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"EASTIMATION OF ANTI-LIPIDEMIC ACTIVITY OF BUTEA MONOSPERMA BY USING DEXAMETHASONE AS AN INDUCER IN WISTAR RATS''

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ABSTRACT

The present study was carried out to evaluate the antilipidemic activity of Butea monosperma aqueous flower extracts in dexamethasone induced diabetic rats for 15 days. Dexamethasone (10 mg/kg) was administered to male wistar albino rats to induce diabetes. These diabetic rats were grouped and treated with the aqueous flower extracts of Butea monosperma and glibenclamide separately for 15 days. Fasting blood glucose estimations and body weight measurements were carried out on 0^{th}, 5^{th}, 10^{th} and 15^{th} day of the experiment. On 15^{th} day the serum was separated and analysed for biochemical parameter variations. The whole pancreas of rat were removed and subjected to histological examination. The Butea monosperma aqueous flower extracts at high dose (400mg/kg) and low dose (200mg/kg) exhibited significant ($p<0.05$ and $p<0.01$) anti-diabetic activity in dexamethasone induced diabetic rats. The extract also showed improvement in parameters like body weight and lipid profile as well as regeneration of β-cells of pancreas in diabetic rats.

KEYWORDS: Butea monosperma, dexamethasone, fasting blood glucose.

INTRODUCTION

WHO defines diabetes mellitus as

A metabolic disorder of multiple etiologies, characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin

secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia (high blood sugar) and other signs, as distinct from a single illness or condition.

Types

There are three main types of diabetes:

- type 1 (accounts for 5-10% of cases),
- type 2 (accounts for about 90% of cases), and
- type 3 gestational diabetes (affects approximately 4% of pregnant women in the United States).

Diabetes Mellitus is a growing epidemic in both developed and developing countries. The spectacular increase in the incidence and prevalence of this chronic disease is destined to have enormous impact on mortality, morbidity and health care resources. The global number of people with diabetes mellitus is expected to be at least 220 million in 2010 reaching 324 million by 2025 (Jayakumar and Nisha, 2005).

As mentioned above, number of people getting affected by diabetes mellitus is increasing because of changes in lifestyle, and it is presenting itself as an epidemic. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries. Modern medicines like Biguanides, Sulphonylureas and Thiozolidinediones are available for the treatment of diabetes. But they also have undesired effects associated with their uses. Alternative medicines particularly herbal medicines are available for the treatment of diabetes.

MATERIAL AND METHODS

Plant material

The aqueous flower extract Butea monosperma was collected from the Innocon pharmaceuticals, Pune.

Chemical and reagents

All the chemicals used for the research work were of analytical grade. Dexamethasone (new neeta chemicals pune), Glibenclamide (daonil 5 mg tablet).

Animals

Wistar strain male albino rats weighing 150–250 g were used for this study. Animals were housed in groups of six in colony cages at an ambient temperature of 25 ± 2°C and 45–55% relative humidity with 12 h light and dark cycle. They had free access to pellet chow (**Nutrivet Life Sciences, Pune; India**) and water ad libitum. The experimentations on animals were approved by the Institutional Animal Ethical Committee (IAEC) under the regulation of **Committee for the Purpose of Control and Supervision of Experiments on Animals** (CPCSEA), New Delhi. The animals were shifted from animal house to the laboratory one hour prior to the start of the experiment and all the experiments were carried out between 9:00- 16:00 hours.

Oral Glucose Tolerance Test in normal rats (M. Anitha et al., 2012)

Normal overnight fasted rats were tested for the Oral Glucose Tolerance Test (OGTT) of the various concentrations of Butea Monosperma extracts and standard drug glibenclamide. Albino Wistar rats of either sex weighing 150-200gm were divided into 4 groups consisting of 6 rats in each group.

Group I - Normal control received 0.9% saline

Group II – aqueous extract of Butea monosperma flower (200mg/kg p.o) Group III - aqueous extract of Butea monosperma flower (400mg/kg p.o)

Group IV - Standard drug glibenclamide (10 mg/kg p.o)

After 30 minutes of drug administration, the rats of all groups were orally treated with 2g/kg of glucose. The blood samples were collected through retro orbital plexux at 0,30,60,90,120 minutes and Blood glucose level was estimated at various time intervals.

Dexamethasone induced diabetes in rats

The animals were weighed and numbered. They were randomized into 5 groups (vehicle control, diabetic control, Butea monosperma test 1, test 2 and standard) with 6 rats in each group. Group 1 was control and given vehicle (2% acacia). Group 2 was diabetic control and received dexamethasone (10mg/kg i.p), Group 3 and 4 were test and received Butea monosperma extract (200 and 400mg/kg p.o. as per b.w.). Group 5 was standard and received Glibenclamide (10 mg/kg p.o).

For induction of diabetes all animal were injected with dexamethasone throughout study except group

1. Treatment of Butea monosperma extract (200 and 400mg/kg p.o.) and glibenclamide to group 3, 4 and 5 were respectively started from day 5 of dexamethasone injection after the confirmation of the hyperglycemic state.

11. Biochemical Estimation

Triglyceride

Total cholesterol

LDL HDL VLDL

On 15th day blood sample from each rats was collected in eppendroff tubes, they were allow to clot and centrifuged immediately to obtain clear serum that used to carry out estimation of biochemical parameters such as triglyceride, total cholesterol, LDL, VLDL and HDL using special kits.

Histopathological Profile

The organs (pancreas) of the experimental animals were fixed in 10% formalin (As prepared 10ml formalin+90 ml water) prior to routine processing in paraffin-embedded blocks. Sections of 5µm thick were cut and stained using hematoxylin-eosin (H & E) stain. The pathological observations of all tissues were performed on gross and microscopic bases. Sections observed under microscope and photomicrographs were taken for histopathological screening.

Statistical analysis

The results were expressed as mean ± SEM. The statistical analysis was done by using Graph Pad prism 5.0. The statistical analysis of all the results was carried out using two way ANOVA followed by Bonferroni test and one way ANOVA followed by Dunnett's test $p<0.05$ was considered as significance.

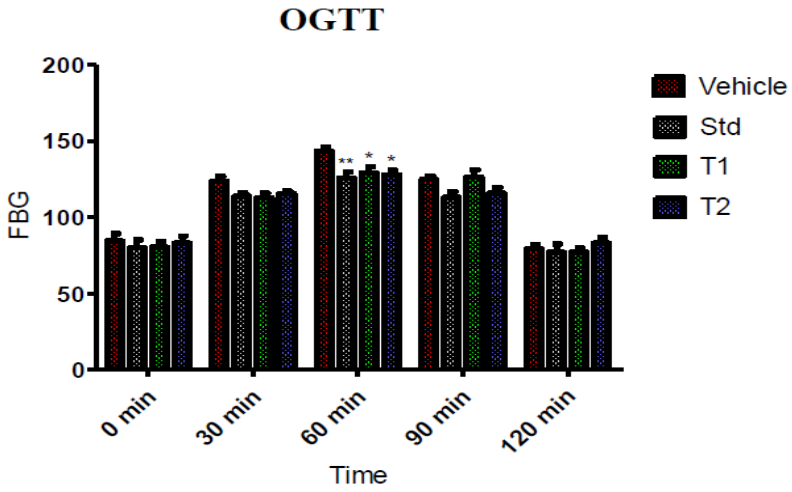
RESULTS

The effect of aqueous extract of Butea monosperma flower and standard drug on blood glucose level were estimated before and after drug treatment at different time intervals 0,30,60,90 and 120 minutes after the glucose load in the normal rats was shown in table. Administration of glucose load significantly increase fasting blood glucose level in all groups. The aqueous extract 200mg/kg and 400mg/kg had significantly ($P<0.05$) reduced blood

glucose when compared with vehicle control group similarly standard drug glibenclamide treated group found more significant (P <0.01) lowering blood glucose level compare to vehicle control groups.

Blood Glucose				
Treatment	**Vehicle**	**Standard**	**Test 1**	**Test 2**
0 min	84.50±4.7	80.00±5.2	80.50±3.7	83.16±4.5
30 min	123.66±3.2	113.66±2.3	113.00±2.8	115.66±1.5
60 min	143.50±2.8	125.83±4.1	129.16±4.1	127.83±2.9
90 min	124.83±2.3	113.16±3.5	126.50±4.7	116.00±3.6
120 min	79.33±3.0	77.16±5.3	77.16±2.7	83.16±3.8

StatisticStatistical significance test was done by Two Way ANOVA followed by Bonferroni posttest (n=6); Values are mean ± SEM of 6 animals per group; *P <0.05 and **P < 0.01 vs vehicle control.



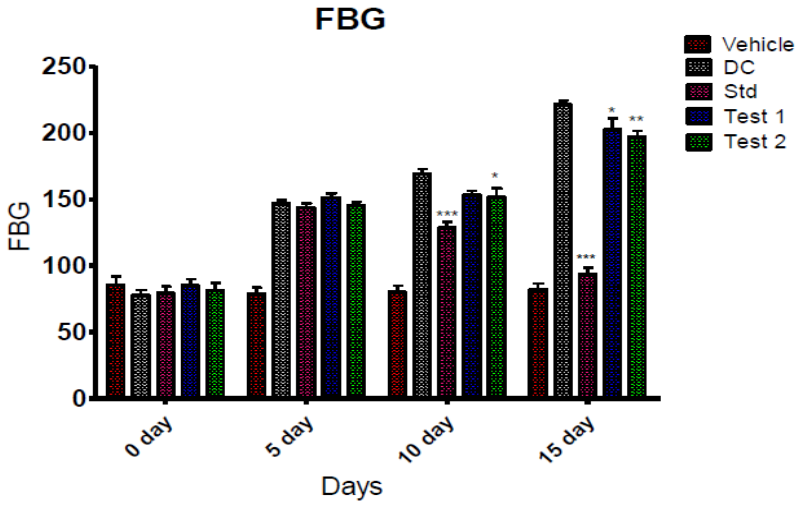
The effect of aqueous extract of Butea monosperma flower on fasting blood glucose level on the 0,5th,10th and 15th days of the study period was shown in table.

Administration of dexamethasone for 15 days found to significantly increase fasting blood glucose level from 130 to 200mg/dl compared to normal control group due to increased insulin resistance.

The aqueous extract 200 mg/kg along with dexamethasone for 15 days had significantly (P< 0.05) reduced fasting blood glucose on 15th days compared to dexamethasone treated group (diabetic control) similarlyaqueous extract 400 mg/kg found more significant(P <0.01) as standarddrug in lowering fasting blood glucose level on the 10th day and 15th day compare to diabetic controlgroups.

Fasting Blood Glucose					
Treatment	**Vehicle**	**Diabetic Control**	**Standard**	**Test 1**	**Test 2**
0 day	85.83±5.8	77.66±4.0	79.33±4.9	85.16±4.7	81.66±5.1
5 day	78.66±4.7	146.83±2.4	143.66±3.0	151.33±3.0	145.66±2.2
10 day	80.16±4.8	169.33±3.2	128.66±4.2	153.33±3.0	151.66±6.6
15 day	82.16±4.5	221.33±3.0	93.33±5.3	202.50±8.6	196.83±4.5

Statistical significance test was done by Two Way ANOVA followed by Bonferronipost test(n=6); Values are mean ± SEM of 6 animals per group; * P <0.05 and **P < 0.01vsdiabetic control (for test groups).*** P < 0.001 vs diabetic control (for standard group



Biochemical parameters

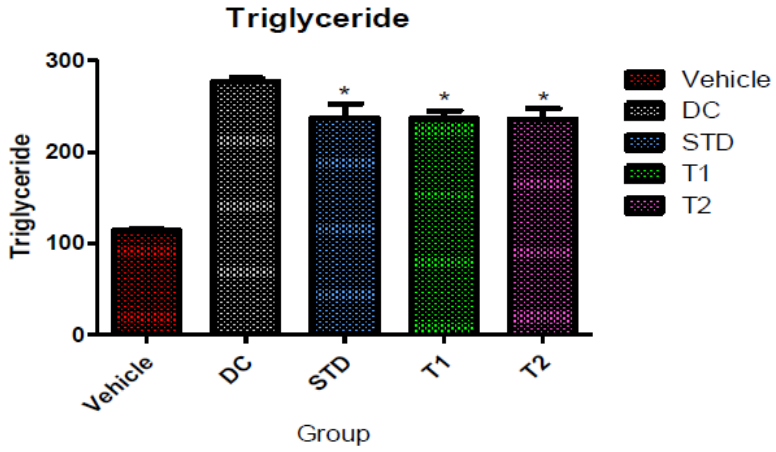
Biochemical parameters of all the groups are estimated on 15th day which shown in table. Animals treated with Dexamethasone significantly increased serum triglycerides, cholesterol, LDL and VLDLwhen compared to normal control group.

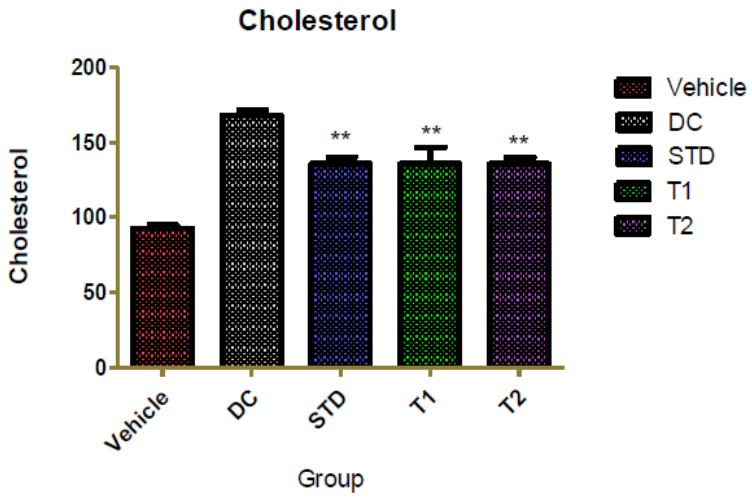
A significant percentage reduction of total cholesterol level, LDL, TGL and VLDL in extracts treated and standard drug glibenclamide was significant (*P < 0.05 and **P < 0.01) to diabetic group. However HDL level increased with extracts and Standard group respectively.

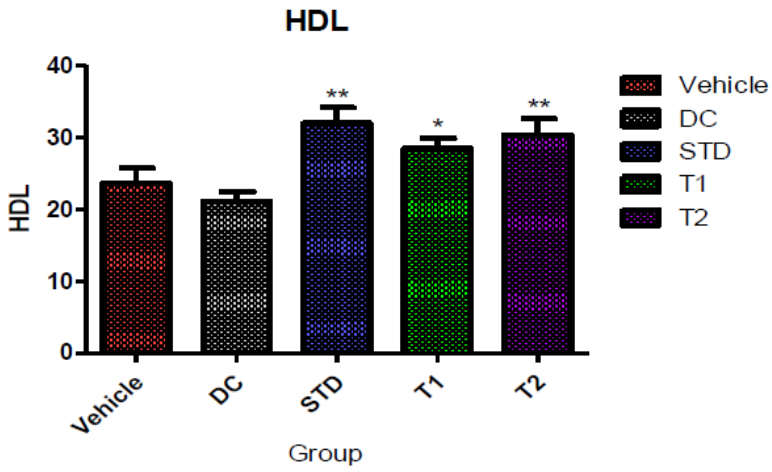
Parameter	Vehicle	Diabetic Control	Standard	Test 1	Test 2
TG	114.00±1.9	277.00±4.1	237.50±15.6	236.83±8.7	235.66±11.7
TC	92.66±2.0	168.33±3.1	135.83±4.4	135.66±11.1	135.66±4.2
HDL	23.66±2.0	21.16±1.3	32.00±2.1	28.50±1.4	30.33±2.3
LDL	40.83±1.5	88.00±2.6	74.00±3.8	84.66±2.5	74.83±3.0
VLDL	10.16±0.6	29.66±1.3	23.66±1.8	23.66±1.4	23.33±2.1

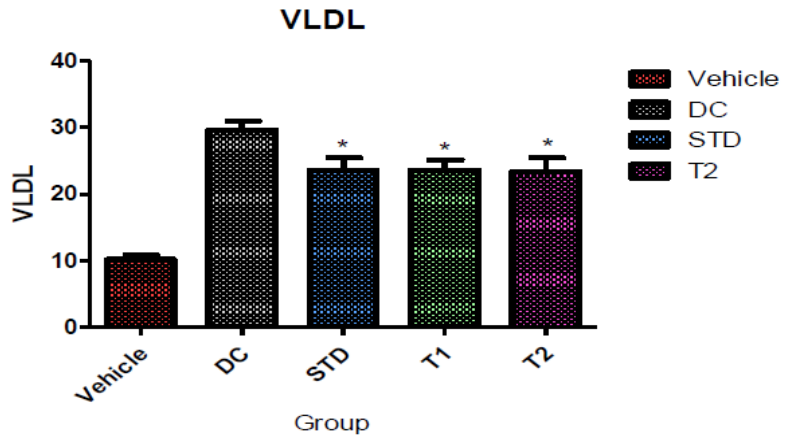
The biochemical parameters in each rats was measured on the 15th day.

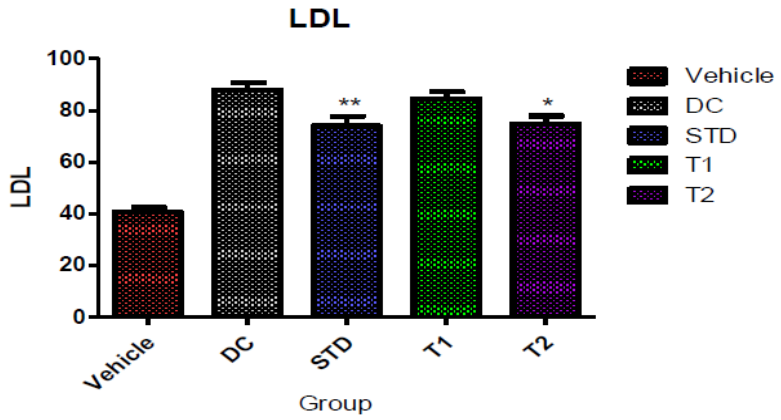
Statistical significance test was done by One way ANOVA followed by Dunnet's multiple comparison test (n=6); Values are mean ± SEM of 6 animals per group; *P < 0.05 and ** P < 0.01 vs diabetic control











Body weight

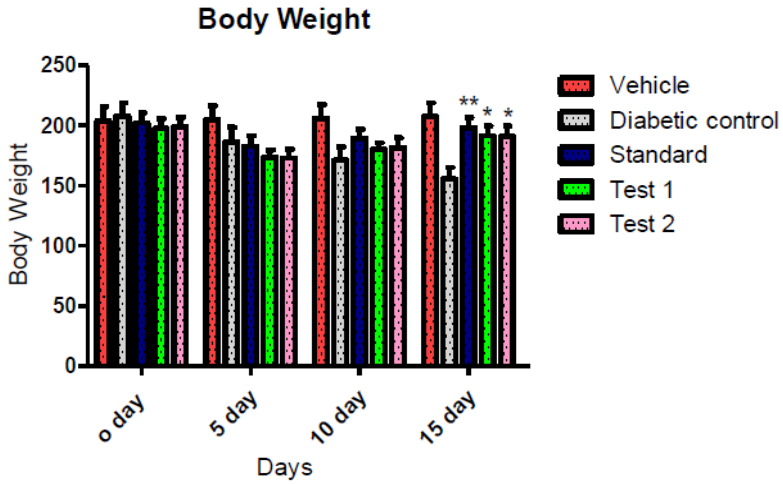
Table showsthe body weight of the normal and treated groups significantly differ from diabetic control on 0 to 15th day. The treated groups animal body weight maintained throughout the experiment compare to diabetic control. Dexamethasone caused weight reduction which was prevented significantly (P< 0.05) by the aqueous extract 200 and 400 mg/kg of Butea monosperma after 15days of treatment compared with diabetic control. Standard Glibenclamide treated animals show more significantly weight reduction (P< 0.01) after 15 day of treatment when compared with diabetic control.

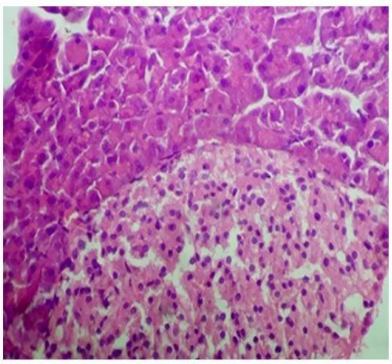
Days	Vehicle	Diabetic control	Standard	Test 1	Test 2
0 day	203.66±12.1	207.83±11.0	202.16±8.4	197.66±8.1	199.00±7.9
5 day	204.66±11.6	186.33±12.1	182.83±8.6	173.66±6.1	173.00±7.6
10 day	206.00±11.6	171.16±10.9	189.3±7.5	180.00±5.7	181.50±8.1
15 day	207.33±11.5	155.50±9.6	198.16±8.6	191.50±8.2	191.00±8.4

Statistical significance test was done by Two Way ANOVA followed by Bonferroni posttest (n=6); Values are mean ± SEM of 6 animals per group; *P <0.05 and **P < 0.01 vsdiabetic control (for test groups).

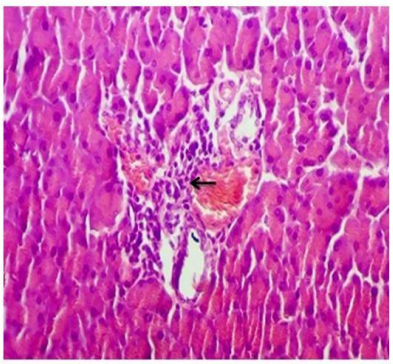
Histopathological changes in Pancreas

The histopatholgical changes in pancreas of all non treated and treated groups.

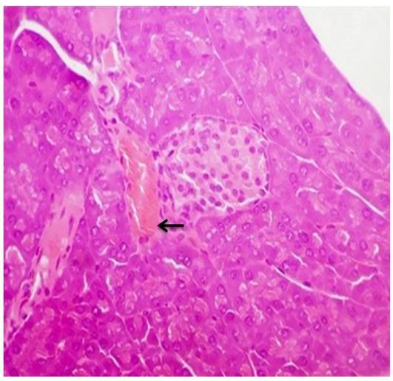




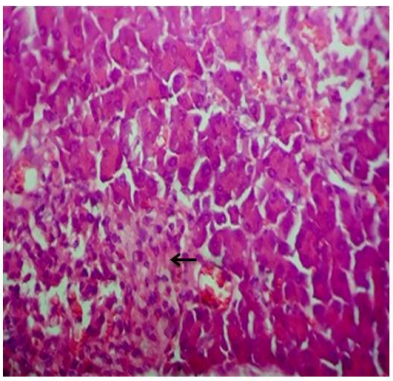
G1: Control- Nothing abnormal detected. **H&E 40X**



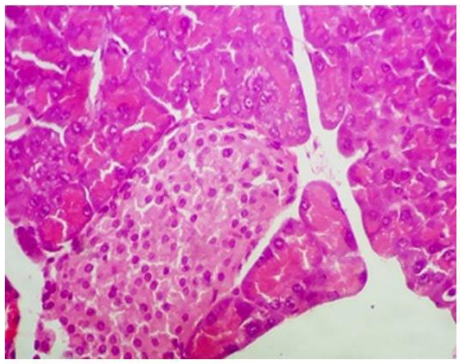
G2: Diabetic- Minimal degeneration of islet of Langerhans, note infiltration of mononuclear cells (arrow.) **H&E 40X**



G3: Standard- Minimal congestion of blood vessels. **H&E 40X**



G4: T1- Note the congestion and degeneration of Islets (arrow). **H&E 40X**



G5 T2- Note recovery of pancratic Islet of langerhans. **H&E 40X**

DISCUSSION AND CONCLUSION

Diabetes (type 2) are rising globally and occupied approximately 90 % of the diabetic patients and it causes serious socioeconomic problems in developing countries. Immunosuppressive drug glucocorticoid like dexamethasone at high dose level causes type-2 diabetes mellitus. Multiple numbers of high doses of dexamethasone represent insulin resistance by augmenting the insulin action on insulin dependent glucose uptake cells. Hepatocyte, skeletal muscle cells and adipose cells are insulin dependent cells. Insulin promotes the expression of GLUT-4 channels there by uptake of glucose increased by insulin dependent cells. At high dose levels of dexamethasone causes protein break down and muscle wasting.

Dexamethasone decreases the consumption of carbohydrates and lipids, decreases the glycogen synthesis and increases the protein break down. We can use different oral hypoglycemic agents and insulin for the treatment but it has various adverse effects so there is need to develop medicinal therapy. Traditionally there are so many medicinal plant which have a potential anti-diabetic activity and have been into practice since ancient times. Common advantages of herbal medicines are effectiveness, safety, affordability and acceptability.

Most of the studies published on these molecules aim to reduce the blood glucose levels but neglect the altered lipid profile, which plays a vital role in long term complications of diabetes. In view of available literature on Butea monosperma, an attempt was made to evaluate the hypoglycemic activity extract of flowers and establish a correlation with its positive effect in reversal of derangement of lipid profile.

As mentioned in the results, at both low and high dose of aqueous extract of Butea monosperma extract in Dexamethasone induced diabetes in rats model showed significant (*P < 0.05 and **P<0.01) decrease in fasting blood glucose levels when compared with untreated dexamethasone induced diabetic rats.

In this study both low and high dose of extract of Butea monosperma showed significant (*P< 0.05 and **P< 0.01) decrease in serum triglyceride, total cholesterol, LDL and VLDL level when compared with untreated dexamethasone induced diabetic rats. Though there was some increase in the HDL levels in low dose extract, high dose extract and glibenclamide treated groups.

Administration of glibenclamide, lower dose and high dose of the extract resulted in a corresponding loss of body weight in diabetic rats. In comparison to untreated diabetic rats the changes in body weight observed were significant (*P<0.05) in low and high dose Butea monosperma extract treated diabetic rats whereas it was more significant (**P<in glibenclamide treated group.

Histopathological data shows the minimal degeneration of islet of Langerhans and infiltration of mononuclear cells in diabetic control group, congestion and degeneration of islet in test 1 (200mg/kg) while the test 2 (400mg/kg) Butea monosperma extract treated group shows significantly recovery of pancreatic islet of Langerhans.

The above finding indicate that the extract of Butea monosperma can be effectively used in insulin resistance conditions. Our studies suggest that aqueous extract of *Butea monosperma* is effective in bringing back glycemic and lipemic levels to normal in dexamethasone induced hyperglycemia, hypercholesterolemia and hypertriglyceridemia in rats. Further studies into these aspects might reveal the actual mechanisms involved in anti-diabetic activity of butea monsperma extract.

Therefore, in conclusion, the current *Butea Monosperma Flower* extract was found to possess anti-diabetic antilipidemic properties, and should be evaluated further for biochemical and pharmacological investigation to understand the mechanism of the antidiabetic effect of *butea monosperma* flower extract.

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