribution of the anti-inflammatory and anti-depressant compounds: Incensole and incensole acetate in genus Boswellia	In the present study, NLC based in-situ gelling intranasal formulation was developed for CBZ. The optimized formulation consisted of CBZ NLC incorporated into in-situ gel formulations prepared using poloxamer 407 and 188 along with chitosan as mucoadhesive polymer. The optimized formulation demonstrated excellent gelling ability, mucoadhesion, and gelling temperature in the range of 30-35°C and exhibited sustained drug release behavior up to 8 hours.
5	Int. J. Pharm.	Isha Kumari,	Boswellia serrata	Boswellia serrata is a globally recognized

	Sci.	Gitika Chaudhary, 2021; Article No. 24, Pages: 161-169	ROXB. EX COLEBR. (Salai): An Ayurvedic Herb with Anti-inflammatory Potential	medicinal plant for its anti-inflammatory and anti-arthritis potential. It is the most common plant species used in the folk medicine system to treat a variety of diseases. It has also been mentioned in many Ayurvedic texts. Boswellia serrata gum resin has many uses including therapeutic as well as non therapeutic.

3. AIM AND OBJECTIVES

3.1. Pharmacological evaluation of standardized extract of boswellia serrata on diabetes induced depression in laboratory rats.

To determine the effect of standardized extract of boswellia serrata (100, 200 and 400mg/kg, p.o) on alloxan induced depression in laboratory rats by following parameters.

3.1.1 In Vivo parameters.

I. Body weight

II. Food intake

III. Water intake

IV. Urine output

V. Fasting Blood Glucose Level

VI. Elevated plus maze test:

i. Time spent in open arm

ii. Number of entries in open arm

iii. Time spent in close arm

iv. Number of entries in close arm

v. Time spent in centre

VII. Forced swim test:

i. Duration of Immobility

VIII. Tail suspension test:

i. Duration of Immobility

3.1.2. Ex-vivo parameters:

1. Oxidative stress (SOD, GSH, MDA, Nitric oxide and Total Protein) in brain
2. Brain monoamines (5-HT and DA)
3. Brain GABA levels

4. Need of Work

Diabetes is widely recognised as one of the leading causes of death and disability worldwide (American Diabetes Association, 2010). The prevalence of diabetes will rise from 6% to over 10% in the next decade (Rosen et al., 2001). In 2000, the World Health Organization (WHO) recorded a total of 171 million people for all age groups worldwide (2.8% of the global population) who have diabetes, and the numbers are expected to rise to 366 million (4.4% of the global population) by 2030 (Wild et al., 2004).

Long-term hyperglycaemia is considered to be one of the most important risk factors for the development of diabetic complications including depression (The Diabetes Control Complications Trial Research Group, 1995). Diabetes has been reported to induce behavioural changes in animals. The development of preclinical diabetes models, with tests of neurobehavioral complications similar to those experienced by humans, can help elucidate the pathophysiology underlying comorbid depressive symptoms and identify specific targets for therapy. Alloxan-induced diabetes has demonstrated consequences for depressive behavior, such as increased immobility in the mouse tail suspension test.

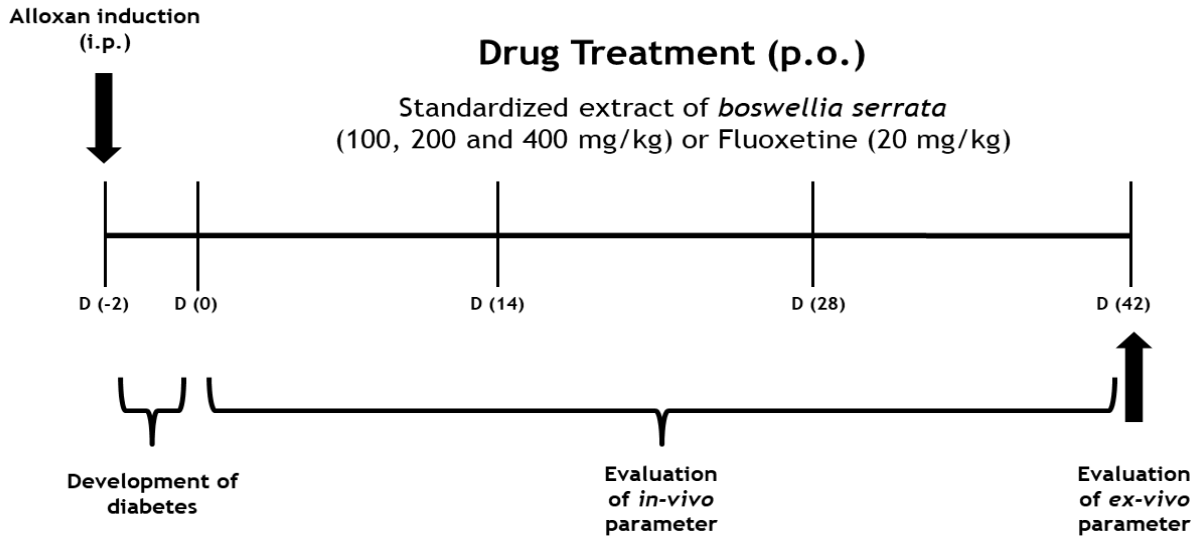
Diabetes-associated depression could be related to the changes in the quality of life imposed by the chronic illness and/or its treatment, or may be a consequence of neurochemical changes induced by the disease. The most frequently studied neurochemical alterations related to depression include changes in the neurotransmitters serotonin, dopamine, noradrenaline, and gamma-aminobutyric acid (GABA). Reduced brain tryptophan levels and decreased brain turnover of cathecolamines and serotonin in diabetic rats suggests involvement of decreased monamine activity in the genesis of diabetes associated depression. Other studies have shown that streptozotocin-induced diabetes unbalances GABA, noradrenaline, serotonin, and monoamine metabolite concentration in the brain (Radahmadi et al., 2006, Gaur et al., 2012, Ho et al., 2012).

Studies show that the active components of B.S. resin have anti-diabetic activity. Boswellic acids is a responsible for anti-depressant action and acetyl-11-keto-betaboswellic acid show the antidiabetic, anti-depressant, anti-inflammatory activity.

Provide information on diabetes induced depression and current treatment with their disadvantages.

Hence, there is need of a therapy which will offer total cure and will be freed of above disadvantages. In the wake of prominent adverse effects of modern medicine, herbal drugs provide a safe therapeutic option for treatment of diabetic complication.

5. Plan of work



Paradigm of experiment

6. Material and Methods

6.1.1. Animals

Sprague Dawley rats weighing 180-200 gm were purchased from Global Bioresearch Solutions Private Limited, H No 251 Nhavi, Tal - Bhor, Dist- Pune. The animals were housed in polypropylene cages and maintained under the environmental condition of temperature 25±1 ºC and relative humidity of 45-55 % under a 12h light: 12 dark cycles. The animals had free access to food pellets (Nav Maharashtra Chakan oil mills Ltd., Pune) and water ad libitum. The Institutional Animal Ethics Committee (IAEC) of new experiments or extensions of ongoing experiments using animals other than non-human primates approved all the experimental protocols under the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA). The protocol approval number is (CPCSEA Reg. No. 2168/PO/Re/S/22/CPCSEA).

6.1.2. Chemicals

Name of chemical	specification	Manufacturer's name	quantity purchased	batch number	storage conditions
Stand. extract of boswellia serrata	I.P.	Natural Remedies Pvt. Ltd., Bangalore	1 kg	BS/11028	2-8 ºC
NADPH	I.P.	Sigma –Aldrich, India	25 mg	---	2-8 ºC
Alloxan	I.P.		25 mg	A7413-25G	
ATP	I.P.		1 gm	---	
Anaesthetic Ether	I.P.	Narson Pharma, Chittor, India	500 ml X 4	AE-06	R.T.
Acetic acid	I.P.	Merck Specialities Pvt. Ltd., Mumbai, India	500 ml	AD4A540152	R.T.
Trichloro acetic acid	I.P.		500 gm	MI9M592253	R.T.
Potassium dihydrogen Phosphate			250 gm	-	R.T.
Potassium hydroxide	I.P.		500 gm	MH9M591251	R.T.
Potassium chloride	I.P.		500 gm	ML9M593064	R.T.
Folin phenol reagent	I.P.		100g	QC2Q620407	R.T.
Chloroform	I.P.		2.5 lit	ll1lf61535	R.T.
EDTA	I.P.	SISCO Research Lab. Pvt. Ltd., Mumbai – 400 099, India	100 gm	T/8 3001162	R.T.
Sodium Phosphate (Dibasic)	I.P.	Himedia Lab. Pvt. Ltd., Mumbai- 400 806, India	500 gm	T-835005	R.T.
Adenosine triphosphate	I.P.		5 gm	0000064674	R.T.
Tris Free Base	I.P.		100 gm	MB029	R.T.
Boric Acid	I.P.		100 gm	MB007	R.T.
Epinephrine	I.P.		5 gm	0000066488	R.T
Tris HCl	I.P.		100 gm	0000049048	R.T.
Sulphanilamide	I.P.	LobaChemi Pvt. Ltd., Mumbai – 400 005	100 gm	GM012210	R.T
Phosphoric acid	I.P.		500 ml	LG012010	R.T
Naphthalamine Diamine HCl	I.P.		10 gm	LB224509	R.T
Magnesium sulphate	I.P.		500 gm	v 209205	R.T
Sodium carbonate	I.P.		500 gm	A 283807	R.T
Sodium pottasium tartrate	I.P.		500 gm	A 566809	R.T
Formaldehyde	I.P.		500 ml	LB 241809	R.T
Ammonium molybdate	I.P.		100 gm	SL29471205	R.T.
Sodium pottasium			500 g	GB 27691109	R.T.

tartrate	I.P.				
Potassium dihydrogen orthophosphate	I.P.		2.5 lit	MCR-6320	R.T.
Sodium sulphite	I.P.	Molychem B-9, MIDC industrial area, Badlapur, dist Thane 421 503, India Research lab fine Mumbai 400(002), India	500 g	01425090612	R.T.
Methanol	I.P.		2.5 lit	MCRT-5162	R.T.
Hydrochloric acid	I.P.	MP Biomedicals India Private Limited, India	AS003	500 gm	R.T
Sodium hydroxide	I.P.		---	500 gm	R.T
Copper sulphate	I.P.		PCT0104-500G	500 gm	R.T.
Sulphuric acid	I.P.	Fisher scientific Powai, Mumbai	AS016	500 ml	R.T
O –Pthalaldehyde	I.P.	Analab fine chemicals Mumbai - 400083 (India)	---	5 gm	R.T
Ninhydrin	I.P.		491200010	10 gm	R.T
n-Heptane	I.P.	3B Black Bio Biotech India Ltd.	3B1159	2.5 lit	R.T
n-butanol	I.P.		3B1102	2.5 lit	R.T
thiobarbituric acid	I.P.		3B1154	100 gm	R.T
Trichloroacetic	I.P.		3B1155	100 gm	R.T
Sucrose	I.P.	Fisher scientific Powai, Mumbai	500 gm	1043/1	R.T.

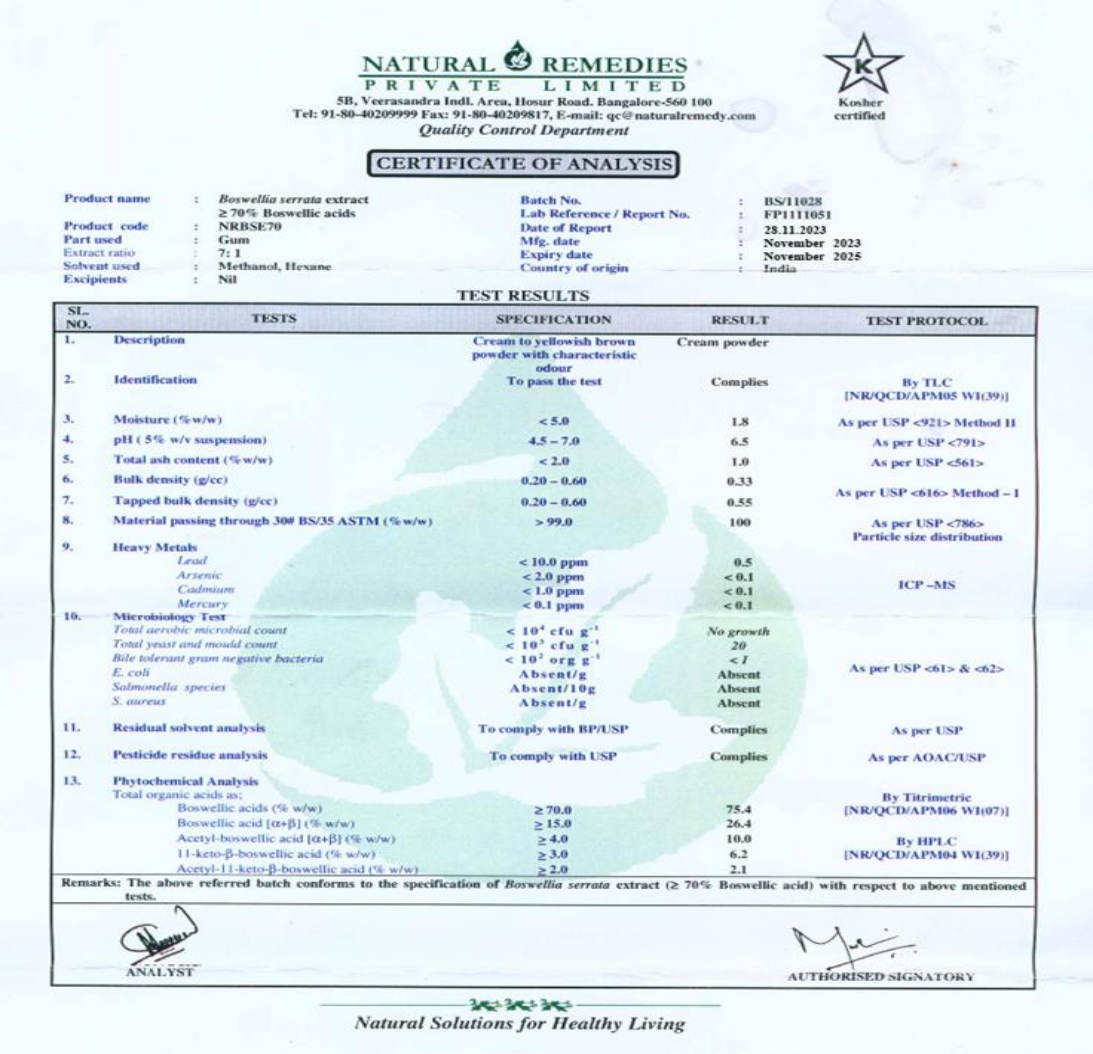
6.1.3. Instruments Used

Name of equipment	Model and make	Manufacturer's name	Address, city, country
Spectrofluorometer	Jasco F-8200	JASCO Benelux B.V.	Veldzigt 2a, 3454 PW de Meern
UV Spectrophotometer	V-630 Sr. No. B157261148	Jasco	Japan
Elevated Plus maze	---	VJ Instruments	Washim, India
Centrifuge	Remi RC4 Lab. Centrifuge	Remi Motors Ltd.	Mumbai – 400 058, India
Glucometer	Freestyle Optium H Blood Glucose monitoring system	Abbott Diabetes Care Ltd.	Rnage Road, Witney, Oxon, UK
Animal weighing electronic balance	CB-220	Contech Instruments Co.	Delhi
Chemical weighing balance	AB-204-S, Metler Tolledo	Classic made	Switzerland
Tissue Homogenizer	RQ-127A	Remi Equipment Pvt. Ltd.	Mumbai, India
Metabolic cage	---	Techniplast	Italy

6.1.1. Preparation and standardization of methanolic extract of boswellia serrate.

The standardised extract of boswellia serrata was obtained from Natural Remedies Pvt. Ltd., Bangalore. The methanolic extract of boswellia serrata contain ≥70% boswellic acids.

Certificate of analysis of standardized extract of boswellia serrata



NATURAL REMEDIES
PRIVATE LIMITED
5B, Veerasandra Indl. Area, Hosur Road. Bangalore-560 100
Tel: 91-80-40209999 Fax: 91-80-40209817, E-mail: qc@naturalremedy.com
Quality Control Department

Kosher certified

CERTIFICATE OF ANALYSIS

Product name	:	*Boswellia serrata* extract ≥ 70% Boswellic acids	Batch No.	:	BS/11028
Product code	:	NRBSE70	Lab Reference / Report No.	:	FP1111051
Part used	:	Gum	Date of Report	:	28.11.2023
Extract ratio	:	7: 1	Mfg. date	:	November 2023
Solvent used	:	Methanol, Hexane	Expiry date	:	November 2025
Excipients	:	Nil	Country of origin	:	India

TEST RESULTS

SL. NO.	TESTS	SPECIFICATION	RESULT	TEST PROTOCOL
1.	Description	Cream to yellowish brown powder with characteristic odour	Cream powder	
2.	Identification	To pass the test	Complies	By TLC [NR/QCD/APM05 W1(39)]
3.	Moisture (% w/w)	< 5.0	1.8	As per USP <921> Method II
4.	pH (5% w/v suspension)	4.5 – 7.0	6.5	As per USP <791>
5.	Total ash content (% w/w)	< 2.0	1.0	As per USP <561>
6.	Bulk density (g/cc)	0.20 – 0.60	0.33	As per USP <616> Method – I
7.	Tapped bulk density (g/cc)	0.20 – 0.60	0.55	
8.	Material passing through 30# BS/35 ASTM (% w/w)	> 99.0	100	As per USP <786> Particle size distribution
9.	Heavy Metals			
	Lead	< 10.0 ppm	0.5	ICP –MS
	Arsenic	< 2.0 ppm	< 0.1	
	Cadmium	< 1.0 ppm	< 0.1	
	Mercury	< 0.1 ppm	< 0.1	
10.	Microbiology Test			
	Total aerobic microbial count	< 10^4 cfu g^{-1}	*No growth*	As per USP <61> & <62>
	Total yeast and mould count	< 10^3 cfu g^{-1}	*20*	
	Bile tolerant gram negative bacteria	< 10^2 org g^{-1}	*< 1*	
	E. coli	Absent/g	Absent	
	Salmonella species	Absent/10g	Absent	
	S. aureus	Absent/g	Absent	
11.	Residual solvent analysis	To comply with BP/USP	Complies	As per USP
12.	Pesticide residue analysis	To comply with USP	Complies	As per AOAC/USP
13.	Phytochemical Analysis			
	Total organic acids as:			By Titrimetric [NR/QCD/APM06 W1(07)]
	Boswellic acids (% w/w)	≥ 70.0	75.4	
	Boswellic acid [α+β] (% w/w)	≥ 15.0	26.4	
	Acetyl-boswellic acid [α+β] (% w/w)	≥ 4.0	10.0	By HPLC [NR/QCD/APM04 W1(39)]
	11-keto-β-boswellic acid (% w/w)	≥ 3.0	6.2	
	Acetyl-11-keto-β-boswellic acid (% w/w)	≥ 2.0	2.1	

Remarks: The above referred batch conforms to the specification of *Boswellia serrata* extract (≥ 70% Boswellic acid) with respect to above mentioned tests.

ANALYST

AUTHORISED SIGNATORY

Natural Solutions for Healthy Living

6.1.4. Preparation of drug solution, storage, volume, and route of administration:

6.1.4.1. Standardized extract of boswellia serrata:

i. Preparation of test drug solution:

a. Drug solution of Standardized extract of boswellia serrata was prepared by using distilled water a vehicle

ii. Storage of drug solution:

a. Standardized extract of boswellia serrata powder was stored in a desiccator. A fresh drug solution was prepared for each day's work. The solution was kept in airtight amber-colored bottles and stored at room temperature until ready for use.

iii. The volume of drug administration:

a. The volume of Standardized extract of boswellia serrata solution to be administered was calculated based upon the body weight of animals.

iv. Route of administration:

a. In the alloxan-induced diabetes mellitus model, the Standardized extract of boswellia serrata solution was administered per oral (p.o.) route.

6.1.4.2. Fluoxetine

i. Preparation of standard drug solution:

a. Solution of fluoxetine was prepared with 1% Sodium-carboxy methylcellulose as the vehicle.

ii. Storage of drug solution:

a. Fluoxetine powder was stored in a refrigerator below 25 ºC. A fresh drug solution was prepared for each day's work.

iii. The volume of drug administration:

a. The volume of fluoxetine solution to be administered was calculated based upon the body weight of animals.

iv. Route of administration:

a. Fluoxetine solution was administered through per oral (p.o.) route.

6.1.5. Alloxan induced diabetes mellitus in laboratory animals

6.1.5.1. Experimental designs.

The animals were divided randomly into groups with six rats per group as follows:

i. Group I: Normal group

a. The rats received only vehicle (Distilled water) p.o., for 6 weeks.

ii. Group II: Diabetes control

a. The rats receive alloxan (150 mg/kg) and only vehicle (Distilled water) p.o.,6 weeks.

iii. Group III: Fluoxetin (10) treated group

a. The rats have received alloxan (150 mg/kg). They were treated with Fluoxetin at a dose of 10mg/kg, p.o., for 6 weeks.

iv. Group IV: Standardized extract of boswellia serrata (100) treated group

a. The rats have received alloxan (150 mg/kg). They were treated with Standardized extract of boswellia serrata at a low dose of 100mg/kg, p.o for 6 weeks.

v. Group V: Standardized extract of boswellia serrata (200) treated group

a. The rats have received alloxan (200mg/kg). They were treated with Standardized extract of boswellia serrata at a medium dose of 50 mg/kg, p.o for 6 weeks.

vi. Group VI: Standardized extract of boswellia serrata (400) treated group

a. The rats have received alloxan (150 mg/kg). They were treated with Standardized extract of boswellia serrata at a high dose of 400mg/kg, p.o for 6 weeks.

6.1.5.2. Induction of diabetes:

A. Preparation of alloxan

i. For each experiment, aliquots of alloxan monohydrate from the same batch were pre-weighed into plastic microfuge tubes, then wrapped in aluminum foil (to protect against light sensitivity) and stored at -20°C with desiccant until use. Alloxan monohydrate dissolved in 0.9% w/v cold normal saline, and the unused contents were discarded after each experiment.

ii. All the required parameters were carried out in all selected rats before diabetes induction.

iii. All animals fasted for 16 hours before alloxan injection.

iv. Diabetes was induced by a single injection of alloxan (150 mg/kg) according to the body weight of animals.

v. 10% sucrose solution was provided after four hours of alloxan injection to avoid hypoglycemic shocks.

vi. Diabetes was confirmed after 48 hours of injection by glucometer.

vii. Glucose level above 250 mg/dl was considered for the study.

viii. Oral treatment with Standardized extract of boswellia serrata (100, 200 and 400mg/kg) or Fluoxetin (10mg/kg) continued for 42 days.

ix. On the 42nd day, animals were sacrificed for ex-vivo parameters.

6.1.5.3. Treatment of Standardized extract of boswellia serrata and fluoxetine

Standardized extract of boswellia serrata doses (100, 200 and 400mg/kg) and fluoxetine (10mg/kg) with different calculated doses based on the animal's body weight were administered per oral from a day of confirmation to 42 days.

The observations were recorded on days 0, 14, 28, and 42 in the morning, and doses were administrated immediately afterward.

6.1. Parameter for assessment of the effect of Standardized extract of Boswellia serrata on alloxan-induced diabetic depression in rats.

6.2.1. In-vivo parameters

6.2.1.1. Body weight, food intake, water intake, and urinary output

i. Rats were weighed daily using animal weighing balance.

ii. Food intake, water intake, and urinary output were determined by using a metabolic cage.

6.2.1.2. Blood parameter

i. The plasma glucose levels were determined daily by using a glucometer.

6.2.1.3. Elevated plus maze test

I. The maze is made of plywood and consists of two open arms 30 X 5 cm and two enclosed arms 30 X 5 X 20 cm. The arms extend from a central 5 X 5cm platform. The maze is dark brown and mounted to the wooded base, raising at 30 cm above the flooring in a dark room. The light of 40W is illuminated above the maze.

II. Before starting the experiment, the rats were handled daily to reduce stress. Two hours after the oral administration of the test drugs, the animal was placed in the center of the maze, facing one of the enclosed arms. After that, the number of entries and time spent in the open and closed arms were recorded during the next 5 min. The following parameters were measured: an arm entry being defined when all four paws are in the arm.

i. Number of open arm entries

ii. Number of closed arm entries

iii. Time spent in open arm

iv. Time spent in closed arm

v. Time spent in a central square

III. At the end of each trial, the apparatus was wiped clean to eliminate any olfactory clues, which might modify the behavior of the next animal. The procedure was conducted preferably in a sound-attenuated room, with observations made from an adjacent room.

6.2.1.4. Tail suspension test

i. In this TST, rats were individually suspended by the tail from an aluminum hook raised 20 cm above the floor using adhesive tape placed 2 cm from the tip of the tail. The rats were positioned such that the base of their tail was aligned with the horizontal plane.

ii. Typically, rats demonstrated escape-oriented behavior interspersed with successively longer bouts of immobility. Test sessions lasted for 6 min, and they were videotaped and subsequently scored by a trained observer.

6.2.1.5. Forced swimming test:

i. The FST apparatus was a transparent Plexiglas cylinder (20 cm diameter, 60.5 cm height). The cylinders were filled with 25±1°C water to a 44.5 cm depth to prevent animals' tails from touching the bottom of the tank. The animals were placed in the cylinders for 5 min. Animals were towel-dried after each exposure and returned to preheated home cages.

ii. Duration of immobility was measured, i.e., immobility is when the rat performs the minimum movement necessary to stay afloat.

6.2.2. Ex-vivo parameters:

6.2.2.1. Determination of brain oxidative stress:

i. All animals were sacrificed at the end of the study, i.e., 42nd day. The brain was immediately isolated.

ii. Tissue homogenate was prepared with 0.1M Tris-HCl buffer (pH 7.4), and supernatant of homogenate was employed to estimate lipid peroxidation (MDA content), superoxide dismutase (SOD), reduced glutathione (GSH), nitric oxide (NO), and total protein.

6.2.2.2.1. Determination of Lipid Peroxidation (MDA content):

i. It was estimated using the method described by Slater and Sawyer (1971). 2.0 ml of the tissue homogenate (supernatant) was added to 2.0 ml of freshly prepared 10% w/v trichloroacetic acid (TCA), and the mixture was allowed to stand in an ice bath for 15 minutes. After 15 minutes, the precipitate was separated by centrifugation, and 2.0 ml of the clear supernatant solution was mixed with 2.0 ml of freshly prepared thiobarbituric acid (TBA).

ii. The resulting solution was heated in a boiling water bath for 10 minutes. It was then immediately cooled in an ice bath for 5 minutes. The color developed was measured at 532 nm against the reagent blank. Different concentrations (0-23nM) of standard malondialdehyde were taken and processed for the standard graph.

iii. The values were expressed as nM of MDA/mg protein.

6.2.2.2.2. Determination of Superoxide Dismutase (SOD)

i. Superoxide dismutase was estimated using the method developed by Misera and Fridovich (1972). 0.5 ml of tissue homogenate was diluted with 0.5 ml of distilled water, to which 0.25 ml of ice-cold ethanol and 0.15 ml of ice-cold chloroform were added. The mixture was mixed well using a cyclo mixer for 5 minutes and centrifuged at 2500 rpm. To 0.5ml of supernatant, 1.5 ml of carbonate buffer, and 0.5 ml of EDTA solution were added.
ii. The reaction was initiated by adding 0.4 ml of epinephrine, and the change in optical density/minute was measured at 480 nm against the reagent blank. SOD activity was expressed as units/mg protein. Change in optical density per minute at 50 % inhibition of epinephrine to adrenochrome transition by the enzyme is taken as the enzyme unit.
iii. The calibration curve was prepared by using 10-125 units of SOD.

6.2.2.2.3. Determination of Reduced glutathione (GSH):

i. Reduced glutathione was determined by the method described by Moron et al. (1979). Equal volumes of tissue homogenate (supernatant) and 20% TCA were mixed. The precipitated fraction was centrifuged, and to 0.25 ml of supernatant, 2 ml of DTNB reagent was added.
ii. The final volume was made up to 3ml with phosphate buffer. The color developed was read at 412 nm against the reagent blank. Different concentrations (10-50 gm) of standard glutathione were taken and processed for the standard graph.
iii. Reduced glutathione was expressed as µg of GSH / mg protein.

6.2.2.2.4. Determination of nitric oxide (NO):

i. The NO level was estimated as nitrite by the acidic Griess reaction after reducing nitrate to nitrite by vanadium trichloride, according to Miranda et al., 2001.
ii. The Griess reaction relies on a simple colorimetric reaction between nitrite, sulfonamide, and N-(1- naphthyl) ethylenediamine to produce a pink azo-product with maximum absorbance at 543 nm.
iii. The concentrations were determined using a standard curve of sodium nitrate, and the results were expressed in µg/mg protein.

6.2.2.2.5. Determination of tissue protein

i. According to the Lowry et al. (1951) method, protein concentration was estimated using BSA (bovine serum albumin) as a standard. Briefly, dilute tissue fraction aliquots (0.1 ml) were taken in a test tube.
ii. To this, 0.8 ml of 0.1 M sodium hydroxide and 5.0 ml Lowry C reagent was added, and the solution was allowed to stand for 15 min. Then 0.5 ml of Folin phenol reagent was added, and the contents were mixed by vortex mixer. The color developed was measured at 660 nm against reagent blank containing distilled water instead of sample.
iii. Different concentrations (40-200 µg) of BSA were taken and processed as above for the standard graph.
iv. The values were expressed as mg of protein/ gm of wet tissue (mg/gm).

6.2.2.2. Determination of brain monoamine

i. The brain was isolated immediately and transferred to a homogenization tube containing 5 ml of 0.01 N hydrochloric acid and homogenized. Brain homogenate was transferred to a bottle containing 8 ml of ice-cold absolute alcohol and kept for 1 hour at 0 ºC. The content was centrifuged for 10 min at 16000 rpm, and the supernatant was collected in a Petri dish. The residue was washed with 3-5 ml of 75% alcohol three times, and washes were combined with supernatant. Contents in the Petri dish were evaporated to dryness at 70-90 0C on a water bath under a stream of air. To the dry mass, 1 ml water and 2 ml chloroform were added and centrifuged at 2000 rpm. The upper phase containing GABA was separated, and 10 ul of it was applied as spot on Whatman paper (No. 41).
ii. The mobile phase consisted of n-butanol (50 ml), acetic acid (12 ml), and water (60 ml). The chamber was saturated for a half-hour with the mobile phase. The paper chromatogram was developed with ascending technique. The paper was dried in hot air and then spread with 0.5% ninhydrin solution in 95% ethanol. The paper was dried for 1 hr at 90 0C. Blue color spot developed on paper was cut and heated with 2 ml ninhydrin solution on a water bath for 5 min. Water (5 ml) was added to the solution and kept for 1h. The supernatant was decanted, and absorbance was measured at 570 nm.
iii. A stock solution of standard GABA, 1 mg/ml, was prepared in 0.01N HCl. Serial dilutions were prepared to get concentrations 1ng/10ul to 1000ng/10ul. To obtain a standard concentration curve for GABA same procedure was followed, replacing brain homogenate with standard GABA solutions (Maynert et al., 1962).

6.2.2.3. Determination of brain 5-hydroxytryptamine:

i. In 2.5 ml of brain homogenate in acidified butanol, 0.1 ml of 0.1 N HCI containing 0.025-10.0/zg of 5HT were added, followed by 0.6 ml of a freshly prepared solution of o-phthalaldehyde (4 mg/100 ml) in 10 N HCI.

ii. After mixing with a vibrating mixer, the tubes were placed in a boiling water bath for 15 min, then removed and cooled in tap water. Fluorescence was measured in a spectrophotofluorometer. Activation and emission wavelengths were set at 360 nm and 470 nm, respectively, to determine the concentration of 5-HT (Green and Curzon, 1968).

6.2.2.4. Determination of brain dopamine

i. The oxidation procedure was reported by Fleming et al. (1965). In 1.25 ml of brain homogenate in 0.1 ml of 0.1N HCI were added and 0.25 ml of 1M phosphate buffer added.

ii. 0.025 ml of 1N iodine in absolute ethanol was added. After mixing, the tubes were allowed to stand exactly two minutes at room temperature, then 0.2 ml of a freshly prepared alkaline sulfite solution (2.5 g of anhydrous Na2SO3 in water and 9 ml of 5N NaOH) was added to each tube.

iii. The tubes were mixed again, then after exactly 1.5 min, 0.2 ml of 5N acetic acid were added, and the tubes were heated in a boiling water bath for 2 min.

iv. The fluorescence was measured after cooling in tap water at activation and emission wavelengths of 378 nm and 335 nm, respectively, to determine dopamine concentration (Schlumpf et al., 1974).

6.2.Statistical Analysis

The arithmetic means±SEM values were calculated for each experiment. The statistical analyses were carried out with the help of Graph pad Prism v 5.0.

7. RESULTS

7.1. Effect of boswellia serrata on diabetes-induced alteration in body weight,

Time (in week)	Body weight (gm) Mean±SEM					
	Normal	Diabetic control	Fluoxetine (10mg/kg)	BS (100mg/kg)	BS (200mg/kg)	BS (400mg/kg)
0	313.67±3.67	312.00±6.61	312.83±11.80	314.33±6.63	310.67±6.35	300.33±5.09
2	324.17±3.47	280.50±5.89$^{###}$	283.17±7.40	288.17±7.89	280.67±9.64	275.33±9.28
4	334.33±4.00	264.50±5.33$^{###}$	294.83±8.02**	276.67±7.41	273.33±13.32	278.50±10.19
6	349.67±3.21	245.33±6.05$^{###}$	306.17±10.99 ***	268.67±5.35	266.83±13.93	297.00±10.98 ***

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{###}P < 0.001$) and For comparison with diabetic control group (**P < 0.01, and ***P < 0.001) on respective days.

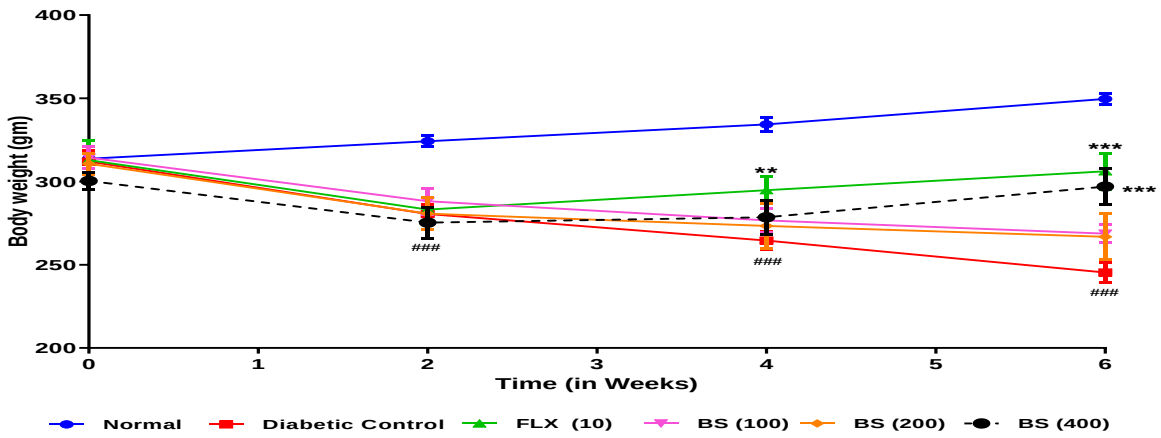


Fig. No. 7.1. Effect of boswellia serrata on diabetes-induced alteration in body weight Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{###}P < 0.001$) and For comparison with diabetic control group (P < 0.01, and ***P < 0.001) on respective days.**

Before induction of diabetes there was no prominent difference in the body weight of diabetic control rats than normal rats. Intraperitoneal administration of alloxan results evidently lessened (P < 0.001) in the diabetic control rats' body weight than normal rats after two weeks. Fluoxetine at a dose of 10mg/kg treated group evidently ameliorated (P < 0.01 and P < 0.001) the lessened body weight of the rats than the diabetic control rats on the 4th and 6th week. Additionally, treatment with Boswellia serrata at a dose of 400mg/kg evidently ameliorated reduced (P < 0.01 and P < 0.001) body weight of the diabetic rats after 4 weeks onwards.

7.2. Effect of boswellia serrata on diabetes-induced alteration in plasma glucose levels:

Time (in week)	Plasma glucose level (mg/dl) Mean±SEM					
	Normal	Diabetic control	Fluoxetine (10mg/kg)	BS (100mg/kg)	BS (200mg/kg)	BS (400mg/kg)
0	66.83±3.88	74.83±4.08	68.67±6.66	73.67±4.88	71.00±5.16	72.50±4.15
2	79.33±1.74	389.5±15.14$^{###}$	328.33±7.00*	340.00±25.20	350.83±8.66	322.67±24.96*
4	79.17±1.30	406.5±13.39$^{###}$	259.67±11.41***	393.33±24.35	354.50±6.78	265.83±25.60***
6	84.5±1.12	415.33±9.81$^{###}$	171.67±15.97***	365.83±23.67	324.50±8.07	236.83±26.48***

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with diabetic control group (*P < 0.05, and ***P < 0.001) and For comparison with normal group ($^{###}$P < 0.001) on respective days.

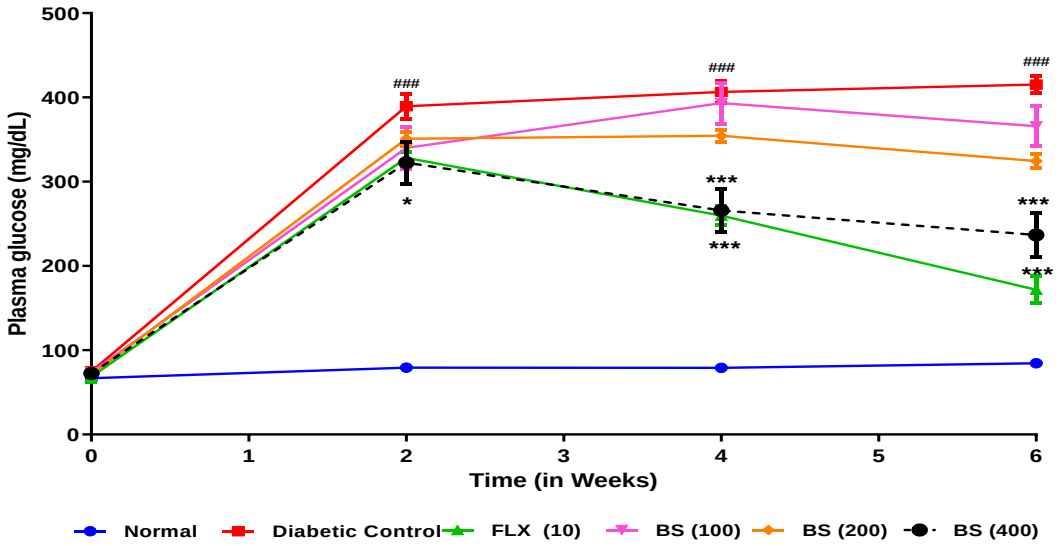


Fig. No. 7.2. Effect of boswellia serrata on diabetes-induced alteration in plasma glucose levels.

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with diabetic control group (*P < 0.05, and ***P < 0.001) and For comparison with normal group ($^{###}$P < 0.001) on respective days

There was no change in serum glucose level in control rats than the normal rats at 0 week. Administration alloxan of control group rats showed a evidently amplified (P < 0.001) in serum glucose level after week 2 than the normal rats. Treatment with Fluoxetine at a dose of 10mg/kg show effective lessened (P < 0.05, P < 0.001 and P < 0.01) in serum glucose level at week 2, 5 and 6 respectively than the diabetic control rats. Whereas, after treatment with Boswellia serrata at a dose of 400mg/kg show meaningful reduced (P < 0.001) in serum glucose level from 4th week onwards. Boswellia serrata (100 and 200mg/kg, p.o.) treated rats failed to produce any effective decrease in serum glucose level.

7.3. Effect of boswellia serrata on diabetes-induced alteration in Intake of food:

Time (in week)	Intake of food (gm) Mean±SEM					
	Normal	Diabetic control	Fluoxetine (10mg/kg)	BS (100mg/kg)	BS (200mg/kg)	BS (400mg/kg)
0	25.25±0.95	26.72±1.59	24.20±1.28	25.03±1.56	23.70±1.64	26.35±1.36
6	25.77±1.65	33.15±1.73$^{###}$	27.32±1.73***	31.77±2.04	31.45±1.49	30.62±0.86**

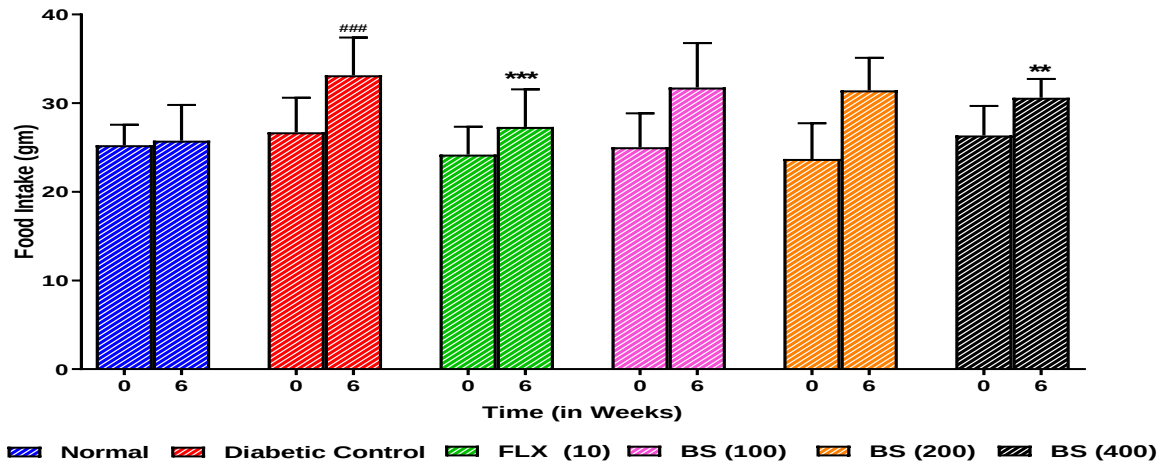


Fig. No 7.3. Effect of boswellia serrata on diabetes-induced alteration in Intake of food.

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{###}P < 0.001$) and For comparison with diabetic control group (**P < 0.01, and ***P < 0.001) on respective days.

There was no prominent difference in the intake of food of diabetic control rats than the non-diabetic rats on week 0. The alloxan-treated control group showed a evidently elevated (P < 0.001) intake of food than normal rats on the 6th week. There was a prominent decrease (P < 0.01) in the amount of intake of food on treatment with Boswellia serrata at a dose of 400mg/kg as compared with diabetic control groups. When compared with diabetic control rats, fluoxetine at a dose of 10mg/kg treated rats also showed a effective decrease (P < 0.001) in the intake of food on the 6th week.

7.4. Effect of boswellia serrata on diabetes-induced alteration in Intake of water.

Time (in week)	Intake of water (ml) Mean±SEM					
	Normal	**Diabetic control**	**Fluoxetine (10mg/kg)**	**BS (100mg/kg)**	**BS (200mg/kg)**	**BS (400mg/kg)**
0	54.33±3.75	53.33±5.70	53.67±3.19	59.33±1.17	51.33±4.59	51.83±4.33
6	56.00±3.76	131.5±4.90$^{###}$	85.50±3.73***	130.50±3.30	119.50±3.32	89.17±3.51***

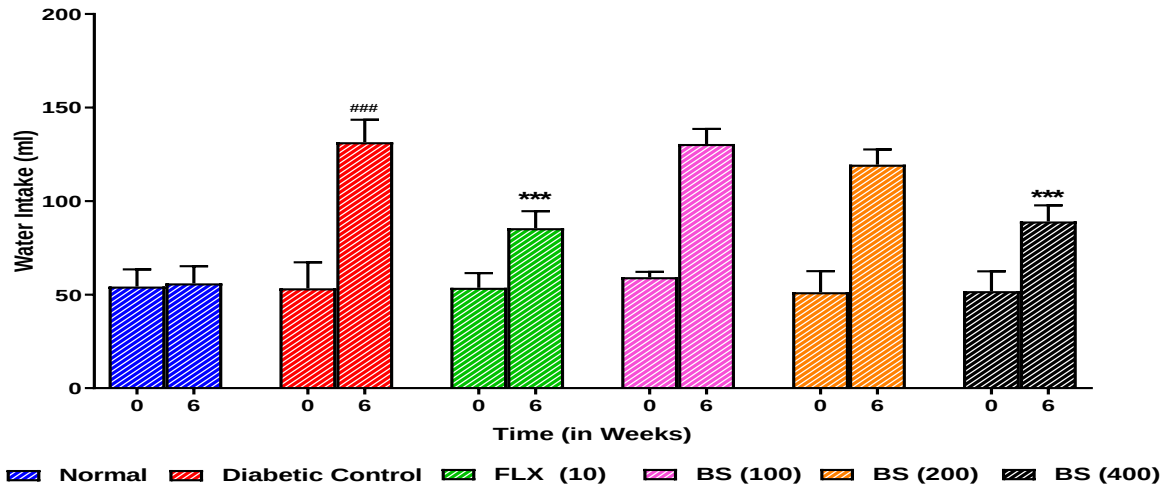


Fig. No. 7.4. Effect of boswellia serrata on diabetes-induced alteration in Intake of water.

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{###}P < 0.001$) and For comparison with diabetic control group (***$P < 0.001$) on respective days.

There was no meaningful difference in the intake of water of diabetic control rats before induction of diabetes on day 0 than normal rats. Diabetic control rats showed evidently elevated ($P < 0.001$) intake of water than the normal rats. Boswellia serrata at a dose of 400mg/kg showed prominent, lessened ($P < 0.001$) in intake of water than diabetic control rats. Additionally, treatment with fluoxetine at a dose of 10mg/kg showed a effective decrease ($P < 0.001$) in intake of water than the diabetic control rats in the 6th week.

7.5. Effect of boswellia serrata on diabetes-induced alteration in Output of urine:

Time (in week)	Output of urine (ml) Mean±SEM					
	Normal	Diabetic control	Fluoxetine (10mg/kg)	BS (100mg/kg)	BS (200mg/kg)	BS (400mg/kg)
0	17.17±1.11	18.50±1.57	17.00±1.37	18.17±1.54	18.00±1.29	18.50±1.23
6	19.50±1.12	45.17±1.08$^{###}$	21.17±1.25***	42.00±1.15	42.00±1.32	27.33±1.23***

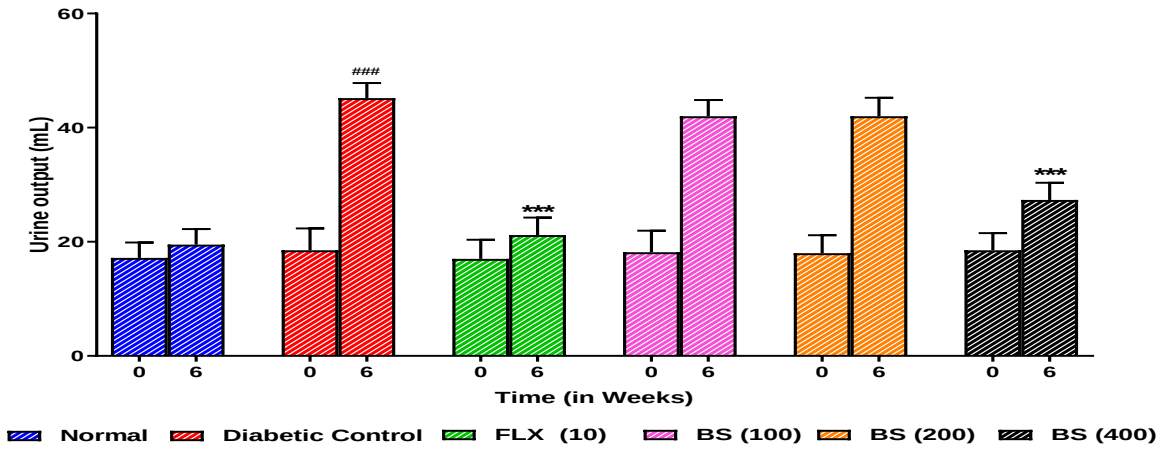


Fig. No. 7.5. Effect of boswellia serrata on diabetes-induced alteration in Output of urine.

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{###}P < 0.001$) and For comparison with diabetic control group (***P < 0.001) on respective days.

There was no prominent difference in the output of urine of the normal rat than diabetic control rats on day 0 before induction of diabetes. On week 6, diabetic control rats showed evidently amplified (P < 0.001) output of urine than the normal rats. Whereas treatment with Boswellia serrata at a dose of 400mg/kg showed evidently lessened (P < 0.001) in output of urine than the diabetic control rats. Fluoxetine at a dose of 10mg/kg treated group showed a meaningful decrease (P < 0.001) in output of urine as compared with diabetic control rats in the 6th week.

7.6. Effect of boswellia serrata on diabetes-induced alteration in duration of immobility during tail suspension test:

Time (in week)	Duration of immobility (sec) Mean±SEM					
	Normal	Diabetic control	Fluoxetine (10mg/kg)	BS (100mg/kg)	BS (200mg/kg)	BS (400mg/kg)
0	154.17±3.31	149.17±4.65	145.33±4.49	142.33±3.04	154.33±2.12	148.67±3.24
2	149.67±4.58	184.5±2.90$^{##}$	167.67±4.11	173.50±3.17	177.33±3.19	175.33±3.14
4	148.33±3.66	237.33±2.11$^{###}$	203.83±2.09***	233.17±3.63	237.83±3.50	209.67±4.00***
6	150.67±4.65	263.67±4.22$^{###}$	185.50±2.66***	266.17±3.68	269.17±2.73	192.67±3.16***

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{###}P < 0.001$) and For comparison with diabetic control group (***P < 0.001) on respective days.

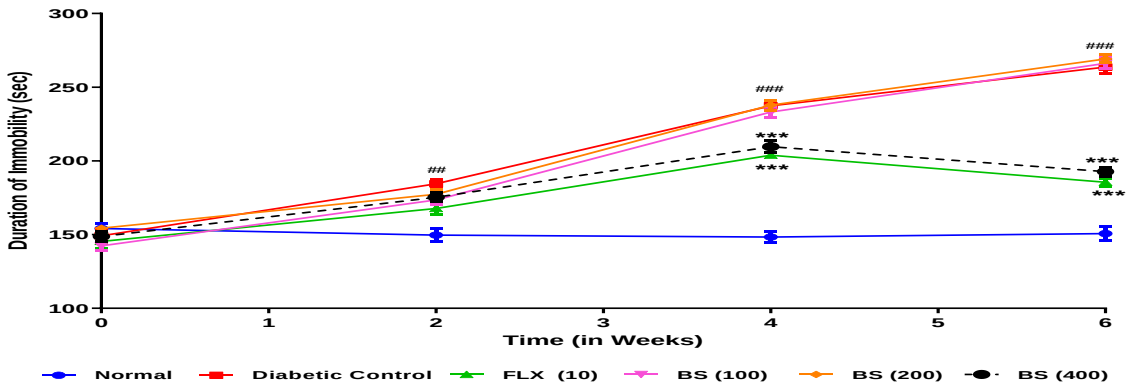


Fig. No. 7.6. Effect of boswellia serrata on diabetes-induced alteration in duration of immobility during tail suspension test.

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{###}P < 0.001$) and For comparison with diabetic control group (***P < 0.001) on respective days.

In tail suspension test, there was effective elevated (P < 0.01, P < 0.001 and P < 0.001) duration of immobility of diabetic control rats after 2nd week of alloxan administration than normal rats. Boswellia serrata at a dose of 400mg/kg evidently attenuated (P < 0.001) this amplified immobility duration than diabetic control rats from 4th week onwards. Treatment with fluoxetine at a dose of 10mg/kg also evidently ameliorated (P < 0.001) the elevated immobility duration than diabetic control rats from 4th week onwards.

7.7. Effect of boswellia serrata on diabetes-induced alteration in duration of immobility in forced swim test.

Time (in week)	Duration of immobility (sec) Mean±SEM					
	Normal	Diabetic control	Fluoxetine (10mg/kg)	BS (100mg/kg)	BS (200mg/kg)	BS (400mg/kg)
0	128.83±4.92	125.17±4.56	130.17±2.64	132.83±4.61	131.17±3.40	134.33±2.69
2	120.17±5.27	181.17±3.21$^{###}$	163.00±5.23*	174.50±4.39	178.17±4.85	164.00±4.84*
4	122.33±4.36	193.67±5.24$^{###}$	147.17±4.38**	190.33±4.50	182.33±4.70	157.83±5.34**
6	124.00±5.73	203.83±2.63$^{###}$	141.67±5.34***	200.33±6.05	188.00±5.34	155.17±5.13***

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{###}P < 0.001$) and For comparison with diabetic control group (*P < 0.05, **P < 0.01, and ***P < 0.001) on respective days.

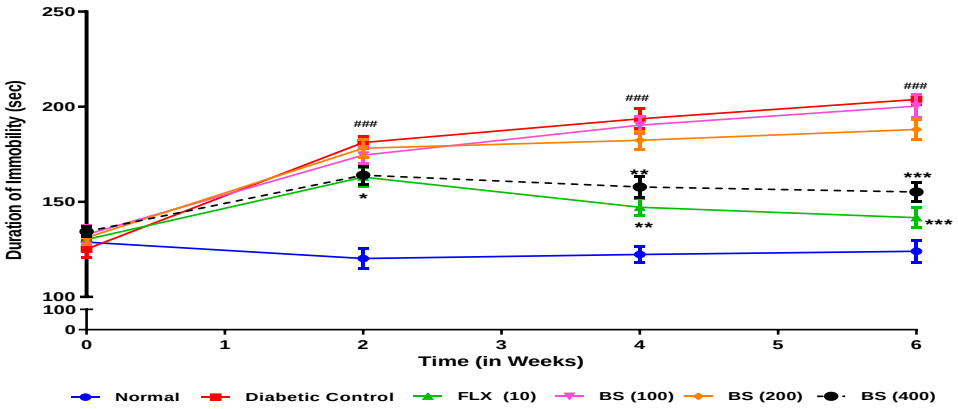


Fig. No. 7.7. Effect of boswellia serrata on diabetes-induced alteration in duration of immobility in a forced swim test.

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{###}P < 0.001$) and For comparison with diabetic control group (*P < 0.05, **P < 0.01, and ***P < 0.001) on respective days.

In forced swim test, the duration of immobility was evidently elevated (P < 0.001) in the diabetic control rats on 2nd weeks of intraperitoneal administration of alloxan than normal non-diabetic rats. This immobility duration was more evidently elevated (P < 0.05, P < 0.01 and P < 0.001, respectively) from 2nd week onward in diabetic control rats. Treatment with Boswellia serrata at a dose of 400mg/kg evidently ameliorated (P < 0.05, P < 0.01, and P < 0.001, respectively) this amplified in immobility duration than diabetic control rats from 2nd week onward. Fluoxetine at a dose of 10mg/kg also showed the evidently attenuation (P < 0.05, P < 0.01 and P < 0.001, respectively) in amplified duration of immobility than diabetic control rats from 2nd week onward.

7.8. Effect of boswellia serrata on diabetes-induced alteration in time spent in open arm in the elevated plus-maze test.

Time (in week)	Time spent in open arm (Sec) Mean±SEM					
	Normal	Diabetic control	Fluoxetine (10mg/kg)	BS (100mg/kg)	BS (200mg/kg)	BS (400mg/kg)
0	169.83±3.56	170.00±2.78	173.50±4.01	172.83±3.66	169.67±3.73	172.50±2.79
2	163.67±4.00	142.83±3.61$^{\#}$	153.67±3.96	135.50±3.89	145.33±3.29	145.83±1.66
4	173.00±1.93	81.33±3.48$^{###}$	129.33±4.22***	76.00±3.70	88.33±5.08	119.50±3.68**
6	172.00±3.39	76.50±4.00$^{###}$	131.17±2.79***	76.33±3.46	85.67±3.77	107.17±2.30**

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{\#}P < 0.05$, $^{###}P < 0.001$) and For comparison with diabetic control group (**P < 0.01, and ***P < 0.001) on respective days.

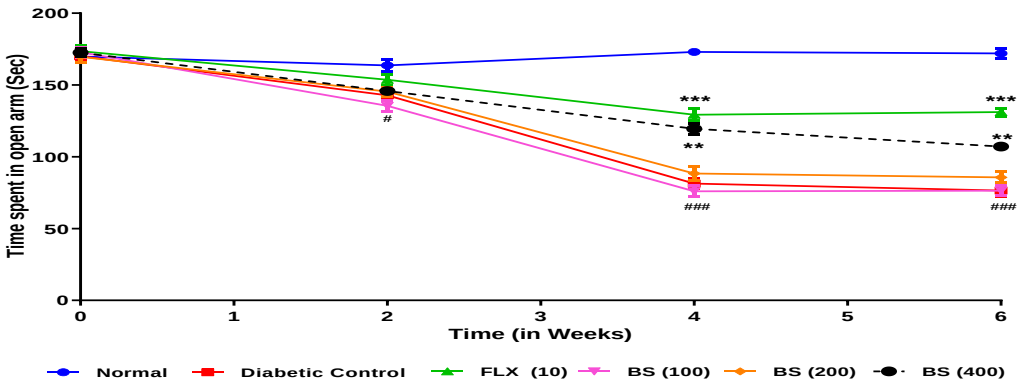


Fig. No. 7.8. Effect of boswellia serrata on diabetes-induced alteration in time spent in open arm in the elevated plus-maze test.

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$) and For comparison with diabetic control group (**P < 0.01, and ***P < 0.001) on respective days.

Time spent by the diabetic control rats in the open arm of the elevated plus-maze was evidently lowered (P < 0.05) after 2nd week of intraperitoneal administration of alloxan than normal non-diabetic rats. Time duration spent in the open arm by the diabetic control rats is still evidently reduced (P < 0.001) up to the 6th week than the 2nd week. After 6 weeks of oral administration of Boswellia serrata (400mg/kg), the time spent in the open arm was evidently elevated (P < 0.01) than diabetic control rats on 4th and 6th weeks. This open arm time duration was evidently elevated (P < 0.001) by fluoxetine at a dose of 10mg/kg treatment on 4th week onwards than diabetic control rats.

7.9. Effect of boswellia serrata on diabetes-induced alteration in time spent in closed arm in the elevated plus-maze test.

Time (in week)	Time spent in the closed arm (Sec) Mean±SEM					
	Normal	Diabetic control	Fluoxetine (10mg/kg)	BS (100mg/kg)	BS (200mg/kg)	BS (400mg/kg)
0	64.50±5.51	62.00±5.99	69.33±3.88	65.17±3.40	66.50±4.49	68.17±6.82
2	70.83±4.40	109.17±5.47$^{\#\#\#}$	94.00±5.51	114.33±5.93	113.83±5.28	96.67±6.39
4	64.17±5.48	151.67±4.32$^{\#\#\#}$	104.83±5.56***	139.00±4.06	147.83±4.74	123.83±3.43**
6	65.33±4.94	146.17±4.08$^{\#\#\#}$	96.67±5.20***	148.50±5.25	151.17±5.03	112.50±5.68***

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{\#\#\#}P < 0.001$) and For comparison with diabetic control group (**P < 0.01, and ***P < 0.001) on respective days.

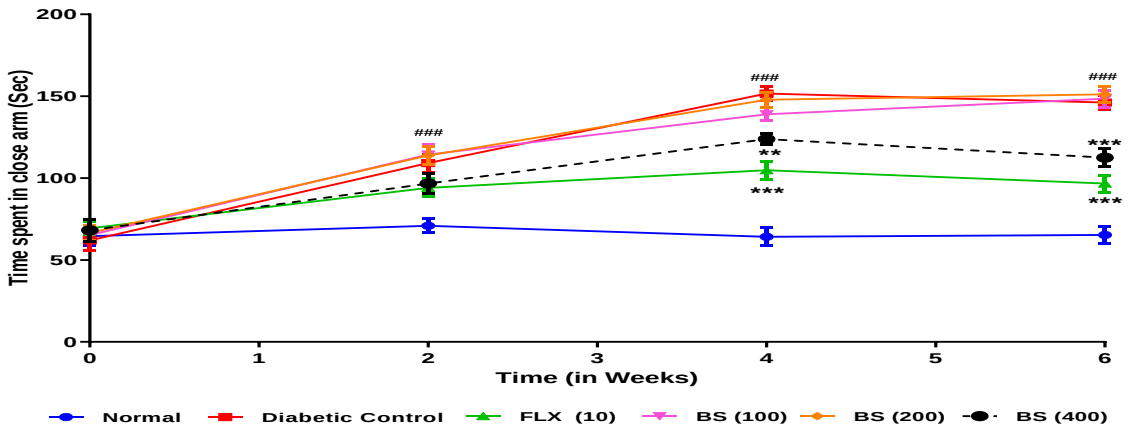


Fig. No. 7.9. Effect of boswellia serrata on diabetes-induced alteration in time spent in close arm in the elevated plus-maze test.

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{###}P < 0.001$) and For comparison with diabetic control group (**P < 0.01, and ***P < 0.001) on respective days.

Time spent in close arm by the diabetic control rats was evidently higher (P < 0.001) than the normal non-diabetic rats from 2nd week onwards. This elevated in the time duration of close arm was evidently (P < 0.01, and P < 0.001, respectively) attenuated by Boswellia serrata at a dose of 400mg/kg treatment from 4th week onwards than diabetic control rats. Fluoxetine at a dose of 10mg/kg also showed the prominent reduced (P < 0.001) in close arm time spent than diabetic control rats.

7.10. Effect of boswellia serrata on diabetes-induced alteration in entries in open arm in the elevated plus-maze test.

Time (in week)	Entries in open arm - Mean±SEM					
	Normal	Diabetic control	Fluoxetine (10mg/kg)	BS (100mg/kg)	BS (200mg/kg)	BS (400mg/kg)
0	45.00±2.80	41.50±2.63	43.50±2.78	43.83±2.33	44.83±2.33	42.33±1.94
2	42.50±0.99	27.50±2.51$^{###}$	31.00±2.71	27.83±1.85	32.17±2.36	33.33±2.74
4	42.17±1.45	17.00±2.16$^{###}$	29.83±1.74***	18.83±2.30	22.33±2.39	27.83±2.18**
6	44.50±2.60	14.83±2.86$^{###}$	33.50±1.98***	18.67±2.68	17.00±1.93	30.33±2.73***

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{###}P < 0.001$) and For comparison with diabetic control group (**P < 0.01, and ***P < 0.001) on respective days.

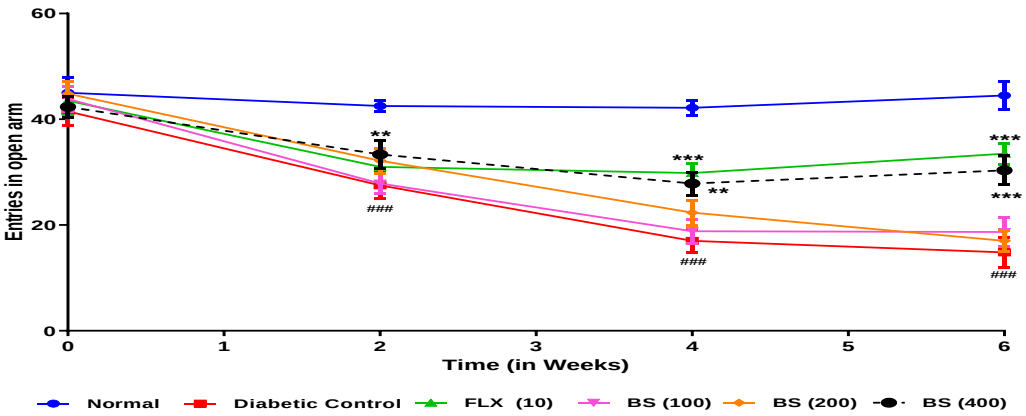


Fig. No. 7.10. Effect of boswellia serrata on diabetes-induced alteration in entries in open arm in the elevated plus-maze test.

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{###}P < 0.001$) and For comparison with diabetic control group (**P < 0.01, and ***P < 0.001) on respective days.

The total number of entries in open arm was evidently lowered (P < 0.001) in diabetic control rats than normal rats after 2nd weeks of intraperitoneal alloxan administration. This lessened in the open arm entries was evidently elevated (P < 0.01, P < 0.01 and P < 0.001) by Boswellia serrata at a dose of 400mg/kg treatment on 2nd week onwards than diabetic control rats. There was prominent amplified (P < 0.001) in the open arm entries by fluoxetine at a dose of 10mg/kg treatment from 4th week onwards than diabetic control rats.

7.11. Effect of boswellia serrata on diabetes-induced alteration in entries in closed arm in the elevated plus-maze test.

Time (in week)	Entries in closed arm - Mean±SEM					
	Normal	Diabetic control	Fluoxetine (10mg/kg)	BS (100mg/kg)	BS (200mg/kg)	BS (400mg/kg)
0	8.00±1.32	7.83±0.95	7.50±0.72	11.00±1.06	9.67±1.33	7.50±1.15
2	8.50±1.26	23.67±1.45$^{###}$	20.33±0.71	24.50±1.31	20.67±0.76	21.67±1.56
4	9.67±1.61	34.17±1.4$^{###}$	21.67±1.43***	33.83±1.19	31.33±1.15	24.67±1.56***
6	9.33±1.33	38.50±1.38$^{###}$	21.00±1.32***	36.83±1.38	35.33±1.20	28.83±1.14***

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{###}P < 0.001$) and For comparison with diabetic control group (***P < 0.001) on respective days.

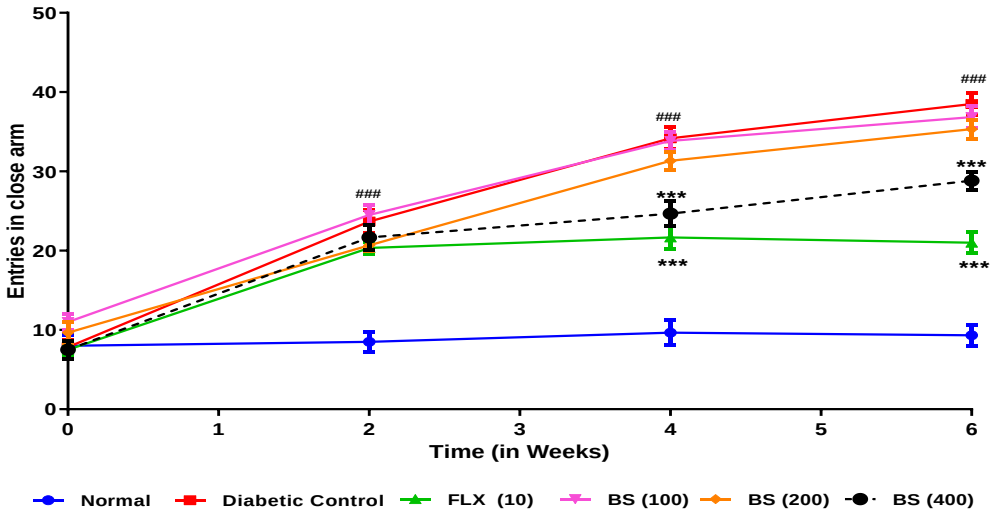


Fig. No. 7.11. Effect of boswellia serrata on diabetes-induced alteration in entries in close arm in the elevated plus-maze test.

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{###}P < 0.001$) and For comparison with diabetic control group (***P < 0.001) on respective days.

There was a effective raise (P < 0.001) in the closed arm entries of diabetic control rats after 2nd week of intraperitoneal alloxan administration than normal non-diabetic control rats. Boswellia serrata at a dose of 400mg/kg treatment evidently attenuated (P < 0.001) this elevated closed arm entries than diabetic control rats from the 4th week onwards. Whereas fluoxetine at a dose of 10mg/kg treatment also evidently reduced (P < 0.001) the number of entries in the closed arm than diabetic control rats.

7.12. Effect of boswellia serrata on diabetes-induced alteration in time spent in the center in the elevated plus-maze test:

Time (in week)	Time spent in center (Sec) Mean±SEM					
	Normal	Diabetic control	Fluoxetine (10mg/kg)	BS (100mg/kg)	BS (200mg/kg)	BS (400mg/kg)
0	53.67±2.22	51.50±1.95	54.83±2.18	54.50±2.93	53.00±2.48	53.17±2.04
2	55.67±3.06	40.67±2.51$^{\#}$	43.50±2.92	42.67±2.40	42.83±1.92	45.50±0.89
4	52.33±2.12	29.17±2.14$^{\#\#\#}$	38.83±1.49**	30.67±2.56	30.67±2.22	36.50±2.35**
6	54.17±1.72	28.17±2.85$^{\#\#\#}$	47.00±2.74***	27.83±2.75	28.17±2.68	41.33±1.28***

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$) and For comparison with diabetic control group (**P < 0.01, and ***P < 0.001) on respective days.

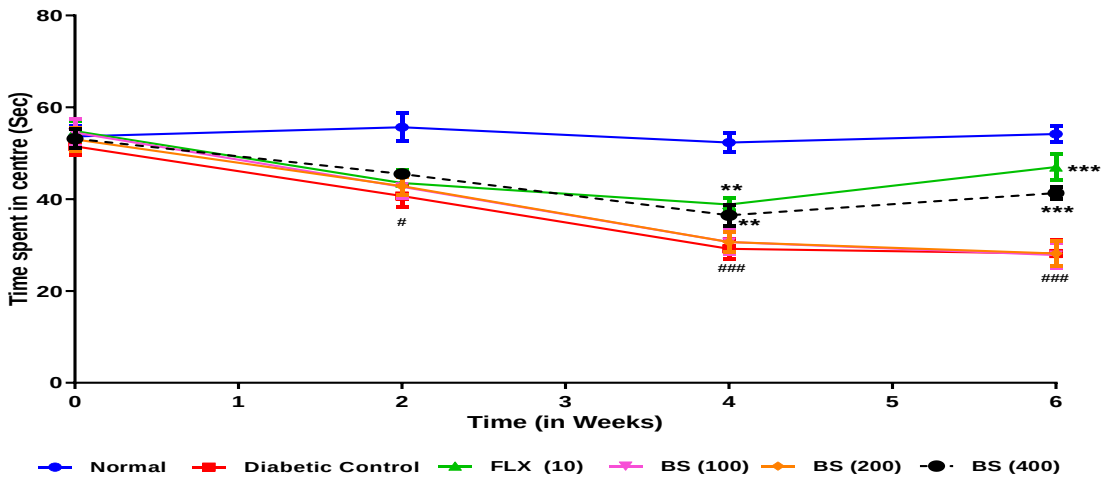


Fig. No. 7.12. Effect of boswellia serrata on diabetes-induced alteration in time spent in the center in open arm in the elevated plus-maze test.

Analysis of data was conducted using Two-Way ANOVA (Bonferroni's post-hoc test). For comparison with normal group ($^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$) and For comparison with diabetic control group (**P < 0.01, and ***P < 0.001) on respective days.

The time spent in the center of the elevated plus-maze by diabetic control rats was evidently lessened (P < 0.05) after 2nd week of alloxan administration than normal non-diabetic rats. There was still a more meaningful decrease (P < 0.001) in the central time duration by diabetic control rats on the 4th week onwards than the 2nd week. Boswellia serrata at a dose of 400mg/kg showed the meaningful elevated (P < 0.01 and P < 0.001, respectively) in central activity than diabetic control rats on 4th week onwards. This reduced in duration of central time spent was evidently elevated (P < 0.01, and P < 0.001) by the fluoxetine at a dose of 10mg/kg treatment than diabetic control rats.

7.13. Effect of boswellia serrata on diabetes-induced alteration in brain GABA levels:

Brain GABA (ng/g of brain tissue) Mean±SEM					
Normal	**Diabetic control**	**Fluoxetine (10mg/kg)**	**BS (100mg/kg)**	**BS (200mg/kg)**	**BS (400mg/kg)**
54.59±2.71	40.46±2.25$^{\#\#\#}$	44.40±3.30	44.13±2.20	42.44±2.84	47.87±2.72***

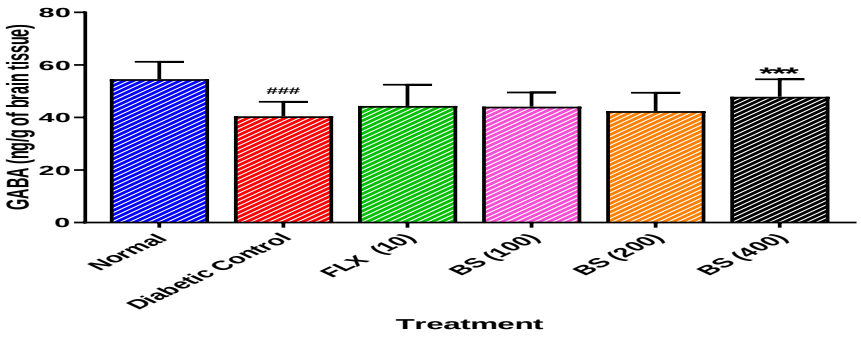


Fig. No. 7.13. Effect of boswellia serrata on diabetes-induced alteration in brain GABA levels.

Analysis of data was conducted using one-way ANOVA (Dunnett's test). For comparison with diabetic control group (***P < 0.001) and For comparison with normal group ($^{###}$P < 0.001).

There was a effective decrease (P < 0.001) in the level of brain GABA after six weeks of intraperitoneal alloxan administration in diabetic control rats than normal rats. This decrease in brain GABA level did not evidently restore by either Boswellia serrata at a dose of 100 and 200mg/kg or fluoxetine at a dose of 10mg/kg compared with diabetic control rats. However, Boswellia serrata at a dose of 400mg/kg treatment showed a prominent raise (P < 0.001) in the levels of brain GABA than diabetic control rats.

7.14. Effect of boswellia serrata on diabetes-induced alteration in brain 5-HT levels:

Brain 5-HT (ng/g of brain tissue) Mean±SEM					
Normal	**Diabetic control**	**Fluoxetine (10mg/kg)**	**BS (100mg/kg)**	**BS (200mg/kg)**	**BS (400mg/kg)**
952.10±17.36	476.60±19.18$^{###}$	856.00±19.2***	454.30±17.03	546.70±20.53	860.00±11.75***

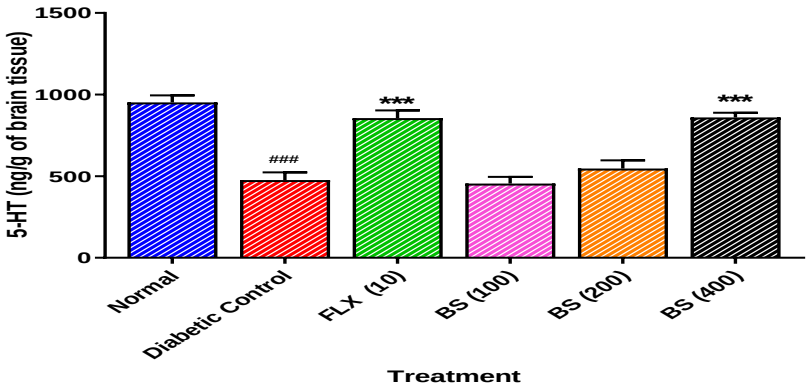


Fig. No. 7.14. Effect of boswellia serrata on diabetes-induced alteration in brain 5-HT levels.

Analysis of data was conducted using one-way ANOVA (Dunnett's test). For comparison with diabetic control group (***P < 0.001) and For comparison with normal group ($^{###}$P < 0.001).

Compared with normal non-diabetic control rats, diabetic control rats showed evidently lessened (P < 0.001) in the brain 5-HT level on the 6th week of alloxan administration. This reduced level of brain 5-HT was evidently amplified (P < 0.001) by the treatment of Boswellia serrata at a dose of 400mg/kg than diabetic control rats. Whereas fluoxetine at a dose of 10mg/kg treatment also evidently elevates (P < 0.001), this lessened brain 5-HT levels than diabetic control rats.

7.15. Effect of boswellia serrata on diabetes-induced alteration in brain dopamine levels.

Dopamine levels in brain (ng/g of brain tissue) Mean±SEM					
Normal	**Diabetic control**	**Fluoxetine (10mg/kg)**	**BS (100mg/kg)**	**BS (200mg/kg)**	**BS (400mg/kg)**
1996.00±33.57	510.40±32.43$^{###}$	1651.00±19.52***	752.70±33.42	920.70±27.47	1551.00±19.52***

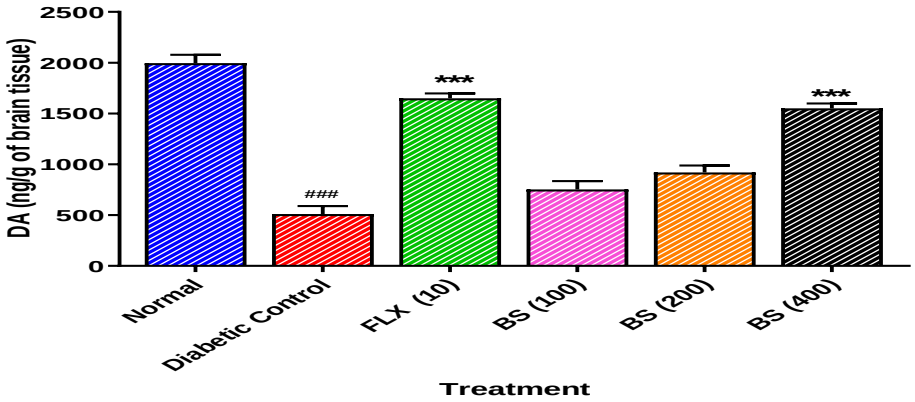


Fig. No. 7.15. Effect of boswellia serrata on diabetes-induced alteration in dopamine levels in brain levels.

Analysis of data was conducted using one-way ANOVA (Dunnett's test). For comparison with normal group ($^{###}$P < 0.001) and For comparison with diabetic control group (***P < 0.001) on respective days.

A effective decrease (P < 0.001) in the level of dopamine levels in brain was observed in the diabetic control rats after six weeks of intraperitoneal alloxan administration than normal non-diabetic rats. This reduced level of dopamine levels in brain was evidently restored (P <

0.001) by six weeks of Boswellia serrata at a dose of 400mg/kg treatment than diabetic control rats. Whereas fluoxetine at a dose of 10mg/kg treated rats also showed a effective raise (P < 0.001) in the levels of dopamine levels in brain than diabetic control rats.

7.16. Effect of boswellia serrata on diabetes-induced alteration in total protein levels in brain level.

Total protein levels in brain (mg/gm) Mean±SEM					
Normal	**Diabetic control**	**Fluoxetine (10mg/kg)**	**BS (100mg/kg)**	**BS (200mg/kg)**	**BS (400mg/kg)**
3.05±0.40	9.37±0.28$^{###}$	3.96±0.36***	8.11±0.28	7.95±0.35	4.15±0.32***

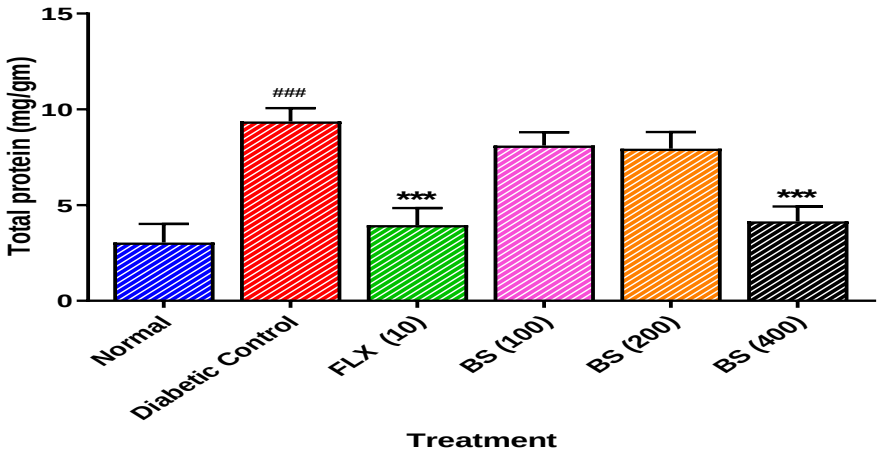


Fig. No. 7.16. Effect of boswellia serrata on diabetes-induced alteration in total protein levels in brain levels.

Analysis of data was conducted using one-way ANOVA (Dunnett's test). For comparison with normal group ($^{###}$P < 0.001) and For comparison with diabetic control group (***P < 0.001) on respective days.

Compared with normal rats, intraperitoneally administered alloxan-induced diabetes control rats show a effective raise (P < 0.001) in the level of total protein in the brain. Treatment with Boswellia serrata at a dose of 400mg/kg shows a prominent (P < 0.001) decrease in total protein levels in brain levels as compared with diabetic control rats. Additionally, fluoxetine at a dose of 10mg/kg treated rats show a prominent reduction (P < 0.001) in total protein levels in brain levels than diabetic control rats. Whereas treatment with Boswellia serrata (100 and 200mg/kg, p.o.) did not show any effective reduction in total protein levels in brain levels compared with diabetic control rats.

7.17. Effect of boswellia serrata on diabetes-induced alteration in brain SOD and GSH level.

Parameter	Brain SOD (U /mg of protein) and GSH µg/mg of protein) levels - Mean±SEM					
	Normal	Diabetic control	Fluoxetine (10mg/kg)	BS (100mg/kg)	BS (200mg/kg)	BS (400mg/kg)
SOD(U /mg of protein)	12.30±0.41	5.84±0.72$^{###}$	10.24±0.55***	6.42±0.53	6.94±0.51	10.18±0.47***
GSH (µg/mg of protein)	2.18±0.14	0.96±0.12$^{###}$	1.79±0.11***	1.09±0.17	1.26±0.20	1.86±0.13***

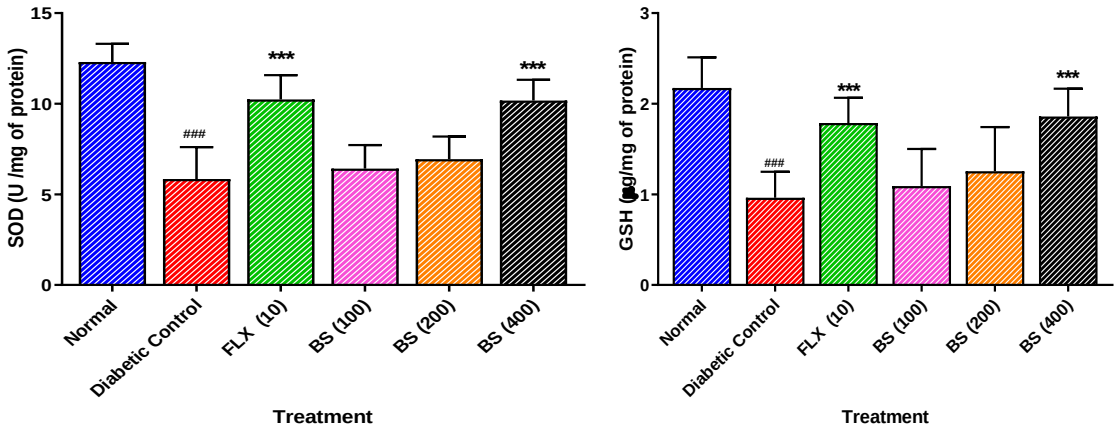


Fig. No. 7.17. Effect of boswellia serrata on diabetes-induced alteration in levels of SOD and GSH in brain.

Analysis of data was conducted using one-way ANOVA (Dunnett's test). For comparison with normal group ($^{###}P < 0.001$) and For comparison with diabetic control group (***P < 0.001).

There was a meaningful decrease (P < 0.001) in levels of SOD and GSH in brain of the diabetic control rats than the normal rats. Treatment with Boswellia serrata at a dose of 400mg/kg show prominent amplified (P < 0.001) in levels of SOD and GSH in brain than the diabetic control rats. Treatment with fluoxetine at a dose of 10mg/kg also showed a meaningful raise (P < 0.001) in brain SOD as well as GSH levels than the diabetic control rats.

7.18. Effect of boswellia serrata on diabetes-induced alteration in brain MDA and nitric oxide level.

Parameter	Brain MDA (nM/mg of protein), nitric oxide (µg/ mg of protein) Mean±SEM					
	Normal	Diabetic control	Fluoxetine (10mg/kg)	BS (100mg/kg)	BS (200mg/kg)	BS (400mg/kg)
MDA (nM/mg of protein)	3.00±0.45	7.62±0.30$^{\#\#\#}$	3.82±0.36***	8.10±0.20	8.01±0.33	4.33±0.27***
Nitric oxide (µg/ mg of protein)	10.62±0.63	71.86±0.86$^{\#\#\#}$	21.53±0.37***	66.36±0.68	65.96±0.84	31.11±0.78***

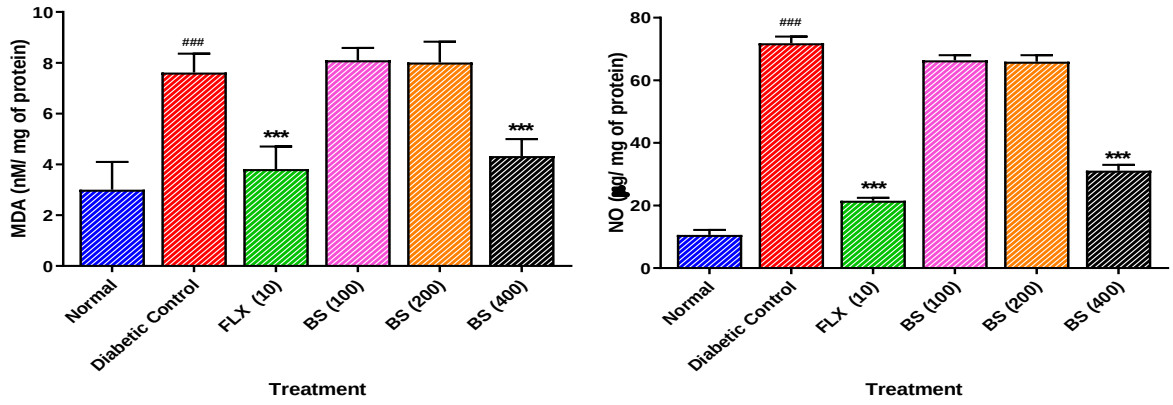


Fig. No. 7.18. Effect of boswellia serrata on diabetes-induced alteration in levels of MDA and NO in brain.

Analysis of data was conducted using one-way ANOVA (Dunnett's test). For comparison with normal group ($^{\#\#\#}P < 0.001$) and For comparison with diabetic control group (***P < 0.001).

Compared with normal rats, diabetic control rats showed evidently elevated (P < 0.001) levels of MDA and NO in brain. There was a effective decrease (P < 0.001) in levels of MDA and NO in brain by treatment of Boswellia serrata at a dose of 400mg/kg than diabetic control rats. Fluoxetine at a dose of 10mg/kg treated rats also show prominent decreases (P < 0.001) in levels of MDA and NO in brain than diabetic control rats.

8. DISCUSSION

Diabetes is a metabolic disorder worldwide experimental surveys suggest that prevalence in both categories' males and females. according to the WHO guidelines, it is categorized into 3 divisions short-term term called as type 1 diabetes, long-term called as type 2 diabetes, and gestational diabetes.

In the present study, we carried out In-Vivo parameters such as body weight, food intake, water intake, urine output, fasting blood glucose level, elevated plus maze test, time spent in the open, time spent in close arm, number of entries in open arm, number of entries in close arm, time spending in center forced swim test, duration of immobility test, tail suspension test.

Ex-Vivo parameters such as oxidative stress, SOD, GSH, MDA, NO, the total protein level in the brain, brain monoamine (5HT, dopamine), and brain GABA level.

8.1. Body Weight

Mechanism: Diabetes often leads to weight loss due to the body's inability to utilize glucose effectively, causing it to break down fat and muscle for energy.

Mechanism: Fluoxetine is a selective serotonin reuptake inhibitor (SSRI), increases serotonin levels in the brain. Serotonin is involved in regulating mood, appetite, and satiety. By enhancing serotonin activity, fluoxetine can affect appetite and metabolism, potentially leading to changes in body weight.

Normal Control: Body weight increased gradually over 6 weeks (313.67 to 349.67 g).

Disease Control: Significant decrease in body weight observed from week 2 onwards compared to normal (280.50 g at 2 weeks to 245.33 g at 6 weeks) (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant improvement in body weight compared to disease control from week 4 (294.83 g) to week 6 (306.17 g) (P < 0.01 and P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg significantly improved body weight from week 4 (278.50 g) to week 6 (297.00 g) compared to disease control (P < 0.001). Lower doses (100 and 200mg/kg) showed less effects.

Boswellia serrata is believed to have anti-inflammatory and potentially glucose-lowering properties. It may be due to prevent break down fat and muscle for energy.

8.2. Plasma Glucose Levels

Mechanism: Diabetes leads to elevated blood glucose levels due to insufficient insulin production or insulin resistance.

The effect of fluoxetine on plasma glucose levels is not entirely direct. It is believed to have an influence through several pathways:

Mood Improvement: By alleviating depression, fluoxetine can lead to better overall health behaviors, including improved adherence to diabetes management practices such as diet, exercise, and medication.

Appetite Regulation: Fluoxetine can reduce appetite, leading to reduced caloric intake, which may help in controlling blood glucose levels.

Stress Reduction: Chronic stress can elevate blood glucose levels. By reducing stress and anxiety, fluoxetine might indirectly contribute to lower blood glucose levels.

Normal Control: Stable plasma glucose levels over 6 weeks (66.83 to 84.5 mg/dl).

Disease Control: Significant increase in plasma glucose levels from week 2 (389.5 mg/dl) to week 6 (415.33 mg/dl) compared to normal (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant reduction in glucose levels compared to disease control from week 2 (328.33 mg/dl) to week 6 (171.67 mg/dl) (P < 0.05 and P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg showed a significant reduction in glucose levels from week 4 (265.83 mg/dl) to week 6 (236.83 mg/dl) (P < 0.001). Lower doses showed less significant effects.

Boswellia serrata might help reduce blood glucose levels through its anti-inflammatory and possibly insulin-sensitizing effects. It may be due to sufficient insulin production or insulin action.

8.3. Food Intake

Mechanism: Diabetes can increase food intake (polyphagia) due to the body's inability to utilize glucose, leading to a state of perceived starvation.

Mechanism: Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) that increases serotonin levels in the brain. Serotonin is a neurotransmitter that regulates mood, appetite, and satiety.

By enhancing serotonin activity, fluoxetine can help suppress appetite and increase feelings of fullness, leading to reduced food intake.

Normal Control: Stable food intake over 6 weeks (25.25 to 25.77 g).

Disease Control: Significant increase in food intake at week 6 (33.15 g) compared to normal (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant reduction in food intake at week 6 (27.32 g) compared to disease control (P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg showed a significant reduction in food intake at week 6 (30.62 g) (P < 0.01). Lower doses showed a slight reduction but were less significant.

Boswellia Serrata may be due to suppress appetite and increase feelings of fullness, leading to reduced food intake.

8.4. Water Intake

Mechanism: Increased water intake (polydipsia) in diabetes is due to high glucose levels causing osmotic diuresis.

Mechanism: fluoxetine's reduction of water intake is likely due to its serotonergic action on the hypothalamus, influencing thirst regulation and behavioral factors that affect drinking habits.

Normal Control: Stable water intake over 6 weeks (54.33 to 56.00 ml).

Disease Control: Significant increase in water intake at week 6 (131.5 ml) compared to normal (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant reduction in water intake at week 6 (85.50 ml) compared to disease control (P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg significantly reduced water intake at week 6 (89.17 ml) (P < 0.001). Lower doses showed less significant effects.

B.S. reduction of water intake may be due to its serotonergic action on the hypothalamus, influencing thirst regulation and behavioral factors that affect drinking habits.

8.5. Urine Output

Mechanism: Increased urine output (polyuria) in diabetes is due to osmotic diuresis caused by high glucose levels.

Mechanism: Reduced urine output, or oliguria, can occur as a side effect of fluoxetine due to several potential mechanisms: Kidney Function, Fluid Retention, Hormonal Changes, and Dehydration.

Normal Control: Stable urine output over 6 weeks (17.17 to 19.50 ml).

Disease Control: Significant increase in urine output at week 6 (45.17 ml) compared to normal (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant reduction in urine output at week 6 (21.17 ml) compared to disease control (P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg significantly reduced urine output at week 6 (27.33 ml) (P < 0.001). Lower doses showed less significant effects.

B.S. significantly reduced urine output may be due to maintenance of blood glucose level, and maintenance body fluid.

8.6. Duration of Immobility in Tail Suspension Test:

Mechanism: Increased immobility is an indicator of depressive-like behavior, which can be exacerbated by diabetes.

Mechanism: fluoxetine typically results in a significant reduction in the duration of immobility. This is interpreted as an antidepressant-like effect, suggesting that the animal is less likely to exhibit behavioral despair.

Normal Control: Stable immobility duration over 6 weeks (154.17 to 150.67 sec).

Disease Control: Significant increase in immobility duration at week 6 (263.67 sec) compared to normal (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant reduction in immobility duration at week 6 (185.50 sec) compared to disease control (P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg significantly reduced immobility duration at week 6 (192.67 sec) (P < 0.001). Lower doses showed less significant effects.

Boswellia serrata might improve mood through its anti-diabetic, anti-depressant and anti-inflammatory effects.

8.7. Duration of Immobility in Forced Swim Test

Mechanism: Similar to the tail suspension test, increased immobility in the forced swim test indicates depressive-like behavior.

Mechanism: fluoxetine typically reduces the duration of immobility in the Forced Swim Test, which is interpreted as an indication of its antidepressant-like activity. This reduction is generally accompanied by an increase in active behaviors such as swimming.

Normal Control: Stable immobility duration over 6 weeks (128.83 to 124.00 sec).

Disease Control: Significant increase in immobility duration at week 6 (203.83 sec) compared to normal (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant reduction in immobility duration at week 6 (141.67 sec) compared to disease control (P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg significantly reduced immobility duration at week 6 (155.17 sec) (P < 0.001). Lower doses showed less significant effects.

B.S. reduces the duration of immobility in the Forced Swim Test, which is interpreted as an indication of its antidepressant-like activity.

8.8. Time Spent in Open Arm in Elevated Plus-Maze Test:

Mechanism: Decreased time in open arms in the Elevated Plus-Maze Test indicates anxiety-like behaviour.

Mechanism: Fluoxetine significant increase in time spent in open arm due to its anxiolytic effects, mediated by increased serotonin levels and resulting in reduced anxiety and increased exploratory behavior.

Normal Control: Stable time spent in open arm over 6 weeks (169.83 to 172.00 sec).

Disease Control: Significant reduction in time spent in open arm at week 6 (76.50 sec) compared to normal (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant increase in time spent in open arm at week 6 (131.17 sec) compared to disease control (P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg significantly increased time spent in open arm at week 6 (107.17 sec) (P < 0.01). Lower doses showed less significant effects.

B.S. significantly increased time spent in open arm due to its anxiolytic effects, mediated by increased serotonin levels and resulting in reduced anxiety and increased exploratory behavior.

8.9. Time Spent in Closed Arm in Elevated Plus-Maze Test

Mechanism: Increase in time spent in the closed arms of the Elevated Plus Maze due to a combination of neurochemical changes, hyperglycemia effects, neuropathy, HPA axis dysregulation, and cognitive impairments associated with diabetes.

Mechanism: fluoxetine can significantly reduce the time spent in the closed arms of the Elevated Plus Maze, indicating its potential to reduce anxiety-like behavior.

Normal Control: Time spent in closed arm in the Elevated Plus-Maze Test remained relatively consistent over 6 weeks, ranging from (64.50±5.51 sec at week 0 to 65.33±4.94 sec) at week 6.

Diabetic Control: Significant increase in time spent in closed arm from week 2 (109.17±5.47 sec) to week 6 (146.17±4.08 sec) compared to normal control (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant reduction in time spent in closed arm at weeks 4 (104.83±5.56 sec) and 6 (96.67±5.20 sec) compared to diabetic control (***P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg showed significant reduction in time spent in closed arm at weeks 4 (123.83±3.43 sec) and 6 (112.50±5.68 sec) compared to diabetic control (**P < 0.01 and ***P < 0.001). Lower doses (100 and 200mg/kg) showed less pronounced effects.

B.S. significantly reduce the time spent in the closed arms of the Elevated Plus Maze, may be due to indicating its potential to reduce anxiety-like behavior.

8.10. Entries in Open Arm in Elevated Plus-Maze Test

Mechanism: diabetic control conditions can lead to a decrease in entries into the open arms of the Elevated Plus Maze due to a combination of increased anxiety, neurological changes, oxidative stress, hormonal imbalances, peripheral neuropathy, metabolic disturbances, and overall behavioral changes associated with chronic illness.

Mechanism: The increase in entries into the open arms of the Elevated Plus Maze after fluoxetine administration is attributed to its anxiolytic effects, primarily through the enhancement of serotonin signalling, which reduces anxiety and promotes exploratory behavior.

Normal Control: Entries in open arm in the Elevated Plus-Maze Test remained relatively stable, from (45.00±2.80 at week 0 to 44.50±2.60) at week 6.

Diabetic Control: Significant decrease in entries in open arm from week 2 (27.50±2.51) to week 6 (14.83±2.86) compared to normal control (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant increase in entries in open arm at weeks 4 (29.83±1.74) and 6 (33.50±1.98) compared to diabetic control (***P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg significantly increased entries in open arm at weeks 4 (27.83±2.18) and 6 (30.33±2.73) compared to diabetic control (**P < 0.01 and ***P < 0.001). Lower doses (100 and 200mg/kg) showed less pronounced effects.

The increase in entries into the open arms of the Elevated Plus Maze after B.S. administration may be due to is its anxiolytic effects, primarily through the enhancement of serotonin signalling, which reduces anxiety and promotes exploratory behavior.

8.11. Entries in Closed Arm in Elevated Plus-Maze Test

Mechanism: The increase in closed arm entries is indicative of anxiety-like behavior, altered risk assessment, or other factors influenced by diabetic conditions.

Mechanism: fluoxetine generally reduces anxiety-like behavior in rodents, as indicated by an increase in entries into the closed arms of tests like the Elevated Plus Maze.

Normal Control: Entries in closed arm in the Elevated Plus-Maze Test showed minimal variation, starting from (8.00±1.32 at week 0 and reaching 9.33±1.33) at week 6.

Diabetic Control: Significant increase in entries in the closed arm from week 2 (23.67±1.45) to week 6 (38.50±1.38) compared to normal control (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant reduction in entries in closed arm at weeks 4 (21.67±1.43) and 6 (21.00±1.32) compared to diabetic control (***P < 0.001).

Test Drug (BS 100, 200, 400mg/kg)**: BS at 400mg/kg significantly reduced entries in the closed arm at weeks 4 (24.67±1.56) and 6 (28.83±1.14) compared to diabetic control (***P < 0.001). Lower doses (100 and 200mg/kg) showed less pronounced effects.

B.S. may be due to reduced anxiety-like behavior in rodents, as indicated by an increase in entries into the closed arms of tests like the Elevated Plus Maze.

8.12. Time Spent in the Center in Elevated Plus-Maze Test

Mechanism: A decrease in time spent in the center of an open field test, for example, could reflect altered anxiety levels or impaired sensory-motor function due to diabetic complications.

Mechanism: The Fluoxetine increase in time spent in the center of the open field after fluoxetine administration is attributed to its anxiolytic and mood-enhancing effects, mediated primarily through increased serotonin activity.

Normal Control: Time spent in the center in the Elevated Plus-Maze Test showed slight fluctuations, starting from (53.67±2.22 sec at week 0 and reaching 54.17±1.72 sec) at week 6.

Diabetic Control: Significant decrease in time spent in the center from week 2 (40.67±2.51) to week 6 (28.17±2.85) compared to normal control (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant increase in time spent in the center at weeks 4 (38.83±1.49) and 6 (47.00±2.74) compared to diabetic control (**P < 0.01 and ***P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg significantly increased time spent in the center at weeks 4 (36.50±2.35) and 6 (41.33±1.28) compared to diabetic control (**P < 0.01 and ***P < 0.001). Lower doses (100 and 200mg/kg) showed less pronounced effects.

B.S. may be due to an increase in time spent in the center of the open field after fluoxetine administration is attributed to its anxiolytic and mood-enhancing effects, mediated primarily through increased serotonin activity.

8.13. GABA (Gamma-Aminobutyric Acid)

Mechanism: The decrease in GABA (gamma-aminobutyric acid) levels in diabetes mellitus can be Insulin Deficiency or Resistance, Oxidative Stress, Glucose Dysregulation, Inflammation, Neurodegeneration, Alterations in GABA Transporters.

Mechanism: Fluoxetine's primary action is on serotonin reuptake inhibition, its effects on GABA levels likely occur through indirect mechanisms involving serotoninergic modulation of GABAergic circuits and neurons. These interactions contribute to the overall neurochemical and behavioral effects observed with fluoxetine treatment.

Normal Control: Brain GABA levels were 54.59±2.71 ng/g of brain tissue.

Disease Control: Significant decrease in GABA levels (40.46±2.25 ng/g) compared to normal (###P < 0.001).

Standard (Fluoxetine 10mg/kg): No significant restoration of GABA levels (44.40±3.30 ng/g).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg significantly increased GABA levels (47.87±2.72 ng/g) compared to disease control (P < 0.001). Lower doses (100 and 200mg/kg) showed less pronounced effects.

B.S. Increased GABA levels may be due to impact disease control by altering neuronal excitability, emotional regulation, cognitive function, sleep patterns.

8.14. 5-HT (Serotonin)

Mechanism: Diabetes can decrease serotonin levels through mechanisms involving insulin deficiency or resistance, impaired tryptophan availability, increased tryptophan degradation, neurodegenerative changes, and effects of hyperglycemia and glucose fluctuations.

Mechanism: fluoxetine increases serotonin levels by blocking the serotonin transporter (SERT), thereby enhancing serotonergic neurotransmission in the brain.

Normal Control: Brain 5-HT levels were (952.10±17.36 ng/g) of brain tissue.

Disease Control: Significant decrease in 5-HT levels (476.60±19.18 ng/g) compared to normal (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant increase in 5-HT levels (856.00±19.2 ng/g) compared to disease control (P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg significantly increased 5-HT levels (860.00±11.75 ng/g) compared to disease control (P < 0.001). Lower doses (100 and 200mg/kg) showed less pronounced effects.

B.S. may be due to increases serotonin levels by blocking the serotonin transporter (SERT), thereby enhancing serotonergic neurotransmission in the brain.

8.15. DA (Dopamine)

Mechanism: Diabetes can decrease dopamine levels through mechanisms involving insulin resistance, neuroinflammation, oxidative stress, and disruption of glucose metabolism in the brain.

Mechanism: Dopamine is a neurotransmitter involved in reward, motivation, memory, attention, and even regulating body movements. it can increase dopamine levels by Serotonin-Dopamine Interaction, Indirect Modulation, Regulation of Neurotransmitter Systems, Chronic Effects.

Normal Control: Brain dopamine levels were (1996.00±33.57 ng/g) of brain tissue.

Disease Control: Significant decrease in dopamine levels (510.40±32.43 ng/g) compared to normal (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant increase in dopamine levels (1651.00±19.52 ng/g) compared to disease control (P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg significantly increased dopamine levels (1551.00±19.52 ng/g) compared to disease control. Lower doses (100 and 200mg/kg) showed less pronounced effects.

B.S. increase dopamine levels may be due to Serotonin-Dopamine Interaction, Indirect Modulation, Regulation of Neurotransmitter Systems, and Chronic Effects.

8.16. TPL (Total Protein Levels)

Mechanism: Diabetes can lead to an increase in total protein levels through Glucose Control, Kidney Function, Liver Response, and Insulin Resistance.

Mechanism: Fluoxetine can lead to a decrease in total protein levels through Liver Enzyme Induction, Nutritional Deficiency, Metabolic Effects, and Renal Effects.

Normal Control: Total protein levels in the brain were 3.05±0.40 mg/g.

Disease Control: Significant increase in total protein levels (9.37±0.28 mg/g) compared to normal (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant decrease in total protein levels (3.96±0.36 mg/g) compared to disease control (P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg significantly decreased total protein levels (4.15±0.32 mg/g) compared to disease control (P < 0.001). Lower doses (100 and 200mg/kg) showed less pronounced effects.

B.S. may be due to a decrease in total protein levels through Liver Enzyme Induction, Nutritional Deficiency, Metabolic Effects, and Renal Effects.

8.17. SOD (Superoxide Dismutase)

Mechanism: The combination of oxidative stress, glycation, inflammatory responses, mitochondrial dysfunction, and genetic factors collectively contribute to the decrease in SOD levels observed in diabetes.

Mechanism: fluoxetine increase the levels of Superoxide Dismutase (SOD) Indirect Antioxidant Activity, Serotonin Pathway, Activation of SOD Gene Expression, Reduction of Oxidative Stress, Neuroprotective Effects.

Normal Control: Normal SOD levels.

Disease Control: Significant decrease in SOD levels compared to normal (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant increase in SOD levels compared to disease control (P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg significantly increased SOD levels compared to disease control (P < 0.001). Lower doses (100 and 200mg/kg) showed less pronounced effects.

B.S. increase the levels of Superoxide Dismutase (SOD) may be due to Indirect Antioxidant Activity, Serotonin Pathway, Activation of SOD Gene Expression, Reduction of Oxidative Stress, Neuroprotective Effects.

8.18. GOD (Glucose Oxidase)

Mechanism: GOD is an enzyme that catalyzes the oxidation of glucose to hydrogen peroxide and gluconic acid.

Mechanism: Fluoxetine increase GOD levels due to its impact on Metabolic Effects, Enzyme Induction or Inhibition, Cellular Signalling, Indirect Effects on Oxidative Stress.

Normal Control: Normal GOD levels.

Disease Control: Significant decrease in GOD levels compared to normal (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant increase in GOD levels compared to disease control (P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg significantly increased GOD levels compared to disease control (P < 0.001). Lower doses (100 and 200mg/kg) showed less pronounced effects.

B.S. increase GOD levels due to its impact on Metabolic Effects, Enzyme Induction or Inhibition, Cellular Signalling, Indirect Effects on Oxidative Stress.

8.19. MDA (Malondialdehyde)

Mechanism: Diabetes can lead to increased levels of malondialdehyde (MDA) due to of oxidative stress and lipid peroxidation, indicating cell membrane damage.

Mechanism: Fluoxetine, a selective serotonin reuptake inhibitor (SSRI) commonly used as an antidepressant, has been observed to decrease malondialdehyde (MDA) levels might be Antioxidant Properties, Neuroprotection, Stress Reduction.

Normal Control: Brain MDA levels were (3.00±0.45 nM/mg) of protein.

Disease Control: Significant increase in MDA levels (7.62±0.30 nM/mg) compared to normal (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant decrease in MDA levels (3.82±0.36 nM/mg) compared to disease control (P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg significantly decreased MDA levels (4.33±0.27 nM/mg) compared to disease control (P < 0.001). Lower doses (100 and 200mg/kg) showed less pronounced effects.

B.S. used as an antidepressant, has been observed to decrease malondialdehyde (MDA) levels might be due to Antioxidant Properties, Neuroprotection, and Stress Reduction.

8.20. NO (Nitric Oxide)

Mechanism: Diabetes can lead to an increase in nitric oxide (NO) levels due to Endothelial Dysfunction, Oxidative Stress, Inflammation, Insulin Resistance.

Mechanism: fluoxetine might decrease NO levels Inhibition of NOS Activity, Altered Neurotransmitter Levels, Oxidative Stress, Effects on Endothelial Cells.

Normal Control: Brain NO levels were (10.62±0.63 µg/mg) of protein.

Disease Control: Significant increase in NO levels (71.86±0.86 µg/mg) compared to normal (###P < 0.001).

Standard (Fluoxetine 10mg/kg): Significant decrease in NO levels (21.53±0.37 µg/mg) compared to disease control (P < 0.001).

Test Drug (BS 100, 200, 400mg/kg): BS at 400mg/kg significantly decreased NO levels (31.11±0.78 µg/mg) compared to disease control (P < 0.001). Lower doses (100 and 200mg/kg) showed less pronounced effects.

B.S. decrease NO levels may be due to Inhibition of NOS Activity, Altered Neurotransmitter Levels, Oxidative Stress, Effects on Endothelial Cells.

Boswellia serrata, particularly at a dose of 400mg/kg, shows significant efficacy in ameliorating various diabetes-induced alterations, including body weight loss, elevated

plasma glucose levels, increased food and water intake, increased urine output, and depressive- and anxiety-like behaviours. However, the effects are generally less potent compared to fluoxetine.

9. SUMMARY AND CONCLUSION

9.1. SUMMARY

In the present study, various in-vivo Such as (Body weight, Food intake, Water intake, Urine output, Fasting blood glucose level, Elevated plus maze test: Time spent in the open arm, Time spent in the closed arm, Number of entries in the open arm, Number of entries in the closed arm, Time spent in the center, Forced swim test: Duration of immobility, Tail suspension test: Duration of immobility) and ex-vivo such as (Oxidative stress markers: Superoxide dismutase (SOD), Glutathione (GSH), Malondialdehyde (MDA), Nitric oxide (NO), Total protein level in the brain, Brain monoamine levels: Serotonin (5HT), Dopamine Brain GABA level) parameters were assessed to evaluate the effects of the test drug on diabetes-induced changes. The selected test has Positive outcomes against were observed in all parameters in the Standard and Test Drug groups. Conversely, the Disease Control group exhibited significant negative changes in all these parameters, reflecting the detrimental effects of diabetes. These findings suggest that the test drug, particularly at higher doses(400mg/kg), has beneficial effects on managing diabetes-related symptoms and behaviors.

9.2. CONCLUSION

The present study investigates the effects of Boswellia serrata (BS) on diabetes-induced physiological and behavioral alterations, particularly in comparison to the standard antidepressant, fluoxetine. Our results demonstrate that BS, especially at a higher dose of 400mg/kg, exhibits significant efficacy in mitigating various diabetes-related changes. Notably, BS administration led to a substantial reduction in plasma glucose levels, a crucial marker of diabetes management. Additionally, BS treatment ameliorated hyperphagia and polydipsia, common symptoms in diabetic conditions, by significantly reducing food and water intake, respectively.

BS exhibited anxiolytic and antidepressant-like activities. In the Elevated Plus-Maze Test, BS increased the time spent in the open arms, indicating reduced anxiety levels. Similarly, in the Forced Swim Test, BS decreased the duration of immobility, reflecting its antidepressant-like

effects. These behavioral improvements suggest that BS modulates neurochemical pathways associated with anxiety and depression, potentially through its influence on serotonin levels. Biochemically, BS demonstrated a pronounced antioxidant effect by significantly lowering malondialdehyde (MDA) and nitric oxide (NO) levels in the brain, markers of oxidative stress and endothelial dysfunction, respectively. In comparison, fluoxetine, a well-established antidepressant, showed greater potency in most parameters, underscoring its efficacy in managing both depressive and diabetic symptoms. However, the significant improvements observed with BS, particularly at higher doses, highlight its potential as a complementary therapeutic agent in diabetes management, offering both glycemic control and neuroprotective benefits.

Future studies should further explore the molecular mechanisms underlying these effects and the potential for clinical applications.

9. REFERENCES

1. Abdel-Barry J, Abdel-Hassan I, Al-Hakiem M. Hypoglycaemic and antihyperglycaemic effects of Trigonella foenum-graecum leaf in normal and alloxan induced diabetic rats. J Ethnopharmacol, 1997; 58(3): 149-55.
2. Acharya SN, Thomasland JE, Basu SK. Fenugreek: an "old world" crop for the "new world". Biodiversity (Tropical, Conservancy), 2006; 7(3& 4): 27-30.
3. Ahlgren SC, Levine JD. Protein kinase C inhibitors decrease hyperalgesia and C-fiber hyperexcitability in the streptozotocin-diabetic rat. J Neurophysiol, 1994; 72: 684-692.
4. Ahsan H, Ali A, Ali R. Oxygen free radicals and systemic autoimmunity. Clin Exp Immunol, 2003; 31: 398-404.
5. Ajabnoor M, Tilmisany A. Effect of Trigonella foenum graceum on blood glucose levels in normal and alloxan-diabetic mice. J Ethnopharmacol, 1988; 22(1): 45.
6. Al-Habori M, Raman A. Antidiabetic and hypocholesterolaemic effects of fenugreek. Phytother Res, 1998; 12(4): 233-42.
7. Al-yassin D, Ibrahim k. A minor haemoglobin fraction and the level of fasting blood glucose. J Faculty Med, 1981; 23: 373-80.
8. Amann B, Tinzmann R, Angelkort B. ACE inhibitors improve diabetic nephropathy through suppression of renal MCP-1. Diabetes Care, 2003; 26: 2421-2425.
9. American Diabetes Association. Diagnosis and classification of diabetes mellitus. J Diabetes Care, 2010; 33: S62-9.

10. Anonymous. The Wealth of India: a dictionary of Indian raw materials & industrial products. New Delhi: Publications & Information Directorate, Council of Scientific & Industrial Research, 1985
11. Arai K, Maguchi S, Fujii S, Ishibashi H, Oikawa K, Taniguchi N. Glycation and inactivation of human Cu-Zn-superoxide dismutase. Identification of the in vitro glycated sites. J Biol Chem, 1987; 262: 16969-16972.
12. Backonja MM, Beydoun A, Edwards KR, Schwartz SL, Fonseca V, Hes M et al. Gabapentin for symptomatic treatment of painful neuropathy in patients with diabetes mellitus. J Am Med Assoc, 1998; 280: 1831-1836.
13. Basch E, Ulbricht C, Kuo G, Szapary P, Smith M. Therapeutic applications of fenugreek. Altern Med Rev, 2003; 8(1): 20-7.
14. Basu SK. Seed production technology for fenugreek (Trigonella foenum-graecum L.) in the Canadian. Master of Science Thesis. Department of Biological Sciences University of Lethbridge, Alberta, Canada, 2006; 202.
15. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes, 1999; 48: 1-9.
16. Bierhaus A, Chevion S, Chevion M, Hofmann M, Quehenberger P, Illmer T, et al. Advanced glycation end product-induced activation of NF-kappaB is suppressed by alpha-lipoic acid in cultured endothelial cells. Diabetes, 1997; 46(9): 1481-90.
17. Boring L, Gosling J, Chensue SW, Kunkel SL, Farese Jr RV, Broxmeyer HE, Charo IF. Impaired monocyte migration and reduced type 1 (Th1) cytokine responses in CC chemokine receptor 2 knockout mice. Journal of Clinical Investigation, 1997; 100: 2552.
18. Broca C, Manteghetti M, Gross R, Baissac Y, Jacob M, Petit P, et al. 4-Hydroxyisoleucine: effects of synthetic and natural analogues on insulin secretion. Eur J Pharmacol, 2000; 390(3): 339-45.
19. Cameron NE, Cotter MA, Robertson S. Essential fatty acid diet supplementation. Effects on peripheral nerve and skeletal muscle function.
20. Carmeliet P, Storkebaum E Vascular and neuronal effects of VEGF in the nervous system: implications for neurological disorders. In: Seminars in cell & developmental biology, 2002; 13: 39-53: Elsevier.
21. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods, 1994; 53: 55–63.
22. Charonis AS, Reger LA, Dege JE, et al. Laminin alterations after in vitro nonenzymatic glycosylation. Diabetes, 1990; 39: 807–814.

23. Cinoshita J.H. A thirty year journey in the polyol pathway. Exp. Res**,** 1990, 50: 567-573.
24. Davis SN and Granner DK Insulin oral agents and the pharmacology of the endocrine pancreas. In: Hardman JG and Limbird LE. Goodman and Gliman's, Pharmacological basis of Therapeutics, 2001; 10th edition, pp: 115-154.
25. DiStefano PS, Friedman B, Radziejewski C, Alexander C, Boland P, Schick CM, et al. The neurotrophins BDNF, NT-3, and NGF display distinct patterns of retrograde axonal transport in peripheral and central neurons. Neuron, 1992; 8-983-993.
26. El-Kamali H, Khalid S. The most common herbal remedies in Central Sudan. Fitoterapia (Milano), 1996; 67(4): 301-6.
27. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal malonaldehyde and related aldehydes. Free Radic Biol Med, 1991; 11: 81-128.
28. Feldman EL, Stevens MJ, Russell JW, Greene DA (1999). Diabetic neuropathy. In: Current Review of Diabetes. Taylor S (Ed). Current Medicine, Philadelphia, pp. 71–83.
29. Fioretto P, Mauer M, Brocco E, Velussi M, Frigato F, Muollo B, Sambataro M, Abaterusso C, Baggio B, Crepaldi G, Nosadini R: Patterns of renal injury in NIDDM patients with microalbuminuria. Diabetologia, 1996; 39: 1569–1576.
30. Fonseca V. Clinical significance of targeting postprandial and fasting hyperglycemia in managing type 2 diabetes mellitus. Curr Med Res opin, 2003; 19: 635-641.
31. Foster S, Tyler V. Tyler's honest herbal: A sensible guide to the use of herbs and related remedies: Routledge, 1999.
32. Fowden L, Pratt HM, Smith A. 4-Hydroxyisoleucine from seed of Trigonella foenum-graecum. Phytochemistry, 1973; 12(7): 1707-11.
33. Gabbay KH, Merola LO, Field RA. Sorbitol pathway: presence in nerve and cord with substrate accumulation in diabetes. Science, 1966; 151: 209-210.
34. Gabr MM, Hussein A, Sherif IO, Ali SI, Mohamed HE. Renal ischemia/reperfusion injury in type II DM: Possible role of proinflammatory cytokines, apoptosis, and nitric oxide. J Physiol Pathophysiol, 2011; 2: 6-17.
35. Gao, D., Li, Q., Gao, Z., & Wang, L. Antidiabetic effects of Corni Fructus extract in streptozotocin-induced diabetic rats. Yonsei medical journal, 2012; 53(4): 691-700.
36. Giugliano D, Ceriello A, Paolisso G. Diabetes mellitus, hypertension and cardiovascular diseases: which role for oxidative stress? Metabolism, 1995; 44: 363–8.
37. Glazner GW, Ishii DN. Insulinlike growth factor gene expression in rat muscle during reinnervation. Muscle Nerve, 1995; 18: 1433–1442.

38. Grimshaw CE. Aldose reductase: model for a new paradigm of enzymic perfection in detoxification catalysts. Biochemistry, 1992; 31: 10139-45.
39. Grover J, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol, 2002; 81(1): 81-100.
40. Hafizur RM, Babiker R, Yagi S, Chishti S, Kabir N, Choudhary MI The antidiabetic effect of Geigeria alata is mediated by enhanced insulin secretion, modulation of β-cell function, and improvement of antioxidant activity in streptozotocin-induced diabetic rats. Journal of Endocrinology, 2012; 214: 329-335.
41. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science, 2003 Aug 08; 301: 805-9. 5634.
42. Osasenaga Macdonald Ighodaroa, Abiola Mohammed Adeosuna, Oluseyi Adeboye Akinloye "Alloxan-induced diabetes, a common model" Science Direct medicina, 2017; 53: 365-374.
43. Rakieten N, Rakieten ML, Nadkarni MV. Studies on the diabetogenic action of alloxan. Diabetes, 1963 Jan; 12(1): 115-9.
44. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia, 2008 Aug; 51(2): 216-26.
45. Kaneto H, Matsuoka TA, Katakami N, et al. Oxidative stress and the JNK pathway are involved in the development of type 1 and type 2 diabetes. Curr Mol Med, 2007 Nov; 7(8): 674-86.
46. Halliwell B. Oxidation of low-density lipoproteins: questions of initiation, propagation, and the effect of antioxidants. J Clin Nutr, 1995; 61: 670-7S.
47. Han M, Li Y, Liu M, Li Y, Cong B Renal neutrophil gelatinase associated lipocalin expression in lipopolysaccharide-induced acute kidney injury in the rat. BMC nephrology, 2012; 13: 25.
48. Haskins K, Bradley B, Powers K. Oxidative stress in type 1 diabetes. Ann N Y Acad Sci, 2003; 1005: 43–54.
49. Hers HG. The mechanism of the transformation of glucose in fructose in the seminal vesicles Biochim Biophys Acta, 1956; 22: 202-203.
50. Lu Q, Yin XX, Wang JY, Gao YY, Pan YM. Effects of Ginkgo biloba on prevention of development of experimental diabetic nephropathy in rats. Acta Pharmacol Sin, 2007; 28(6): 818-28.

51. Madar Z, Stark A. New legume sources as therapeutic agents. British Journal of Nutrition, 2007; 88(S3): 287-92.
52. Mauer SM, Steffes MW, Brown DM: The kidney in diabetes. Am J Med, 1981; 70: 603–612.
53. McCance DR, Dyer DG, Dunn JA, Bailie KE, Thorpe SR, Baynes JW, et al. Maillard reaction products and their relation to complications in insulin-dependent diabetes mellitus. J Clin Invest, 1993; 91: 2470-2478.
54. Misera HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for SOD. J Biol Chem, 1972; 247: 3170–5.
55. Mishkinsky J, Joseph B, Sulman F. Hypoglycaemic effect of trigonelline. Lancet, 1967; 2: 1311-2.
56. Schmidt-Ott KM, Mori K, Kalandadze A, Li J-Y, Paragas N, Nicholas T, Devarajan P, Barasch J (2006) Neutrophil gelatinase-associated lipocalin-mediated iron traffic in kidney epithelia. Current opinion in nephrology and hypertension, 15: 442-449.
57. Sefi M, Fetoui H, Soudani N, Chtourou Y, Makni M, Zeghal N. Artemisia campestris leaf extract alleviates early diabetic nephropathy in rats by inhibiting protein oxidation and nitric oxide end products. Pathol Res Pract, 2012; 208(3): 157-62.
58. Shah S, Bodhankar S, Bhonde R, Mohan V. Hypoglycemic activity of the combination of active ingredients isolated from Trigonella foenumgraecum in alloxan induced diabetic mice. Pharmacologyonline, 2006; 1: 65-82.
59. Shapiro K, Gong W. Natural products used for diabetes. J Am Pharm Assoc, 2002; 42(2): 217-26.
60. Shapiro K, Gong W. Natural products used for diabetes. J Am Pharm Assoc, 2002; 42(2): 217-26.
61. Sharma R. Effect of fenugreek seeds and leaves on blood glucose and serum insulin responses in human subjects. Nutr Res, 1986; 6(12): 1353.
62. Slater TF, Sawyer BC. The stimulatory effects of carbon tetrachloride and other halogenoalkanes or peroxidative reactions in rat liver fractions in vitro. Biochem J., 1971; 123: 805–14.
63. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med, 1993; 329: 977-86.

64. The Diabetes Control Complications Trial Research Group. The effect of intensive diabetes therapy on the development and progression of neuropathy in the Diabetes Control and Complications Trial. Ann Int Med, 1995; 122: 561–8.
65. Thomalley PJ. Glycation in diabetic neuropathy: characteristics, consequences, causes, and therapeutic options. Int Rev Neurobiol, 2002; 50: 37-57.
66. Thomalley PJ, Langborg A, Minhas HS. Formation of glyoxal, methylglyoxal and 3 deoxyglucosone in the glycation of proteins by glucose. Biochem J., 1999; 344 Pt 1: 109-116.
67. Thornberry NA, Lazebnik Y. Caspases: enemies within. Science, 1998; 281: 1312-1316.
68. Tilton RG, Chang K, Pugliese G, Eades DM, Province MA, Sherman WR, et al. Prevention of hemodynamic and vascular albumin filtration changes in diabetic rats by aldose reductase inhibitors. Diabetes, 1989; 38: 1258-1270.
69. Togashi Y, Miyamoto Y. Urinary cystatin C as a biomarker for diabetic nephropathy and its immunohistochemical localization in kidney in Zucker diabetic fatty (ZDF) rats. Exp Toxicol Pathol, 2012.
70. Torrejais MM, Soares JC, Matheus SMM, Cassel FD, Mello JM, Basso NA. Histochemical and SEM evaluation of the neuromuscular junctions from alcoholic rats. Tissue Cell, 2002; 34: 117-123.
71. Vague P, Coste TC, Jannot MF, Raccah D, Tsimaratos M. C-Peptide Na+, K + - ATPase and diabetes. Exp Diabesity Res, 2004; 5: 37–50.
72. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol, 2007; 39: 44-84.
73. Visnagri, A., Kandhare, A. D., Shiva Kumar, V., Rajmane, A. R., Mohammad, A., Ghosh, P., ... & Bodhankar, S. L. Elucidation of ameliorative effect of Co-enzyme Q10 in streptozotocin-induced diabetic neuropathic perturbation by modulation of electrophysiological, biochemical and behavioral markers. Biomedicine & Aging Pathology, 2012.
74. Wada T, Furuichi K, Sakai N, Iwata Y, Yoshimoto K, Shimizu M, Takeda S-I, Takasawa K, Yoshimura M, Kida H. Up-regulation of monocyte chemoattractant protein-1 in tubulointerstitial lesions of human diabetic nephropathy. Kidney international, 2000; 58: 1492-1499.
75. Wahren J, Ekberg K, Jörnvall H. C-peptide is a bioactive peptide. Diabetologia, 2007; 50: 503–509.

76. Wang Q, Ouazzani J, Sasaki N, Potier P. A Practical Synthesis of (2S, 3R, 4S)-4-Hydroxyisoleucine, A Potent Insulinotropic-Amino Acid from Fenugreek. Eur J Org Chem, 2002; 834–9.
77. Waris G, Alam K. Attenuated antigenicity of ribonucleoproteins modified by reactive oxygen species. Biochem Molec Biol Int, 1998; 45: 33-45.
78. Watkins PJ. Clinical observations and experiments in diabetic neuropathy. Diabetologia, 1992; 35: 2-11.
79. Wild SH, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care, 2004.
80. Wolff SP. Diabetes mellitus and free radicals: free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. Br Med Bull, 1993; 49: 642.
81. Yagihashi S, Kamijo M, Baba M, Yagihashi N, Nagai K. Effect of aminoguanidine on functional and structural abnormalities in peripheral nerve of STZ-induced diabetic rats. Diabetes, 1992; 41: 47-52.
82. Yagihashi S. Pathogenetic mechanisms of diabetic neuropathy: lessons from animal models. J Peripher Nerv Syst, 1997; 2: 113-132.
83. Yagihashi, S., Yamagishi, S. I., & Wada, R. (2007). Pathology and pathogenetic mechanisms of diabetic neuropathy: correlation with clinical signs and symptoms. Diabetes research and clinical practice, 77(3): S184-S189.
84. Yasuda H, Sonobe M, Hatanaka I, Yamashita M, Miyamoto Y, Terada M et al. A new prostaglandin E1 analogue (TFC-612) prevents a decrease in motor nerve conduction velocity in streptozocin-diabetic rats. Biochem Biophys Res Commun, 1988; 150: 225-230.
85. Yusuf S, Teo KK, Pogue J, Dyal L, Copland I et al. Telmisartan, Ramipril or both in patients at high risk for vascular events. The ON TARGET Investigators. N Engl J Med, 2008; 358: 1547-1559.
86. Lambert O, Bourin M. SNRIs: mechanism of action and clinical features. Expert Rev Neurother, 2002 Nov; 2(6): 849-58. [PubMed]
87. Asnis GM, Henderson MA. Levomilnacipran for the treatment of major depressive disorder: a review. Neuropsychiatr Dis Treat, 2015; 11: 125-35.
88. Horst WD, Preskorn SH. Mechanisms of action and clinical characteristics of three atypical antidepressants: venlafaxine, nefazodone, bupropion. J Affect Disord, 1998 Dec; 51(3): 237-54.

89. Hickie IB, Rogers NL. Novel melatonin-based therapies: potential advances in the treatment of major depression. Lancet, 2011 Aug 13; 378(9791): 621-31.
90. Fiedorowicz JG, Swartz KL. The role of monoamine oxidase inhibitors in current psychiatric practice. J Psychiatr Pract, 2004 Jul; 10(4): 239-48.
91. Kaminsky BM, Bostwick JR, Guthrie SK. Alternate Routes of Administration of Antidepressant and Antipsychotic Medications. Ann Pharmacother, 2015 Jul; 49(7): 808-17.
92. Settimo L, Taylor D. Evaluating the dose-dependent mechanism of action of trazodone by estimation of occupancies for different brain neurotransmitter targets. J Psychopharmacol, 2018 Jan; 32(1): 96-104.
93. Glauser J. Tricyclic antidepressant poisoning. Cleve Clin J Med, 2000 Oct; 67(10): 704-6, 709-13, 717-9.
94. Lenzen S. The mechanisms of alloxan and streptozotocin induced diabetes. Diabetologia 2008; 51(2): 216–26.
95. Dunn JS, McLetchie NG. Experimental alloxan diabetes in the rat. Lancet, 1943; 242(September (6265)): 384–7.
96. Gomori G, Goldner MG. Acute nature of alloxan damage. Proc Soc Exp Biol Med, 1945; 58(March (3)): 232–3.
97. Goldner MG, Gomori G. Studies on the mechanism of alloxan diabetes. Endocrinology, 1944; 35(October (4)): 241–8.
98. Mullen S. Major depressive disorder in children and adolescents. Ment Health Clin, 2018 Nov; 8(6): 275-283.
99. Bahar MA, Kamp J, Borgsteede SD, Hak E, Wilffert B. The impact of CYP2D6 mediated drug-drug interaction: a systematic review on a combination of metoprolol and paroxetine/fluoxetine. Br J Clin Pharmacol, 2018 Dec; 84(12): 2704-2715.
100. Edinoff AN, Fort JM, Woo JJ, Causey CD, Burroughs CR, Cornett EM, Kaye AM, Kaye AD. Selective Serotonin Reuptake Inhibitors and Clozapine: Clinically Relevant Interactions and Considerations. Neurol Int, 2021 Sep 01; 13(3): 445-463.
101. Wei A, Peng J, Gu Z, Li J. QTc prolongation and torsades de pointes due to a coadministration of fluoxetine and amiodarone in a patient with implantable cardioverter-defibrillator: Case report and review of the literature. Medicine (Baltimore), 2017 Dec; 96(49): e9071.
102. Dent LA, Orrock MW. Warfarin-fluoxetine and diazepam-fluoxetine interaction. Pharmacotherapy, 1997 Jan-Feb; 17(1): 170-2.

103. Mazhar F, Akram S, Haider N, Ahmed R. Overlapping of Serotonin Syndrome with Neuroleptic Malignant Syndrome due to Linezolid-Fluoxetine and Olanzapine-Metoclopramide Interactions: A Case Report of Two Serious Adverse Drug Effects Caused by Medication Reconciliation Failure on Hospital Admission. Case Rep Med, 2016; 2016: 7128909.
104. Tisdale JE. Drug-induced QT interval prolongation and torsades de pointes: Role of the pharmacist in risk assessment, prevention and management. Can Pharm J (Ott), 2016 May; 149(3): 139-52.
105. Selph SS, McDonagh MS. Depression in Children and Adolescents: Evaluation and Treatment. Am Fam Physician, 2019 Nov 15; 100(10): 609-617.
106. Edinoff AN, Akuly HA, Hanna TA, Ochoa CO, Patti SJ, Ghaffar YA, Kaye AD, Viswanath O, Urits I, Boyer AG, Cornett EM, Kaye AM. Selective Serotonin Reuptake Inhibitors and Adverse Effects: A Narrative Review. Neurol Int, 2021 Aug 05; 13(3): 387-401.
107. Riggin L, Frankel Z, Moretti M, Pupco A, Koren G. The fetal safety of fluoxetine: a systematic review and meta-analysis. J Obstet Gynaecol Can, 2013 Apr; 35(4): 362-369.
108. Anderson KN, Lind JN, Simeone RM, Bobo WV, Mitchell AA, Riehle-Colarusso T, Polen KN, Reefhuis J. Maternal Use of Specific Antidepressant Medications During Early Pregnancy and the Risk of Selected Birth Defects. JAMA Psychiatry, 2020 Dec 01; 77(12): 1246-1255.
109. Agrawal R, Almoghrabi A, Attar BM, Gandhi S. Fluoxetine-induced Stevens-Johnson syndrome and liver injury. J Clin Pharm Ther, 2019 Feb; 44(1): 115-118.
110. Akaltun İ, Ayaydin H. Fluoxetine-related mania in an adolescent girl diagnosed with selective mutism: A case report. Asia Pac Psychiatry, 2020 Mar; 12(1): e12367.
111. Costagliola C, Parmeggiani F, Semeraro F, Sebastiani A. Selective serotonin reuptake inhibitors: a review of its effects on intraocular pressure. Curr Neuropharmacol, 2008 Dec; 6(4): 293-310.
112. Gandhi S, Shariff SZ, Al-Jaishi A, Reiss JP, Mamdani MM, Hackam DG, Li L, McArthur E, Weir MA, Garg AX. Second-Generation Antidepressants and Hyponatremia Risk: A Population-Based Cohort Study of Older Adults. Am J Kidney Dis, 2017 Jan; 69(1): 87-96.
113. Seshadri M, Thalitaya MD, Simon T, Odelola C, Enow H. Antidepressants and Hyponatremia in a patient with Colectomy - a Case Report. Psychiatr Danub, 2017 Sep; 29(Suppl 3): 610-614.

114. Jing E, Straw-Wilson K. Sexual dysfunction in selective serotonin reuptake inhibitors (SSRIs) and potential solutions: A narrative literature review. Ment Health Clin, 2016 Jul; 6(4): 191-196.
115. Patel DD, Galarneau D. Serotonin Syndrome With Fluoxetine: Two Case Reports. Ochsner J., 2016 Winter; 16(4): 554-557.
116. Kurt A. Jellinger, "The pathobiological basis of depression in Parkinson disease" Journal of Neural Transmission, 20 October 2022, 129: 1397–1418.
117. Henderson MA. Levomilnacipran for the treatment of major depressive disorder: a review. Neuropsychiatr Dis Treat, 2015; 11: 125-35.
118. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science, 2003 Aug 08; 301(5634): 805-9.
119.