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 antidepressant/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 antidepressant/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 adrenocorticotropic 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. 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 gastrointestinal 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 healing, 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 antioxidants such as gallic acid, gallicin, quercetagenin, 6-hydroxykaempferol-O-hexoside, patuletin-O-hexoside and quercetin were found in the extracts of TEL, where the quercetagenin was identified as the strongest antioxidant.[^47,48] Epidemiological studies have suggested that antioxidants rich plants play a protective role in health and against diseases and their consumption lowered risk of cancer, heart disease and hypertension 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 inflamed and ulcerated tissues, cancer and anti-septics. Flavonoids have protective role against allergies, inflammation, free radical, platelet aggregation, microbes, ulcer, hepatotoxins, viruses and tumors.\textsuperscript{49} Saponins show anti-inflammatory, coagulant, antidiabetic, antioxidant, aldose reductase inhibitory activity and cholesterol binding properties.\textsuperscript{50} The folklore uses of TEL include its use in anaemia, irregular menstruation, abdominal pain, muscular pain, bone pain, indigestion, colic cough, dysentery and rheumatism.\textsuperscript{36,40} The present study was aimed to evaluate the antidepressant/antistress activity of methanolic extract of \textit{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 \textit{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 \textit{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.\textsuperscript{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.\textsuperscript{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 F254, 20 cm; Merck) were prewashed with methanol and activated in oven at 100°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 (10μl) were loaded on the TLC plates with the help of linomat 5 applicator and processed for HPTLC analysis. Results were analyzed in HPTLC monitor by using CAMAG software for digitization and photo documentation. Pr-ecoated aluminum silica gel 60 F254 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°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°C). 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.

<table>
<thead>
<tr>
<th>Compounds extract</th>
<th>Identification Test</th>
<th>TE leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendroff test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mayer’s test</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Wagner’s test</td>
<td>+</td>
</tr>
<tr>
<td>Foam Test</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>Legal’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Shinoda test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Sodium hydroxide test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Lead acetate test</td>
<td>+</td>
</tr>
<tr>
<td>FeCl2 test</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Molisch’s test</td>
<td>+</td>
</tr>
</tbody>
</table>

+ 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.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Start Position</th>
<th>End Position</th>
<th>Area%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.04 Rf</td>
<td>0.11 Rf</td>
<td>60.03 %</td>
</tr>
<tr>
<td>2.</td>
<td>0.11 Rf</td>
<td>0.14 Rf</td>
<td>5.17 %</td>
</tr>
<tr>
<td>3.</td>
<td>0.16 Rf</td>
<td>0.18 Rf</td>
<td>5.02 %</td>
</tr>
<tr>
<td>4.</td>
<td>0.19 Rf</td>
<td>0.23 Rf</td>
<td>11.69 %</td>
</tr>
<tr>
<td>5.</td>
<td>0.31 Rf</td>
<td>0.36 Rf</td>
<td>2.68 %</td>
</tr>
<tr>
<td>6.</td>
<td>0.54 Rf</td>
<td>0.61 Rf</td>
<td>15.41 %</td>
</tr>
</tbody>
</table>

Figure 1: HPTLC Finger Print of *Tegetes erecta* (Scanned 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 comparison 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).](image)

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 250 mg/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).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Relative Organs weights (%)</th>
<th></th>
<th>Brain</th>
<th>Heart</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.55±0.16</td>
<td>0.87±0.02</td>
<td>1.77±0.02</td>
<td>0.89±0.02</td>
<td>1.63±0.20</td>
</tr>
<tr>
<td>Treated-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(250mg/kg)</td>
<td>6.56±0.15</td>
<td>0.87±0.02</td>
<td>1.77±0.04</td>
<td>0.88±0.03</td>
<td>1.62±0.21</td>
</tr>
<tr>
<td>(500mg/kg)</td>
<td>6.58±0.153</td>
<td>0.88±0.02</td>
<td>1.75±0.07</td>
<td>0.88±0.06</td>
<td>1.62±0.21</td>
</tr>
<tr>
<td>(500mg/kg)</td>
<td>6.74±0.15</td>
<td>0.89±0.02</td>
<td>1.75±0.09</td>
<td>0.87±0.03</td>
<td>1.62±0.03</td>
</tr>
</tbody>
</table>
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 (%), Eosinophils 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).

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters Studied</th>
<th>Control Group (250mg/kg)</th>
<th>TEL-Treated Groups (500mg/kg)</th>
<th>(2000mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>WBC (m/mm³)</td>
<td>6.94±0.10</td>
<td>6.92±0.07</td>
<td>6.81±0.12</td>
</tr>
<tr>
<td>2.</td>
<td>RBC (m/mm³)</td>
<td>8.99±0.04</td>
<td>8.75±0.03</td>
<td>8.53±0.09</td>
</tr>
<tr>
<td>3.</td>
<td>Platelets (%)</td>
<td>513±1.71</td>
<td>511±1.75</td>
<td>510±1.81</td>
</tr>
<tr>
<td>4.</td>
<td>HGB (g/dl)</td>
<td>13.7±0.18</td>
<td>13.7±0.18</td>
<td>13.4±0.18</td>
</tr>
<tr>
<td>5.</td>
<td>MCV (fL)</td>
<td>48.3±0.23</td>
<td>48.2±0.26</td>
<td>48.1±0.28</td>
</tr>
<tr>
<td>6.</td>
<td>HCT (%)</td>
<td>42.2±0.18</td>
<td>42.2±0.18</td>
<td>42.2±0.18</td>
</tr>
<tr>
<td>7.</td>
<td>MCHC (g/dl)</td>
<td>31.2±0.23</td>
<td>31.2±0.21</td>
<td>31.1±0.20</td>
</tr>
<tr>
<td>8.</td>
<td>Polymorphs</td>
<td>31.1±0.30</td>
<td>31.0±0.31</td>
<td>30.9±0.29</td>
</tr>
<tr>
<td>9.</td>
<td>Lymphocytes</td>
<td>38.2±0.13</td>
<td>38.4±0.13</td>
<td>38.7±0.13</td>
</tr>
<tr>
<td>10.</td>
<td>Monocytes (%)</td>
<td>3.6±0.17</td>
<td>3.6±0.17</td>
<td>3.7±0.17</td>
</tr>
<tr>
<td>11.</td>
<td>Eosinophils</td>
<td>9.1±0.11</td>
<td>9.1±0.11</td>
<td>9.0±0.11</td>
</tr>
<tr>
<td>12.</td>
<td>Basophils</td>
<td>1.0±0.21</td>
<td>1.1±0.21</td>
<td>1.1±0.21</td>
</tr>
</tbody>
</table>

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. for 14 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.\textsuperscript{[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.\textsuperscript{[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 \textit{Tribulus terrestris} and \textit{Alchornea cordifolia}.\textsuperscript{[66]} Anoxia of the central nervous system (CNS) cells plays a major role in the development of CNS disorders.\textsuperscript{[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.\textsuperscript{[68]} The Plant, TEL, improves anoxia stress tolerance, may allow cellular functions of CNS at low level of oxygen (O\textsubscript{2}) supply, and thus appears to decrease O\textsubscript{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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[73,74]} Reported studies have shown that the inhibition of anxiolytic-like effects, GABA/BDZ receptor antagonist and 5-HT\textsubscript{A} by Tagetes lucida extract in plus-maze model.\textsuperscript{[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.\textsuperscript{[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.\(^7\)\(^7\),\(^7\)\(^8\) The body has very limited reserve of platelets, so they can be rapidly depleted.\(^7\)\(^9\) Lymphocytes are the main effector cells of the immune system\(^8\)\(^0\) 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**


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.


