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ANTIDEPRESSANT AND ANTISTRESS ACTIVITY OF METHANOLIC EXTRACT OF TAGETES ERECTA LEAVES AND ITS ACUTE TOXICITY PROFILE IN MICE

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ABSTRACT

Different parts of plant *Tagetes erecta* Linn. (Asteraceae) have been traditionally used for a number of disorders including fever, epileptic fits, astringent and as a carminative to relieve flatulence or abdominal pain or distension. The aim of the study was to evaluate antidrepressant/antistress activity of methanolic extract of *Tagetes erecta* leaves (TEL) and its acute toxicity profile including haematological/histopathological aspects, and phytochemical analysis. The mice were administered with vehicle (10 ml/kg saline, served as control), the extract of TEL (250 and 500 mg/kg body weight/rat/p.o.) and Standard drug, Diazepam (2 mg/kg/rat) for 7 days. Forced Swimming Test (FST), Tail Suspension Test (TST) and Anoxic Tolerance Test (ATT) were conducted 60 min after the last administration of TEL extract to determine the antidrepressant/antistress effects. Acute toxicity profile including haematological and histopathological aspects was carried out after 14 days treatment of TEL extract in mice. Results showed that TEL extract (at 250 and 500 mg/kg doses) significantly ($p < 0.01$ and 0.001) reduced the duration of immobility in FST and TST without affecting locomotor activity in open field test. It was also observed that TEL extract significantly increased ($p < 0.05$) anoxia stress tolerance time in a dose dependant manner as compared to control, but,

less than in Diazepam treatment. The TEL extract did not show any toxic effects, and body/organ weights, histological characteristics and haematological parameters were normal after 14 days of treatment. High performance thin layer chromatography-ultra violet (HPTLC-UV) analysis indicated the presence of alkaloids, flavonoids, tannins, saponin, carbohydrates and glycosides as the active chemical constituents of TEL extract. In conclusion, TEL extract administration significantly reduced the immobility in FST and TST and increased anoxia stress tolerance in ATT in mice. It is also nontoxic in acute toxicity test. This may validate its traditional use in management of stress disorder.

KEYWORDS: Forced swimming test, Tail suspension test, Anoxic stress tolerance test, Acute toxicity profile, *Tagetes erecta* Linn.

INTRODUCTION

There is arguably nothing more ubiquitous than psychological stress and virtually all diseases are affected by it. The term stress is defined by Hans Selye (1976) as the sum of all the nonspecific changes caused by function or damage and a state of threatened homeostasis.[1] Stress triggers a wide range of body changes called General Adaptation Syndrome (GAS), the stimuli, which produce GAS are called the stressors including cold, heat, infection and toxins.[2,3] Psychological stress has been used by researchers to examine a variety of stress related phenomena including fear[4], anxiety[5], post-traumatic stress disorder[6], and learning and memory.[7] Chronic unpredictable stress model has been used to examine depression[8], obesity[9], atherosclerosis[10] and Alzheimer's disease.[11] Restraint stress in animals results in tissue damage.[12] Stress responses are composed of alterations in behavior, autonomic function and the secretion of hormones including adrenocorticotropin hormone, corticosterone, oxytocin, prolactin and rennin.[13] The Gamma aminobutyric acid-A receptor (GABA-AR) has been known to be closely involved in the acute stress response and clinically relevant anxiolytic drugs such as benzodiazepines act on this receptor.[14] All the antagonist of the Corticotrophin-releasing factor-1 (CRF1) receptor inhibits the adrenocorticotrophic hormone (ACTH)-corticosterone response to stressors.[15] Benzodiazepine drugs such as diazepam, chlordiazepoxide, alprazolam and midazolam have been used as antidepressants.[16]

Recently, the use of complementary and alternative medicines is increasing over worldwide because of Allopath (symptonic drugs of chemical composition) limits the clinical utility due to problem of tolerance and physical dependence on their prolonged use.[17] Therefore, the

search for novel pharmacotherapy from medicinal plants for psychiatric illness has been progressed significantly. Therefore, there is a need of an effective herbal anti-stress agent in the therapy of stress.[18] Several herbal drugs have been introduced for decreasing anxiety and stress in many emotional and physical disorders and gastric ulcers. Reported studies have been shown that the number of plants such as, *Alchornea cordifolia*[19], green tea[20], *Sida cardifolia*[21], *Ginkgo biloba* Leaves[22], *Centella asiatica* Linn.[23], *Argyreia speciosa*[24], and *Cinnamomum tamala* Nees and Eberm[25] possess antistress and antidepressant-like properties. FST, TST, ATT and gastric ulcer tests in mice are the behavioural tests useful in the screening of potential antidepressant/antistress drugs, and assessing of other manipulations that are expected to affect depression related behaviours.[19,22,24,26,27]

The plant TEL is locally known as Genda Phul (Marigold) and belongs to the family Asteraceae (Compositae). It is a stout, branching herb, native of Mexico and other warmer parts of America and neutralized elsewhere in the tropics and subtropics including India and Bangladesh.[28, 29] The parts of the plant are used in colds and bronchitis[28,30], various gastro-intestinal disorders[31], carbuncles, eye infection, muscular spasms[32], radiation dermatitis associated with breast cancer therapy[33] and as an anti-nociceptive and anti-inflammatory effects.[34] Marigold therapy was first described in the paediatric (children and their diseases) literature as a treatment for plantar hyperkeratotic lesions in 1980.[35] The flowers are useful in epileptic fits[36], fevers, as an astringent and as a carminative.[28,30] The flowers and leaves extracts also show antibacterial, antimicrobial, insecticidal, wound haeling, hepatoprotective, antioxidant and antidiabetic activities.[27,29,38-40] *Tagetes erectus is* rich in the xanthophylls, lutein, which occurs acylated with fatty acids.[41,42] The phytochemical constituent, carotenoids have shown excellent antioxidant properties while α- and β-carotene, xanthophylls and retinoids have been reported to inhibit some types of cancers.[43,44] The phytochemical studies on different parts of TEL have been shown various chemical constituents such as thiophenes, alkaloids, saponins, flavonoids, carotenoids and triterpenoids.[45,46] The antioxidents such as gallic acid, gallicin, quercetagetin, 6-hydroxykaempferol-O-hexoside, patuletin-O-hexoside and quercetin were found in the extracts of TEL, where the quercetagetin was identified as the strongest antioxidant.[47,48] Epidemiological studies have suggested that antioxidents rich plants play a protective role in health and against diseases and their consumption lowered risk of cancer, heart disease and hypertention and stroke. The curative properties of medicinal properties are due to presence of different constituents such as alkaloids, saponins, flavonoids, glycosides, phenols and

sterols. Tannins are useful in treatment of inflammed and ulcerated tissues, cancer and anti-septics. Flavonoids have protective role against allergies, inflammation, free radical, platelet aggregation, microbes, ulcer, hepatotxins, viruses and tumors.[49] Saponins show anti-inflammatory, coagulant, antidiabetic, antioxient, aldose reductase inhibitory activity and cholesterol binding properties.[50] The folkfore uses of TEL include its use in anaemia, irregular menstruation, abdominal pain, muscular pain, bone pain, indigestion, colic cough, dysentery and rheumatism.[36,40] The present study was aimed to evaluate the antidepressant/antistress activity of methanolic extract of *Tagetes erecta* leaves in mice. In addition, study also reveals its preliminary phytochemical screening and acute toxicity profile.

MATERIALS AND METHODS

Chemicals

Diazepam was obtained from Sigma Chemical Co., USA. All other chemicals were available locally.

Plant Material

Leaves (500g) of *Tagetes erecta* Linn were collected in the months of Oct-Dec, 2011 from local areas of Lucknow (India). The plant specimen was authenticated from botany department of National Botanical Research Institute (NBRI), Lucknow (voucher specimen no. NBRI/CIF/259/2011). The collected leaves were dried in shade, crushed to coarse powder and used for further studies.

Preparation of extract

The powdered leaves (500g) of *Tagetes erecta* were loaded in Soxhlet extractor and defatted with petroleum ether (40-60C). The marc (the residue) was dried and extracted with methanol (50% v/v) in a same extractor up to three cycles. Finally, the extracts were concentrated to semi solid mass using rotary evaporator under vacuum. The traces of solvent were removed by keeping the dried extract in to a desiccator as per method described earlier.[51]

Preliminary phytochemical screening

The extract of TEL was subjected to preliminary phytochemical screening for identification of the phytoconstituents viz. alkaloids, saponins, carbohydrates, glycosides, flavonoids and tannins.[52]

High-performance thin layer chromatography(HPTLC)

HPTLC was performed for determination of flavonoids and to confirm the different phytoconstituents in plant extract using HPTLC instrument and CAMAG software (Germany). Thin layer chromatography (TLC) plates (silica gel>60 F_{254}, 20 cm; Merck) were prewashed with methanol and activated in oven at 100^{o}C for 10 min. Pre coated silica gel acted as stationary phase in the experiments and Rf values were determined by developing the spots. Samples (10 µl) from plant extract (1mg/ml) were spotted on pre-coated plates using Linomat 5 Applicator system (CAMAG). Mobile phase for TEL extract was used as ethyl acetate: formic acid: glacial acetic acid: water (100: 11: 11: 26) for separation of flavonoid. Samples (10ul) were loaded on the TLC plates with the help of linomat 5 applicator and processed for HPTLC analysis. Results were analysized in HPTLC monitor by using CAMAG software for digitization and photo documentation. Pr-ecoated aluminum silica gel 60 F_{254} plates were used as stationary phase and Hexane: ethyl acetate (93:7) was used as mobile phase for development of chromatogram.

Animals

Adult male mice (20-25g Body weight) used in this study was obtained from the Institute's breeding colony. They were acclimatized to uniform husbandry conditions (22 ± 3°C, 12 h light: 12 h dark cycle) for 1 week prior to the experiment. Animals were fed with a pellet diet supplied by Hindustan Lever Ltd., Bangalore, India and access to water *ad libitum*. All animals were handled in accordance with the standard guide for the care and use of laboratory animals, and experimental protocol was approved by Institutional Animal Ethics Committee (IAEC, approval No. CPCSEA/IAEC/IAEC/col./06).

Pharmacological screening methods

Forced swim test (FST)

FST is one of the most frequently used behavioral models for screening an antistress like activity in rodents. Male mice were randomly divided into 4 groups, Group I (Control, 10 ml/kg Saline, p.o.), Group II and III (TEL extract 250 and 500 mg/kg body weight, p.o., respectively) and Group IV (2 mg/kg Standard drug, diazepam /rat/day, i.p.) were treated for 7 days. On the seventh day, one hour after oral treatment and 30 min after intraperitoneal administration of the Standard drug, all the mice were subjected to swimming endurance test. Briefly, mice were individually forced to swim in open glass chamber (25x15x25 cm3) containing fresh water to a height of 15 cm and maintained at 26±1^{o}C. At this height of

water, animals were not able to support themselves by touching the bottom or the sidewalls of the chambers with their hind-paws or tail. Mice were considered to be immobile when they ceased struggling and remained floating motionless in water, making only those movements necessary to keep their head above water.[24]

Tail-Suspension Test (TST)

Male mice were randomly divided into 4 groups containing five animals each, Group I (Control, 10 ml/kg Saline, p.o.), Group II and III (TEL extract 250 and 500 mg/kg body weight, p.o., respectively) and Group IV (2mg/kg Standard drug, diazepam /rat/day, i.p.) were treated for 7 days. The TST was conducted on the 7th day within 6 min of drug administration which is commonly employed behavioral model for screening antidepressant activity in mice. Each mouse was individually suspended to the edge of a table, 50cm above the floor, by adhesive tape placed approximately 1cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded manually during 6 min of testing period. Animals were considered to be immobile when it did not show any body movement, hung passively and completely motionless.[26]

Anoxic Tolerance Test (ATT)

The mice were randomly divided into four groups of five animals each. Group I (Control, 10 ml/kg Saline, p.o.), Group II and III (TEL extract 250 and 500 mg/kg body weight, p.o., respectively) and Group IV (2mg/kg Standard drug, diazepam /rat/day, i.p.) were treated for 7 days. On the seventh day, the mice were subjected to ATT by keeping them in a confined airtight 250 ml glass jar. The time taken for the mice to exhibit the first clonic convulsion was taken as the end point. The animals were removed immediately from the vessel for recovery and resuscitated if needed.[19]

Acute toxicity profile

For acute toxicity study, 24 male mice were randomly divided into four groups containing six animals in each. Animals in Group I was given vehicle only (saline water, 10ml/kg body weight, p.o.), served as control. Animals in Groups II (250mg/kg), III(500 mg/kg) and IV (2000 mg/kg) were administered orally with TEL extract for 14 days. Food/water intake and gross behavioural changes were recorded daily for 1-2 hr after the drug administration daily for toxic effects (if any). Autopsy of animals from control and treated groups was done on day 15. Blood samples were collected from tail region before autopsy in pre-coated ethylene diamine tetra acetic acid (EDTA) vials for haematology. The vital body organs, viz. brain,

liver, heart, spleen and kidneys, were dissected out, freed from connective tissues/blood clots, weighed and fixed in Bouin's solution (24 h) for histology purpose.

Haematological studies

Blood samples from control and treated animals were processed in MS-9 Haematology Analyzer(Germany) to study haematological parameters, viz. packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC) counts, platelets counts, haematocrit (HCT), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), polymorphs, neutrophil, lymphocytes and monocytes.

Histopathology

Bouin's fixed tissues from brain, liver, heart, spleen and kidneys were dehydrated in graded ethanol series, cleared in xylene, infiltrated and embedded in moulton paraffin wax (at 58^0C). Tissues blocks were cut at 5 µm and stained with routine haematoxylin and eosin staining, and the slides were examined under Olympus Trinocular Microscope (Olympus BX 51, Tokiyo, Japan) and photomicrographed at X200 Magnification.

Statistical analysis

The values were represented as mean ± SEM for six mice each group. Analysis of variance (ANOVA) test was followed by individual comparison by Newman–Keuls test using Prism Pad Software for the determination of level of significance.

RESULTS

Preliminary phytochemical Analysis

Methanolic extract of TEL showed the presence of alkaloids, saponins, flavonoids, tannins and carbohydrates and glycosides (Table 1).

Table 1: Phytochemical Screening Tests for methanolic extract of TE leaves.

Compounds extract	Identification Test	TE leaves
Alkaloids	Dragendroff test	+
	Mayer's test	-
Glycosides	Wagner's test	+
Foam Test		+
Flavanoids	Legal's test	+
	Shinoda test	+
	Sodium hydroxide test	-
	Lead acetate test	+
FeCl2 test		+
Carbohydrate	Molisch's test	+

+ present, - Absent.

Separation of active compounds by HPTLC

Compounds from methanolic extract of TEL were detected using the solvent system n-hexane:ethyl acetate (93:7 ratio) and analyzed in HPTLC. Plant extract showed six compounds having an Rf value of 0.11, 0.14, 0.18, 0.23, 0.36, 0.61 and λ max at 254 nm. The compound 1 was the major compound with 60.03% area (Table 2, Figures 1 and 2).

Table 2: Rf values and Area% by HPTLC screening of TEL extract.

Peak	Start Position	End Position	Area%
1.	0.04 Rf	0.11 Rf	60.03 %
2.	0.11 Rf	0.14 Rf	5.17 %
3.	0.16 Rf	0.18 Rf	5.02 %
4.	0.19 Rf	0.23 Rf	11.69 %
5.	0.31 Rf	0.36 Rf	2.68 %
6.	0.54 Rf	0.61 Rf	15.41 %

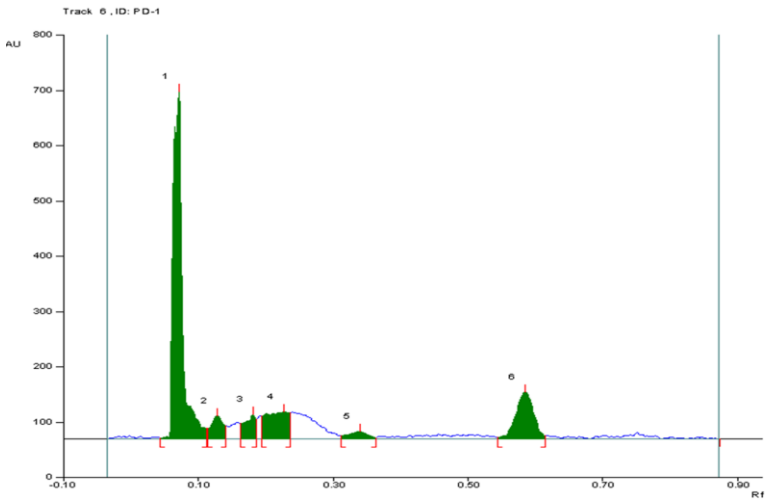

Figure 1: HPTLC Finger Print of *Tegetes erecta* (Scaned wave length -254 nm).

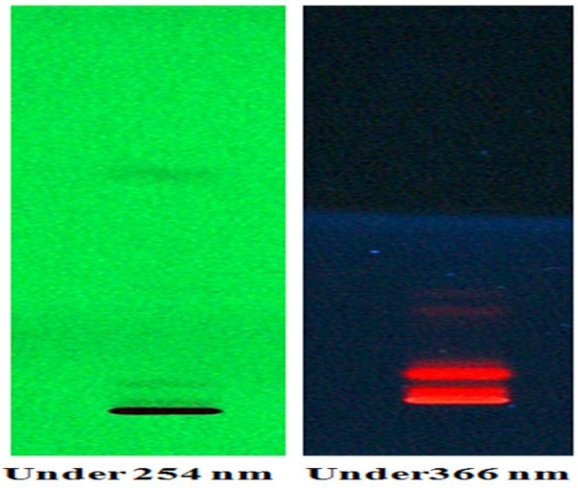

Figure 2: HPTLC plates of *Tagetes erecta* derivatised at wavelengths of 254 nm and 366 nm.

Effect of TEL extract on FST

The treatment groups had a significant increase in restraint stress measured by the FST in comparision to control group. The methanolic extract of TEL induced a significant decrease ($p<0.01$) in immobility time of animals in a dose dependant manner. The immobility time was reduced in the TEL treated groups after 7 days treatment (i.e. 6.84 sec) as compared to vehicle treated group (i.e. 18.46 sec). However, Diazepam treated mice produced a significant decrease (2.40 sec, $p <0.001$) in immobility time as compared to control (18.46 sec) and TEL extract treated(6.84-11.2 sec) animals (Figure 3).

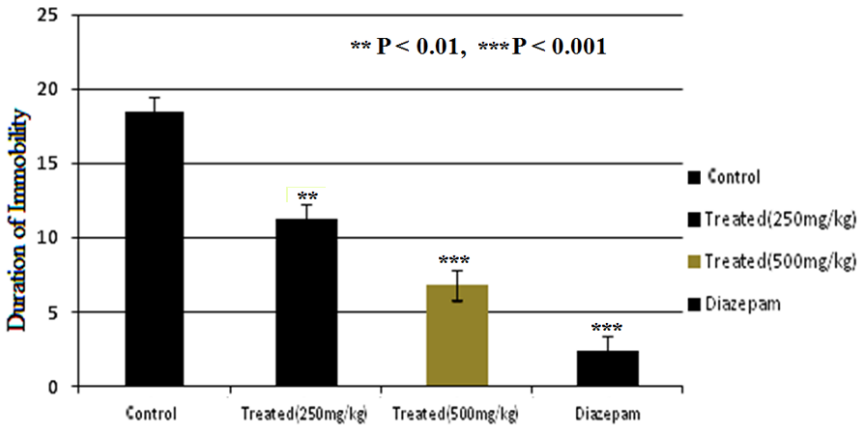

Figure 3: Effect of *Tagetes erecta* leaves extract on Forced Swim Test in mice (P < 0.01, ***P < 0.001, Control vs. Treated).**

Effect of TEL extract on ATT

In stressed treated mice, an increase in anoxia tolerance time was observed. The effect of TEL extract on anoxia stress tolerance in mice was found to be in a dose dependant manner. Treatment with methanolic extract at 250 and 500 mg/kg doses for 7 days caused significant ($p<0.05$) increase in the anoxia stress tolerance time (24.5 min and 30.22 min respectively.) when compared to stressed control animals (i.e. 20.04 min). Diazepam treated mice increased the anoxia tolerance time as compared to extract treated animals (i.e. 33.24 min)(Figure 4).

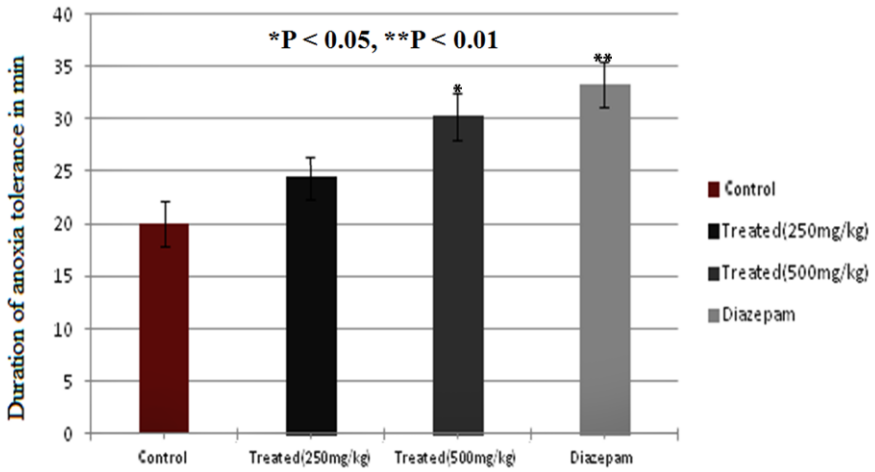

Figure 4: Showing effects of *Tagetes erecta leaves* extract on Anoxia Stress Tolerance test in mice (*P < 0.05, **P <0.01, Control vs. Treated).

Effect of TE Extract on TST

Mice treated with methanolic extract of TEL show dose dependent decrease in mean duration of immobility time as compared to control. The antistress effect of Standard drug, diazepam was observed to be 152 sec as compared to control (226 sec) which showed highly significant decrease ($p<0.001$) as compared to control. Treatment of methanolic extract of TEL at 250mg/kg showed insignificant decrease (NS), but, at dose of 500mg/kg body weight it showed significant decrease ($P<0.01$) in immobility time as compared to controls. The results are represented in figure 5.

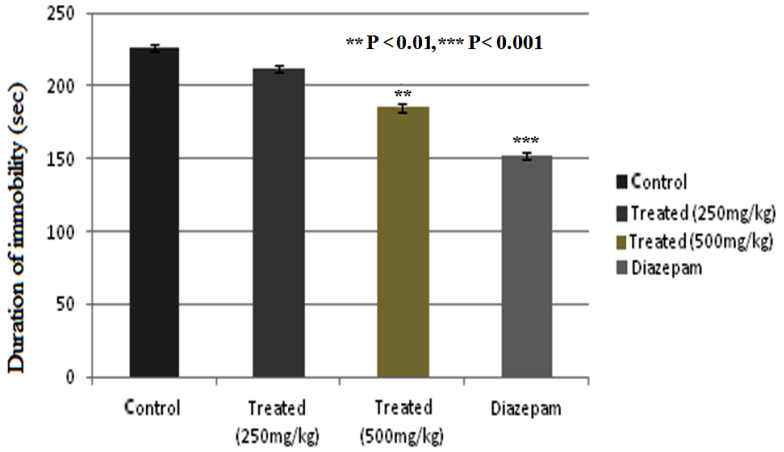

Figure 5: Showing effect of *Tagetes erecta* extract on Tail Suspension Test in mice (P <0.01; ***P < 0.001; control vs. treated).**

Toxicity Studies

Administration of methanolic extract of TE leaves in mice at doses 250mg/kg, 500 mg/kg and 2000 mg/kg body weight produced no significant changes in gross behavioural aspects. No mortality was observed during 1–3 hr of toxicity study in treated mice as compared to controls. The acute toxicity study indicates that the methanolic extract of TEL is safe up to a dose of 2000 mg/kg body weight by oral route.

Effect of TEL extract on organ weights, hematology and histopathology

There were no significant differences in weight of principal organs viz. Liver, spleen, brain, heart and kidney of treated as compared to that of the controls and all animals exhibited a body weight gain during the 14 days treatment period (Table 3).

Table 3: Effect of acute treatment of methanolic extract of *Tagetes erecta* leaves on the weight of principal organs in albino mice (n=5 number of animals, Values are expressed as mean ± SEM).

Groups	Relative Organs weights (%) Liver	Spleen	Brain	Heart	Kidney
Control	6.55±0.16	0.87±0.02	1.77±0.02	0.89±0.02	1.63±0.20
Treated-					
(250mg/kg)	6.56±0.15	0.87±0.02	1.77±0.04	0.88±0.03	1.62±0.21
(500mg/kg)	6.58±0.153	0.88±0.02	1.75±0.07	0.88±0.06	1.62±0.21
(500mg/kg)	6.74±0.15	0.89±0.02	1.75±0.09	0.87±0.03	1.62±0.03

The results of the hematology parameters, viz. WBC (m/mm³), RBC (m/mm³), Platelets (%), HGB (g/dl), MCV (fL), HCT (%), MCHC (g/dl), Polymorphs, Lymphocytes, Monocytes (%), Eosinophlis and basophils showed no significant changes in treated mice (at doses 250mg/kg, 500 mg/kg and 2000 mg/kg body weight) as compared to controls (Table 4).

Table 4: Effect of Methanolic extract of *Tagetes erecta* leaves on hematological parameters in mice (n= 5 number of animals; Values are expressed as Mean ± SEM).

S.No.	Parameters Studied	Control Group	TEL-Treated Groups (250mg/kg)	(500mg/kg)	(2000mg/kg)
1.	WBC (m/mm³)	6.94±1.09	6.92±1.07	6.81±1.12	6.07±0.15
2.	RBC (m/mm³)	8.99±0.04	8.75±0.03	8.53±0.09	8.58±0.29
3.	Platelets (%)	513±1.71	511±1.75	510±1.81	509±2.21
4.	HGB (g/dl)	13.7±0.18	13.7±0.18	13.4±0.18	13.1±0.20
5.	MCV (fL)	48.3±0.23	48.2±0.26	48.1±0.28	47.9±0.22
6.	HCT (%)	42.2±0.18	42.21±0.2	42.2±0.18	41.9±0.21
7.	MCHC (g/dl)	31.2±0.23	31.2±0.21	31.1±0.20	31.4±0.21
8.	Polymorphs	31.1±0.30	31.0±0.31	30.9±0.29	30.7±0.20
9.	Lymphocytes	58.2±0.13	58.4±0.13	58.7±0.13	59.8±0.07
10.	Monocytes(%)	3.6±0.17	3.6 ±0.17	3.7±0.17	3.9±0.15
11.	Eosinophlis	9.1±0.11	9.1 ±0.11	9.0±0.11	8.98±0.08
12.	Basophils	1.0±0.21	1.1 ±0.21	1.1±0.21	1.10±0.06

No marked histopathological changes were observed in vital organs viz. liver, brain, spleen, heart and kidney of treated mice (at 250, 500 and 2000 mg/kg doses of TEL extract/day, p.o. for14 days) as compared to controls (Figure 6).

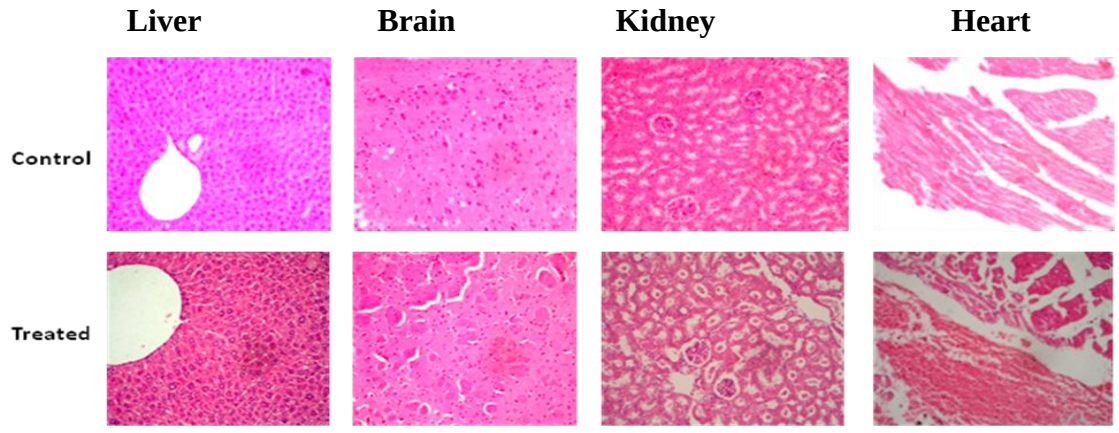

Figure 6. Histology of liver, brain, kidney, heart and spleen from control group of mice showing normal histoarchitecture. Treatment with methanolic extract of TEL for 14 days did not show any deleterious effects in liver, brain, kidney, heart and spleen respectively at 2000mg/kg dose level as compared to controls. H-E staining; x400 magnification for all figures.

DISCUSSION

The phytochemical screening of TEL extract revealed the presence of alkaloids, flavonoids, tannins, saponins, carbohydrates and glycosides which show concurrence with the previous study of Ramya et al., (2012) who demonstrated the presence of alkaloids, flavonoids, steroids, tannins and phenolic compounds as the major secondary metabolites in leaf extract.[53] Presence of compounds like, beta-sitosterol, daucosterol and gallic acid in the extract of TEL have also been reported previously.[54] The antistress and antidepressant potential of *Tagetes erecta* can be attributed to its alkaloids[55] and flavonoids[56] which are present in methanolic extract. In addition, carbohydrates, saponins and glycosides present in TEL extract that offer protection to cellular component.[57] The separation of active compounds by HPTLC, showed the presence of six compounds at λ max 254nm, and compound 1 was the major compound with 360.03% area and Rf value of 0.11.

The results of the present study show that the methanolic extract of TEL possess significant anti-stress activity based on observations of FST, ATT and TST in stressed albino mice. The high dose of TEL extract (500mg/kg) increases the swimming endurance time and tail suspension time as compared to lower dose (250 mg/kg). It was evident by decreased immobility time and increased anoxia stress tolerance by TEL extract. The immobility displayed in rodents by stress such as FST and TST that reflect a state of despair or lowered mood and may reflect depression-like disorders in humans. Further, this immobility time has been shown to be reduced by treatment with antistress/antidepressant drugs, Imipramine hydrochloride and phenobarbitone sodium.[23,58] A significant correlation was found between the clinical efficacy of antidepressant drugs and their potency in both models. It has been recently shown that the regulation of adrenergic receptor may be the major mechanism which increases plasma levels of adrenaline and noradrenaline and decreases monoamine oxidase levels in brain during swimming endurance test (FST).[59,60] The FST and TST results indicate clearly that the TEL extract has the properties whereby it increases the physical endurance as well as the overall performance in mice exhibiting significant anti-stress activity. TST is based on the observations in rodents mostly in mice although gerbils and rats have been used after initial escape behavior, develop an immobile position when subjected to inescapable stressful situation.[61,62] It has been suggested that acute antistress drugs decreases the immobility time in FST and TST, thus, using immobility time as an indicator of a state of lowered mood or hopelessness in animals. This behavioral model has been proposed for the screening of new antidepressant compounds and used further as a tool for preclinical

screening of antidepressant agents, and neurobiological and genetic mechanisms underlying stress and antidepressant responses.[59,63,64] In ATT, the anoxia tolerance time significantly increases in TEL extract-treated animals. This prolongation of mean time to convulsion, demonstrate antistress property which can be attributed to its powerful anti-oxidant and free radical scavenging activities.[65] Previous study have also been shown an increase in anoxia stress tolerance time and swimming endurance time as compared to control group with ethanolic extract of *Tribulus terrestris* and *Alchornea cordifolia*.[66] Anoxia of the central nervous system (CNS) cells plays a major role in the development of CNS disorders.[67] It may be due to increased utilization of the ATP-PC pathway, increased levels of muscle glycogen (a storage form of glucose that can provide energy for more prolonged activities), or decreased concentrations of muscle lactic acid and ammonia (two toxic by-products of muscular effort). This may be attributed to the anti-oxidant effect of plant extracts. The food and water intake by the stress induced rats were decreased in comparison with that of the normal control and TEL treated rats. It may be due to the decreased release of cortisol, a major stress hormone that appears to promote abdominal fat indicating its primary connection between stress and weight gain.[68] The Plant, TEL, improves anoxia stress tolerance, may allow cellular functions of CNS at low level of oxygen (O_2) supply, and thus appears to decrease O_2 demand during anoxia. The increase in anoxia tolerance time by TEL is a good indicator for its usefulness in various brain disorders. Benzodiazepines (BDZ) such as diazepam reduce stress and anxiety responses by acting on high-affinity receptor sites present in the CNS. Because of this effect, they are one of the most frequently used class of psychotropic drugs worldwide.[69] Other central receptors such as BDZ, peripheral-type binding sites (PBR) have also been identified in the endocrine steroidogenic tissues, immune organs, and in cells such as macrophages and lymphocytes.[70-72] BDZ did not affect the brain and hypothalamic 5-hydroxytryptamine receptors (5-HT) and plasma corticosterone in control rats. But, attenuated stress-induced elevation of brain and hypothalamic 5-HT and plasma corticosterone level induced by stress justifying its anti-stress action.[73,74] Reported studies have shown that the inhibition of anxiolytic-like effects, GABA/BDZ receptor antagonist and 5-HT_A by Tagetes lucida extract in plus-maze model.[75]

In acute toxicity study, TEL extract did not affect the body /organs weights, pheripheral blood parameters and histological characteristics of body organs after 14 days of treatment. Analysis of blood parameters is relevant to risk evaluation as changes in the haematological system have higher predictive value for various studies.[76] Methanolic extract of TEL did not

differ the values for Hb, WBC, HCT, MCHC, platelets, lymphocytes, eosinophils, basophils and monocytes when compared with control group after 14 days of treatment. The slight increase in haematological values (lymphocytes, monocytes and basophils) have been demonstrated to be an improvement in disease progression.[77,78] The body has very limited reserve of platelets, so they can be rapidly depleted.[79] Lymphocytes are the main effector cells of the immune system[80] and any alteration in its number may affect the effector cells of the immune system. The decrease in haemoglobin, HCT and MCHC which causes anemia or other disturbances, but, these parameters were statistically equal to those of control group, discarding the possibility of anemia by TEL treatment. The histological staining of vital organs did not show any apparent damage to the kidney, liver, heart, spleen in all the treated groups when compared with the controls. Therefore, the plant extract is non-toxic up to 2000 mg/kg dose for durations of 14 days treatment period.

CONCLUSION

The present study with extract of TEL showed significant antidepressant-like anti-stress activity as evidenced by the FST and TST that decreases immobility time and prolonged convulsion/anoxia tolerance time in ATT. Further, this plant extract did not show any toxic sign in acute toxicity study where haematological parameters and histology of vital organs remained unaffected, similar to in controls. This indicates that TEL extract has a potential clinical application for the management of stress disorders. However, isolation, characterization and identification of active phytoconstituents for antidepressant/anti-stress activity and neuropharmacological disorders need to be investigated.

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REFERENCES

1. Selye H. Stress in Health and Disease Butterworth, London. Aquaculture. 1976; 28: 241-249.
2. Selye H. Experimental evidence supporting the conception of adaptation Energy. Am J Physiol, 1938; 123: 758-765.
3. Panossian A, Wikman G, Wagner H. Plant adaptogens III. Earlier and more recent aspects and concepts on their mode of action. Phytomedicine, 1999; 6: 287-300.
4. Uribe-Mariño A, Francisco A, Castiblanco-Urbina MA, Twardowschy A, Salgado-Rohner CJ, Crippa JA, Hallak JE, Zuardi AW, Coimbra NC. Anti-aversive effects of cannabidiol on innate fear-induced behaviors evoked by an ethological model of panic

attacks based on a prey vs the wild snake Epicrates cenchria crassus confrontation paradigm. Neuropsychopharmacology, 2012; 37: 412-421.

5. Sotnikov SV, Markt PO, Umriukhin AE, Landgraf R. Genetic predisposition to anxiety-related behavior predicts predator odor response. Behavioural Brain Res, 2011; 225: 230-234.
6. Zoladz PR, Conrad CD, Fleshner M, Diamond DM. Acute episodes of predator exposure in conjunction with chronic social instability as anmanimal model of post-traumatic stress disorder. The Int J on the Biology of Stress, 2008; 11: 259-281.
7. Park CR, Zoladz PR, Conrad CD, Fleshner M, Diamond DM. Acute predator stress impairs the consolidation and retrieval of hippocampusdependent memory in male and female rats. Learning & memory, 2008; 15: 271-280.
8. You Z, Luo C, Zhang W, Chen Y, He J, Zhao Q, Zuo R, Wu Y. Pro- and anti-inflammatory cytokines expression in rat's brain and spleen exposed to chronic mild stress: Involvement in depression. Behav Brain Res, 2011; 225: 135–141.
9. Bose M, Oliván B, Laferrère B. Stress and obesity: the role of the hypothalamic-pituitary-adrenal axis in metabolic disease. Current opinion in endocrinol diabetes obesity, 2009; 16: 340-346.
10. Gu H, Tang C, Peng K, Sun H, Yang Y. Effects of chronic mild stress on the development of atherosclerosis and expression of toll-like receptor 4 signaling pathway in adolescent apolipoprotein E knockout mice. J Biomed Biotechnol, 2009; 61: 38-79.
11. Carroll J, Iba M, Bangasser D. Chronic Stress Exacerbates Tau Pathology, Neurodegeneration, and Cognitive Performance through a Corticotropin-Releasing Factor Receptor-Dependent Mechanism in a Transgenic Mouse Model of Tauopathy. J Neuroscience, 2011; 40: 14436-14449.
12. Kovacheva-Ivanova S, Bakalova R, Ribavov SR. Immobiliation stress enhances lipid peroxidation in rat lungs. Material and methods. Gen Physiol Biophys, 1994; 13: 469-482.
13. Van de Kar LD, Blair ML. Forebrain pathways mediating stress induced hormone secretion. Front. Neuroendocrinology, 1999; 20: 1-48.
14. Rudolph U, Mohler H. GABA-based therapeutic approaches: GABAA receptor subtype functions. Curr Opinion Pharmacol, 2000; 6: 18-23.
15. Gully D, Geslin M, Serva L, Fontaine E, Roger P, Lair C, Darre V, Marcy C, Rouby PE, Simiand J. 4-(2-Chloro-4-methoxy-5-methylphenyl)- N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]5-methyl-N-(2-propynyl)-1,3-thiazol-2-amine hydrochloride

(SSR125543A): a potent and selective corticotrophin-releasing factor(1) receptor antagonist: I. Biochemical and pharmacological characterization. J Pharmacol Exptl Therapeutics, 2002; 301: 322-332.

16. Groenink L, Van der Gugten J, Zethof TJJ, Van der Heyden JAM, Olivier B. Neuroendocrine effects of diazepam and flesinoxan in the stress-induced hyperthermia test in mice. Pharmacol Biochem Behav, 1996; 54: 249-254.
17. Gray TS, Carney ME, Magnuson DJ. Direct projections from the central amygdaloid nucleus to the hypothalamic paraventricular nucleus: possible role in stress-induced adrenocortico- tropin release. Neuroendocrinol, 1989; 50: 433-446.
18. Habbu PV, Mahadevan KM, Kulkarni PV, Daulatsingh C, Veerapur VP, Shastry R. Adoptogenic and *in vitro* antioxidant activity of flavanoids and other fractions of *Argyreia speciosa* (Burm. F) Boj. in acute and chronic stress paradigms in rodents. Ind J Exptl Biol, 2010; 48: 53-60.
19. Ishola IO, Ashorobi RB, Adoluwa O. Evalution of antistress potential and phytochemical constituents of aqueous root extract of Alchornae cordifolia. Asian J Sci Res, 2008; 1: 476-480.
20. *Salim* S, *Rajaje* A. Effect of *green* and black *teas* on immobilization induced stress in male wister albino rats. Int J Pharmacol, 2009; 5: 137-145.
21. Sumnath M, Mustafa SS. Antistress, adaptogenic activity of sida cordifolia, Indian J Pharmacol Sci, 2009; 71: 323-324.
22. Kalkunte SS, Singh AP, Chaves FC, Gianfagna TJ, Pundir VS, Jaiswal AK, Vorsa N, Sharma S. Antidepressant and Antistress Activity of GC-MS characterized Lipophilic Extracts of Ginkgo biloba Leaves. Phytother. Res, 2007; 21: 1061 –1065.
23. Selvi PT, Senthil Kumar M, Kathiravan T, Rajesh R, Megala J, Sravani S. Antistress activity of aqueous extract of leaves of *Centella asiatica*. Linn by *in vivo* methods. Asian J Res Pharm Sci, 2012; 2: 91-94.
24. Patel NB, Galani VJ, Patel BG. Antistress activity of *Argyreia speciosa* roots in experimental animals. J Ayurveda Intgr Med, 2011; 2: 129-136.
25. Upadhyay G, Khoshla S, Kosuru R, Singh S. Anxiolytic, antidepressant, and antistress activities of the aqueous extract of *Cinnamomum tamala* Nees and Eberm in rats. Ind J Pharmacol, 2016; 48: 555-561.
26. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology (Berl), 1985; 85: 367-370.

27. Can A, Dao DT, Terrillion CE, Piantadosi SC, Bhat S, Gould TD. The Tail Suspension Test. J Vis Exp, 2012; 59: e3769, DOI:10.3791/3769.
28. Kirtikar KR, Basu BD. Indian medicinal plants. Lalit Mohan Basu, Allahabad, India, 1987.
29. Karwani G, Sisodia SS. *Tagetes erecta* plant: Review with significant pharmacological activities. World Journal of Pharmaceutical Sciences, 2015; 1180-1183.
30. Ghani A. Medicinal plants of Bangladesh: Chemical constituents and uses. Asiatic Society of Bangladesh, Dhaka, 1998.
31. Kirtikar KR, Basu BD. Indian medicinal plants. International Book Publishers. Delhi, 1993.
32. Caius J. "The Medical and Poisonous Compsoitae of India." J Bombay Natural History Society, 1940; 41: 90-91.
33. Pommier P, Gomez F, Sunyach MP, D'Hombres A, Carrie C, Montbarbon X. Phase III randomized trial of *Calendula officinalis* compared with trolamine for the prevention of acute dermatitis during irradiation for breast cancer. J Clin Oncol, 2004; l22: 1447-1453.
34. Shinde NV, Kanase KG, Shilimkar VC, Undale VR, Bhosale AV. Antinociceptive and Anti-Inflammatory Effects of Solvent Extracts of *Tagetes erectus* Linn. Tropical J Pharmaceutical Res, 2009; 8: 325-329.
35. Hadfield RA,. Vlahovic TC, Tariq Khan M. The Use of Marigold Therapy for Podiatric skin Conditions. The Foot & Ankle Journal, 2008; 1(7): 1.
36. Shetty L J, Harikiran H, Fernandes J. Pharmacological evaluation of ethanolic extract of flowers of Tagetes erecta on epilepsy. J Pharmacy Res, 2009; 2: 1035-1038.
37. Salinas-Sánchez DO, Aldana-Llanos L, Valdés-Estrada ME, Gutiérrez-Ochoa M, Valladares-Cisneros G, Rodríguez-Flores E. Insecticidal activity of Tagetes erecta extract on Spodostera Frugipera (LEPIDOPTERA: NOCTUIDAE), Florida Entomologist, 2012; 95: 428-432.
38. Verma P, Verma A. Evaluation of antibacterial activity of different parts of *Tagetes erecta.* Int J Pharm Life Sci. (IJPLS), 2012; 3: 1766-1768.
39. Padalia H, Chanda S. Antimicrobial Efficacy of Different Solvent Extracts of Tagetes erecta L. Flower, Alone and in Combination with Antibiotics. Appli Micro Open Access, 2015; 1: 1000106. doi:10.4172/2471-9315.1000106.
40. Shetty L, Sakr FM, Al-Obaidy K, Patel MJ, Shareef H. A brief review on medicinal plant *Tagetes erecta* Linn. J Applied Pharmaceutical Sci, 2015; 5: 91-95.

41. Gregory GK, Chen TS, Philip T. Quantitative analysis of lutein esters in Marigold flowers *Tagetes erecta* by high performance liquid chromatography. J Food Sci, 1986; 51: 1093-1094.
42. Philip T, Berry JW. Nature of lutein acylation in marigold (*Tagetes erecta*) flowers. J Food Sci, 1975; 40: 1089-1090.
43. Mathews-Roth MM. Antitumor activity of beta-carotene, canthaxanthin and phytoeno. Oncology, 1982; 39: 33-37.
44. Moon RC, Mc Cormick DL, Mehta RG. Inhibition of carcinogenesis by retinoids. Cancer Res, 1983; 43: 2469-2475.
45. Faizi S, Naz A. Palmitoleate(9Z-Hexadeca-9-enoate)esters of oleanane triterpanoids from the golden flowers of *Tagetes erecta*: isolation and autoxidation products. Helv Chim Acta, 2004; 87: 46-56.
46. Munhoza VM, Longhinia R, Souzab JRP, Zequic JAC, Leite Mellod EVS, Lopesa GC, Melloa JCP. Extraction of flavonoids from Tagetes patula: process optimization and screening for biological activity. Rev Bras Farmacogn, 2014; 24: 576-583.
47. Gong Y, Liu X, He WH, Xu HG, Yuan F, Gao YX. Investigation into the antioxidant activity and chemical composition of alcoholic extracts from defatted marigold (*Tagetes erecta* L.) residue. Fitoterapia, 2012; 83: 481-489.
48. Wang W, Xu H, Chen H, Tai K, Liu F, Gao Y. In vitro antioxidant, anti-diabetic and antilipemic potentialsof quercetagetin extracted from marigold (*Tagetes erecta* L.) inflorescence residues. J Food Sci Technol, 2016; 53: 2614–2624
49. Patel DK, Kumar R, Prasad SK, Hemlatha S. Pedalium murex Linn (Pedaliaceae) fruits: A comparative antioxidant activity of its different fractions. Asian Pac J Trop Biomed, 2011; 1: 395-400.
50. Patel DK, Patel K, Dhanabal SP. Phytochemical standardization of Aloe vera extract by HPTLC technique. J Acute Disease, 2012; doi: 10.1016/S2221-6189(13)60054-2.
51. Kiranmai M, Kazim SM, Ibrahim M. Combined wound healng activity of Gymnema Sylvestere and *tagetes erecta* linn. Int J Pharmaceutical Applications, 2011; 2: 135-114.
52. Ashokkumar P, Rajkumar, Kanimozhi. Phytochemical screening and antimicrobial activity from five Indian medicinal plants against human pathogens. Mid Eastern J Sci Res, 2010; 5: 157-162.
53. Ramya R, Mahna S, Bhanumathi, Samanta P, Bhat SK. Analysis of phytochemical composition and bacteriostatic activity of Tagetes sp. IRJP, 2012; 3: 114-115.

54. Zhang Y, Zhang TT. Studies on the chemical constituents from the stem and leaves of Tagetes erecta. Zhong Yao Cai, 2010; 33: 1412-1414.
55. Perviz S, Khan H, Pervaiz A. Plant Alkaloids as an Emerging Therapeutic Alternative for the Treatment of Depression. Front Pharmacol, 2016; 7: 28. doi: 10.3389/fphar.2016.00028
56. Dimpfel W. Rat electropharmacograms of the flavonoids rutin and quercetin in comparison to those of moclobemide and clinically used reference drugs suggest antidepressive and/or neuroprotective action. Phytomedicine, 2009; 16: 287-294.
57. Rastrelli L, Saravia A, Hernandez CR, Desimone F. Anti-imflammatory activity-guide fractionation of Gnaophalium strminewn. Pharm Biol, 1998; 30: 315-319.
58. Ipek Y, Fazilet A. Effects of desipramine and tramadol in a chronic mild stress model in mice are altered by yohimbine but not by pindolol. Eur J Pharmacol, 2005; 514: 165–174.
59. Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacological Sciences, 2002; 23: 238-245.
60. Singh N, Misra N, Srivastava AK, Dixit KS, Gupta GP. Effect of antistress plants on biochemical changes during stress reaction. Ind J Pharmacol, 1991; 23: 137-142.
61. Varty GB, Cohen-Williams ME, Hunter JC. The antidepressant- like effects neurokinin NK1 receptor antagonists in a gerbil tail suspension test. Behavioural Pharmacol, 2003; 14: 87-89.
62. Chermat R, Thierry B, Mico JA, Steru L, Simon P. Adaptation of the tail suspension test to the rat. J Pharmacol, 1986; 17: 348-350.
63. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: A primary screening test for antidepressants. Arch Int Pharmacodyn Ther, 1977; 229: 327-336.
64. Guadarrama-Cruz G, Alarcon-Aguilar FJ, Lezama-Velasco R, Vazquez-Palacios G, Bonilla-Jaime H. Antidepressant-like effects of *Tagetes lucida* Cav. in the forced swimming test. J Ethnopharmacol, 2008; 120: 277-281.
65. Oke JM, Hamburger. Screening of some Nigeria medicinal plants for anti-oxidants activity using 2,2, diphenyl picryl-hydrazyl radical. Afr J Biomed Res, 2001; 5: 77-79.
66. *Shivakumar H, Javed T, Prakash T, Rao* RN, Swamy BJ, *Goud* AV. *Adaptogenic activity* of *ethanolic extract* of *Tribulus terrestris* L. J Nat Remedies, 2006; 6*:* 87-95.
67. Ruiz I, Offermanns J, Lanctot KL, Busto U. Comparative study on benzodiazepine use in Canada and Chile. J Clin Pharmacol, 1993; 33: 124-125.

68. Venkatesh G, Saranya K, Sri madhuri Y, Venkatesvaralu V, Ranjith kumar P. Effect of *Terminalia chebula* on urinary metabolites and its antistress activity in rat models. *J* Innovative trends in Pharmaceutical Sci, 2011; 2: 122-133.
69. Papadopoulos V. Peripheral-type benzodiazepine/diazepam binding inhibitor receptor: biological role in steroidogenic cell function. Endocrinol Review, 1993; 14: 222-240.
70. Schreiber AA, Frei K, Lichtensteiger W, Schlumpf M. The effect of prenatal diazepam exposure on TNF-α production by rat splenocytes. Agents Actions, 1993; 38: 265-272.
71. Zavala F, Tupin V, Descamps-Latscha B. In vivo treatment with benzodiazepines inhibits murine phagocyte oxidative metabolism and production of Interleukin-1 tumor necrosis factor and Interleukin-6. J Pharmacol Exptl Therapeutics, 1990; 225: 442-450.
72. Feldman S, Conforti N, Weidenfeld J. Limbic pathway and hypothalamic neurotransmitters mediating adrenocortical responses to neural stimuli. Neurosciences Biobehavioural Review, 1995; 19: 235-240.
73. Grossman A, Costa A. The regulation of hypothalamic CRH: Impact of in vitro studies on the control of the stress response. Functoinal Neurol, 1993; 8: 325-334.
74. Van de Kar LD. Neuroendocrine pharmacology of serotonergic (5-HT) neurons. Annual Review Pharrnacol Toxicol, 1991; 31: 289-320.
75. Pérez-Ortega G, González-Trujano ME, Ángeles-López GE, Brindis F, Vibrans H, Reyes-Chilpa R. Tageteslucida Cav. Ethnobotany, phytochemistry and pharmacology of its tranquilizing properties. J Ethnopharmacol, 2016; 181: 221-228.
76. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G. Concordance of toxicity of pharmaceuticals in humans and in animals. Regulatory Toxicol Pharmacol, 2000; 32: 56-67.
77. Chang KH, Stevenson MM. Malarial anaemia: mechanisms and implications of insufficient erythropoiesis during blood-stage malaria. Int J Parasitol, 2004; 34: 1501-1516.
78. Weatherall DJ, Miller LH, Baruch DI, Marsh K, Doumbo OK, Casals-Pascual C, Roberts DJ. Malaria and the red cell. Hematology Am Soc Hematol Educ Program, 2002; 35-57.
79. Wagner DD, Burger PC. Platelets in inflammation and thrombosis, Arterioscler Thromb Vasc Biol, 2003; 23: 2131-2137.
80. Mcknight DC, Mills RG, Bray JJ, Crag PA. Human Physiology, Churchill Livingstone, 1999.