Evalution of the Antidepressant Activity of Ethanolic Extract of Tagetes Erecta Flower Using Albino Mice

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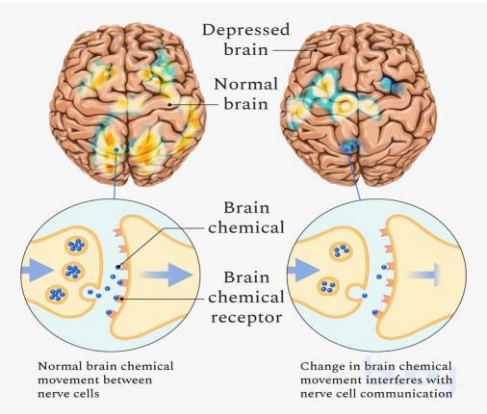
Abstract- **Different parts of plant Tagetes erecta (Asteraceae) have been habitually used for a number of disorder including fits, epileptic, fever, astringent as a carminative to relieve abdominal pain or distension or flatulence. The aim of study was to estimate antidepressants activity of ethanolic extract Tagetes erecta flower identification of Phytochemical analysis. The mice were administered with vehicle (12 ml/kg saline as a control), the extract of TEF (250 and 500 mg/kg body weight/rat/p.o.) and Standard drug, Diazepam (3mg/kg/rat) for 7 days., Tail Suspension Test (TST), Forced Swimming Test (FST), Hole Board Test (HBT) Actophotometer and Anoxic Tolerance Test (ATT) were conducted 70 min after the last administration of TEF extract determine to the antidepressant effects. Results showed that TEF herbal extract (500 and 250 mg/kg dose) significantly values (p< 0.01 and 0.0001) reduced duration of immobility test in TST and FST without affecting the locomotor action on open field test. It was also observed that Tagetes erecta flower extract significantly more (p< .05) anoxia stress acceptance time in a dose dependant & compared to effect control, but less than Diazepam treatment. The Tagetes erecta flower extract did not prove any noxious effects, and organ, weights, histological and hematological parameters were normal observed after 14 days treatment. In summary, TEF herbal extract administration considerably reduced the immobility in FST and TST.**

Index -Terms **Forced swimming test, Tail suspension test, Anoxic stress tolerance test, Hole Board Test, Tagetes erecta flower.**

INTRODUCTION

Now a day's of depression is a very major health problem of whole world. About 355 million people suffer from this disease, and it will become to the second cause of death till 2020. [1]
Depression is a condition of psychological or emotional illness. It is characterized by feelings of sadness. Depression can be modify individual's thinking ability and also affects social behavior or activities and sense of physical well-being. Peoples of different age group can be affected by the depression including young, old and children. It can be genetic, run in families & generally start between the age of 15 and 35 years. [2-5]
Few of the common factors that cause the depression are hereditary (genetics), trauma and high levels of pressure, mental illnesses such as substance abuse, schizophrenia, and postpartum (after childbirth) depression. Some other reason that contribute to depression are serious medical situation such as cardiac disease, alcohol and drug abuse, cancer and HIV use of certain medications, individuals person with low self-esteem, trauma and increased levels of stress due to financial problems, breakup of a relationship or loss of a loved one.
Depression can be reduced by day to day exercise, healthy diet, and stable relationships. They help keep stress low and thereby reduce the chances of feeling depressed again. With correct treatment, a depressed person can return to a happier life. [6]



This term is stress defined by Hans Selye (1976-1977) as the sum of all the unfocused changes caused by the role or damage homeostasis. Stress triggers is a broad range of body changes is called as General Adaptation Syndrome (GAS), thestimuli, which produce General Adaptation Syndrome are called stressors with cold, toxins, infection and heat.[7,8] Psychological pressure has been used by the researchers to observe a variety of tension related phenomenon with fear[9], post-traumatic tension disorder[10], and learning memory.[11] Chronic random stress model has been used to the observe depression[12], Alzheimer's atherosclerosis[13] disease[14] obesity, and Restraint stress in animals response in tissue damage.[15] The Gamma aminobutyric acid-A receptor (GABA-AR) was study known as closely involved in severe stress response.[16] All antagonist of the Corticotrophin-releasing factor-1 (CRF1) receptor inhibit the adrenocorticotrophic hormone (ACTH)-corticosterone reaction to stressors.[17] Benzodiazepine categories drugs such as a, chlordiazepoxide, midazolam, alprazolam and diazepam have been used as different types of antidepressants.[18]

some herbal drugs have been introduced for decreasing stress and anxiety in many emotional and physical disorders and gastric ulcers.[19]

Herbal Plant *Tagetes erecta* flower (TEF) is locally is known as a Genda Phul (Marigold) from belonging family Asteraceae (Compositae). It is a branching herb, native of Mexico and other heater parts of America & neutralized somewhere else in the tropics & subtropics including in India & Bangladesh.[20,21] Different parts of plant are used in bronchitis and colds[22] , carbuncles, eye infection, various gastro- intestinal disorders,[23]muscular spasms[24]. Marigold therapy was primary described in the paediatric literature treatment for plantar hyperkeratotic lesion in 1980.[25] Flowers are usefulin epileptic fits[26]and fevers, as an astringent, carminative. The flowers extracts use as a antibacterial, activities. antimicrobial, insecticidal, antidiabetic hepatoprotective, antioxidant, wound haeling and insecticidal [27-28] The Phytochemical study on different-different parts of *Tagetes erecta* flower. (TEF) have been publicized various chemical constituents alkaloids, saponins, thiophenes, carotenoids flavonoids, and triterpenoids.[29,30] The antioxidents such as gallicin, gallic acid, 6-hydroxykaempferol-O-hexoside, quercetagetin, quercetin, and patuletin-O-hexoside were originate in the extracts of Merigold, where the quercetagetin was identified as a present strongest antioxidant.[31,32]

MATERIALS AND METHODS

Chemicals

Diazepam drug was obtained from Divya Enterprises Chemical Co., lucknow. All other chemicals were available l in BNCP campus.

Plant Material

Flower (1kg) of TEF were collect in the months of Feb-March, 2021 from Herbal garden of BNCP, Lucknow, U.P (India). The plant was authenticated by CDRI, Lucknow. The collected flower were dried crushed powder and used for further studies.

Preparation of extract

The powdered flower (1kg) of *Tagetes erecta* were loaded in the Soxhlet extract and defatted with petroleum ether (35-55^{0}C). The marc (the residue) was dried extracted with ethanolic extraction was carried five cycles until exhaustion. This extract was dried under decrease pressure at 50°C using a Rotary Vacuum evaporator. This crude extract was weigh & kept in a air tight container protected from light.

Experimental Animals: Mice of either sex will be selected for the study and followed by acclimatization for 14 days by maintaining standard environment conditions with pellet standard feed & water. The experiment will be carried out by the CPCSEA guidelines and Institutional animal ethical committee. Then all animals will be randomized into 5 groups with 6 animals in each group.

Pharmacological screening methods Forced swim test (FST)

FST is one of the most frequently use behavioral models activity in rodents. Mice were at random divided into 5 groups, Group I (Control, 10 ml/kg Normal Saline, p.o.), Group II (Disease control stress) Group III (2 mg/kg Standard drug, diazepam /day, i.p.) Group IV and V (HD=500mg/kg & LD=250mg/kg TEF extract 250 and 500 mg/kg body weight, p.o., respectively) were treated for7 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 the swimming test. Briefly, mice were separately forced to the swim in open glass chamber (25x15x25 cm^3) containing fresh water to a height of 15cm and maintained at 26±1^{o}C.

Tail-Suspension Test (TST)

Mice were at random separated into 5 groups contain five animals each, Group I (Control, 10 ml/kg Normal Saline, p.o.), Group II (Disease control, stress) Group III (2mg/kg Standard drug, diazepam /day, i.p.) Group IV & V (TEF extract 250 and 500 mg/kg body weight, p.o., respectively) were treated for 7 days. The TST was conducted on the 7th day within 6 min of drug administration which is usually employed behavioral model for showing antidepressant activity in mice. Each mouse was separately suspended to the edge of table, 50cm above the ground, by adhesive tape located approximately 1cm from the tip of the tail. The total period of immobility induce by tail suspension was record physically during 6 min of testing period..

Haematological studies

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

Histopathological evaluation: The tissue of each mice will be removed immediately and washed in saline, blotted between filter paper fold to dryness and weighed. Then the brain will be in the formalin buffer (pH – 7.4) for histopathological changes in organ anatomy.

Statistical analysis: The results will be comparing with the control group. The result will be analyzed statistical using one-way analysis of various (ANOVA). The result will be found to be significantly at P<0.01. All tests were conducting using Graph Pad software, Inc., a privately held California corporation.

RESULTS

Preliminary Phytochemical Analysis

Eethanolic extract of showed the presence of saponins, flavonoids, tannins and carbohydrates and glycosides, alkaloids.

Table 1: Phytochemical Screening Tests for Eethanolic extract of TEF.

Compounds extract	Identification Test	TE flower
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.

Effect of TEF extract on FST

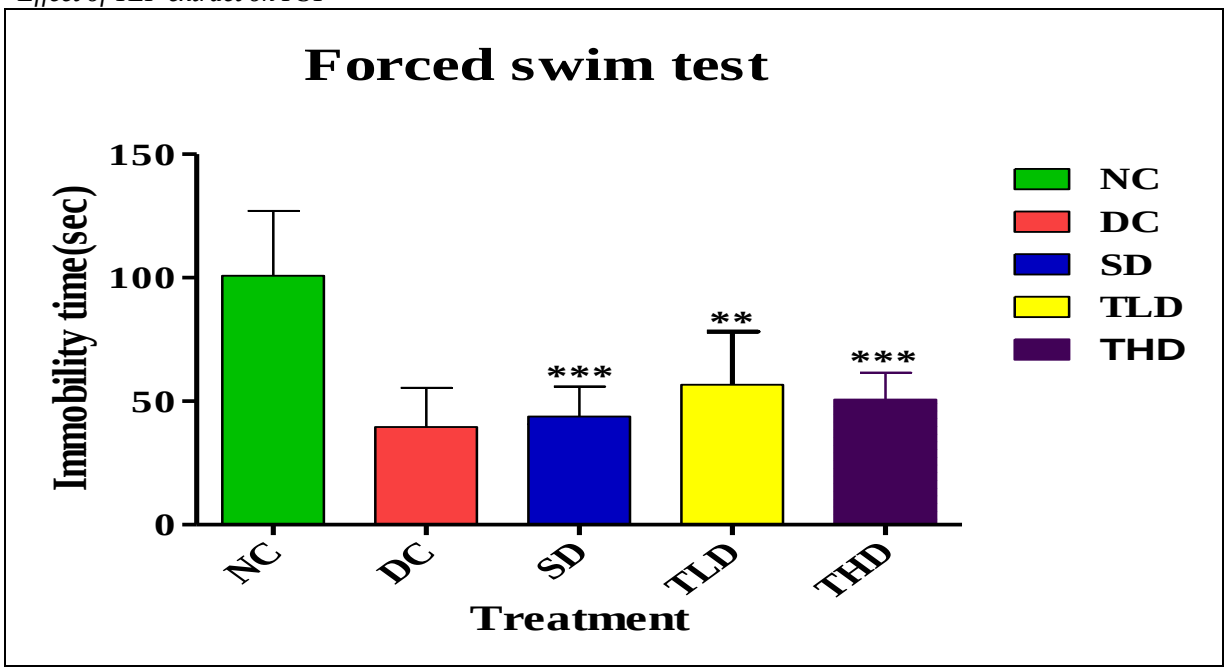


Figure: Effect of *Tagetes erecta* Flower extract on Forced Swim Test in mice (**P <0.01, ***P < 0.001, Control vs. Treated).

Effect of TEF Extract on TST

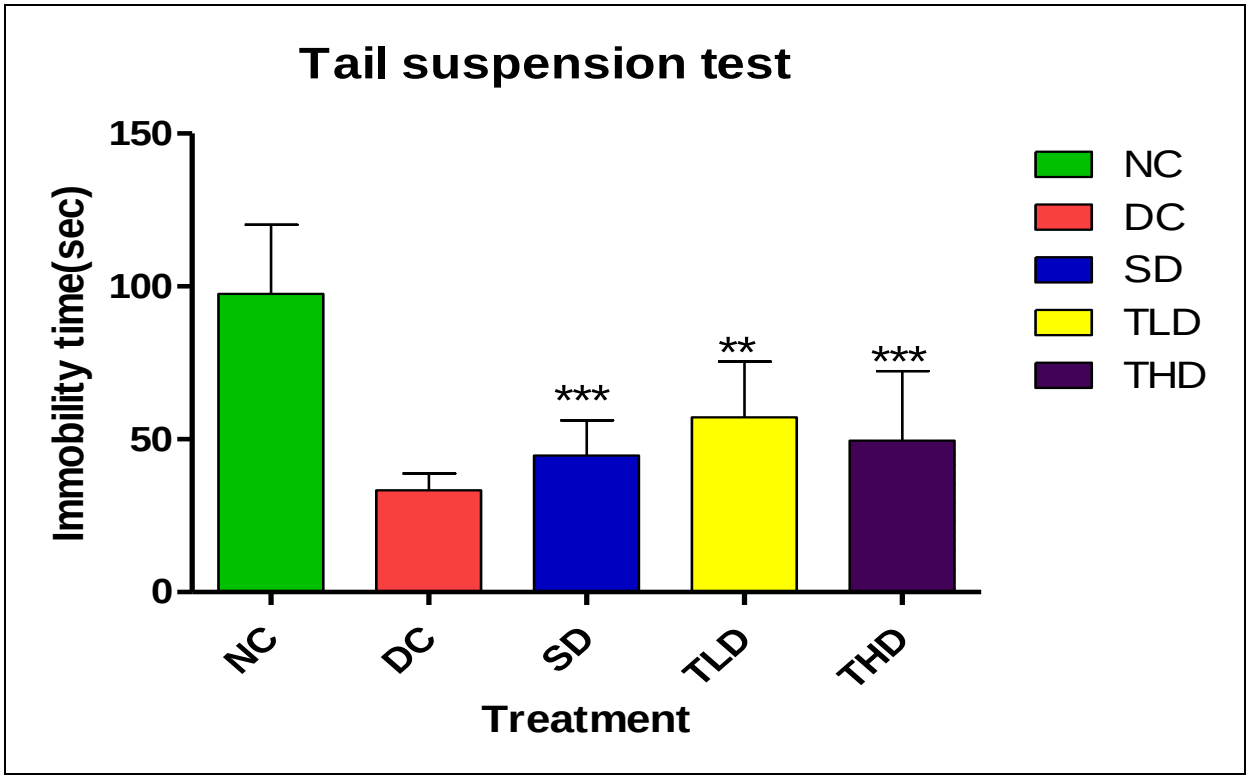


Figure: Showing effect of *Tagetes erecta* extract on Tail Suspension Test in mice (**P <0.01; ***P < 0.001; control vs. treated).

Effect of TEL extract on HBT

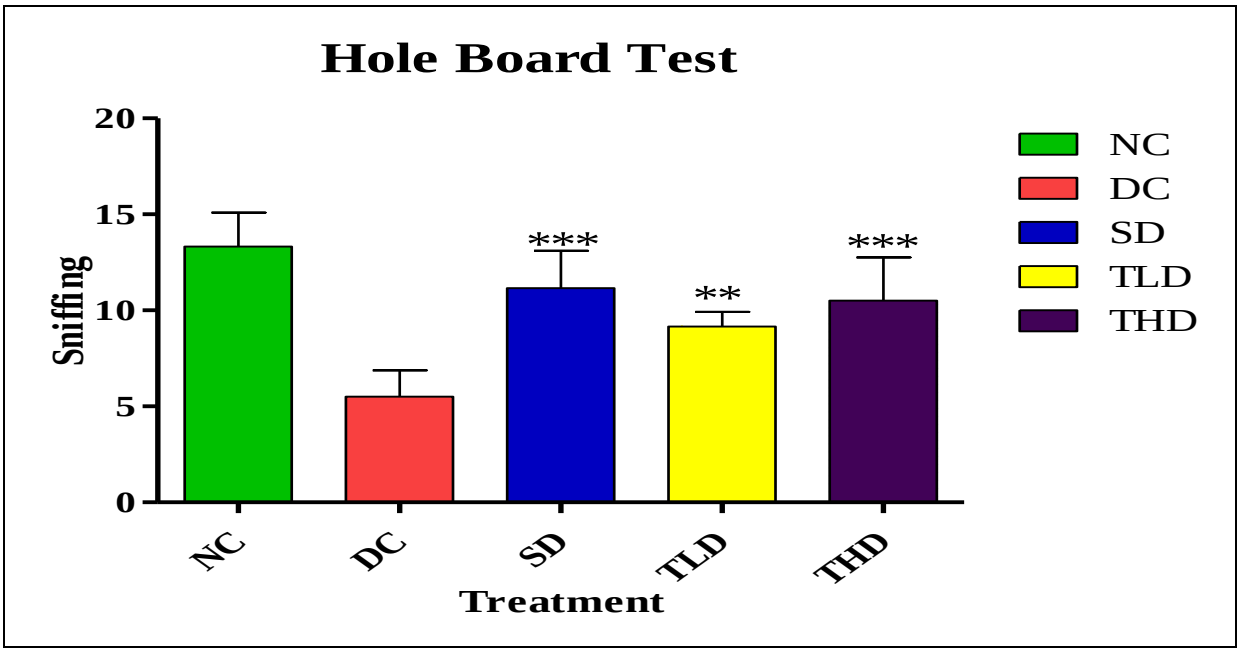


Figure: Showing effect of *Tagetes erecta* extract on Hole board test Test in mice (**P <0.01; ***P < 0.001; control vs. treated).

Effect of TEL extract on Actophotometer

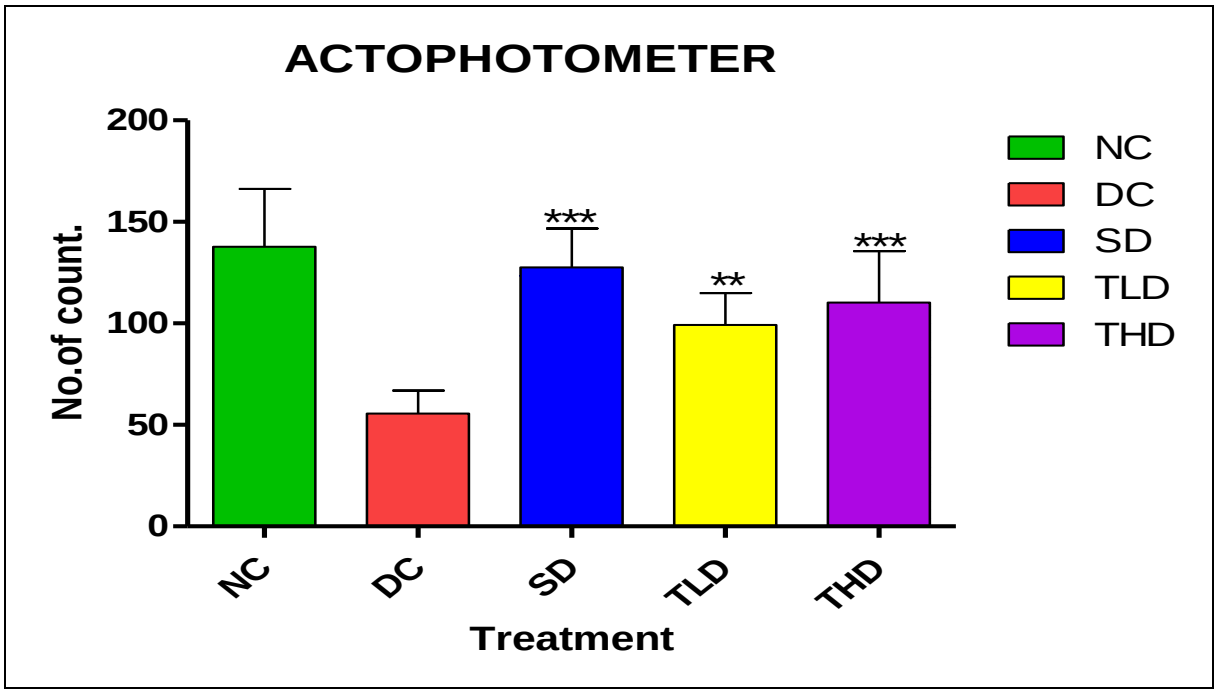


Figure: Effect of *Tagetes erecta* flower extract on Actophotometer in mice (**P <0.01, ***P < 0.001, Control vs. Treated).

Histopathological evaluation

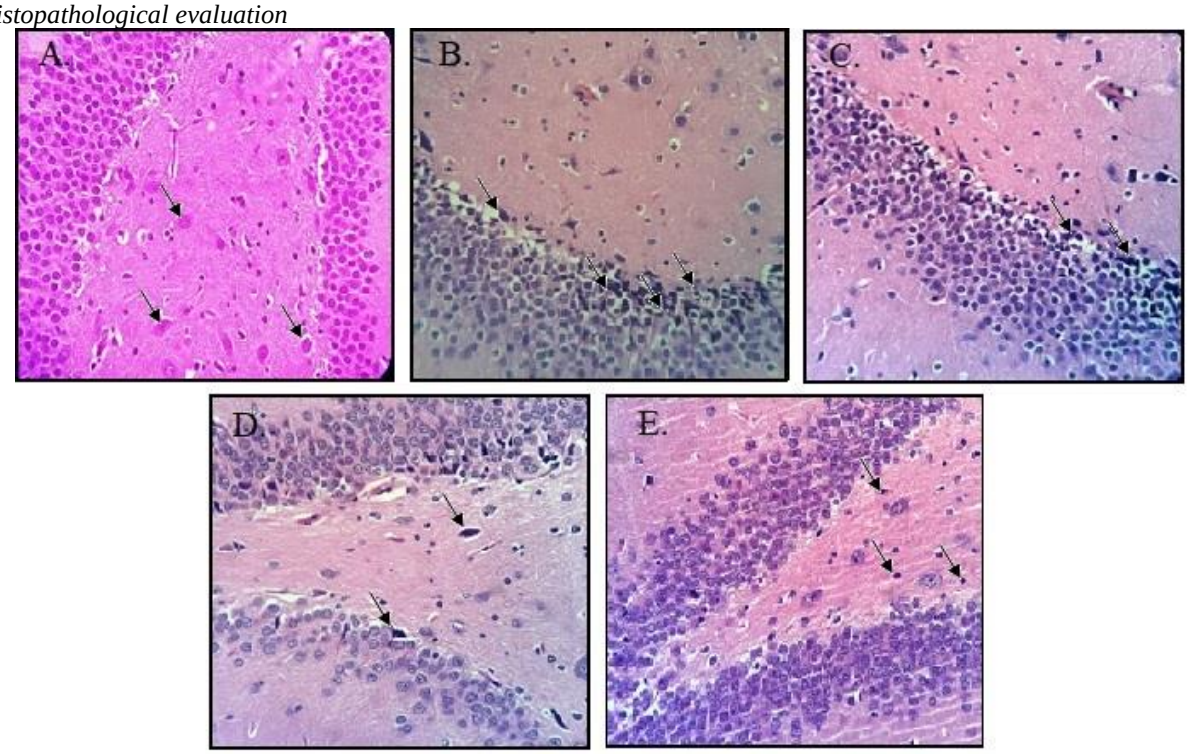


Fig. A BRAIN CORTEX NORMAL B. HIPPOCAMPUS Degeneration of granular neurons of Dentatus gyrus. (arrow) C. HIPPOCAMPUS: Degeneration of granular neurons of the dentate gyrus (arrow) but most of the cells are protected. D HIPPOCAMPUS: Majority of neurons are protected. E HIPPOCAMPUS: Protected dentate gyrus.

DISCUSSION

The phytochemical screening of TEF extract revealed the presence of saponins, , carbohydrates, flavonoids, glycosides, alkaloids and tannins which show concurrence with the prior study of Ramya et al., (2013) who confirmed the presence of phenolic, steroids, tannins, flavonoids and alkaloids, compounds as the major secondary metabolites in flower extract.[33] Present of compounds like, daucosterol beta-sitosterol, gallic acid in the extract of TEL have also been reported previously.[34] The antidepressant potential of Tagetes erecta can be recognized to the flavonoids and alkaloids[35] which are present in Ethanolic extract. In adding, saponins, carbohydrates, and glycosides present in TEF extract that offer safety to cellular component.

RESULT

The results of the there study show that the Ethanolic extract of TEF possess important anti-depressant activity based on observations of TST, HBT and FST in stressed albino mice. The high dose of Herbal extract (500mg/kg) increases the swimming endurance time, hole board test and tail suspension time as compared to lower dose (250 mg/kg). It was clear by decreased immobility time herbal extract. The immobility display in rodents by depress such as TST and FST that reflect to despair or lowered mood and reflect to the depression-like disorders in humans. Further,this immobility has been shown to be time reduced by treatment with antidepressant drugs, phenobarbitone sodium and Imipramine hydrochloride. A important correlation was found to be clinical efficacy of antidepressant drugs and their potency in all models. It has been recently shown that the rule 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). The FST and TST results indicate clearly that the TEF extract has the properties whereby it increases the physical survival as well as the overall show in mice exhibiting important anti-stress activity. TST is based on the observations in rodent mostly in mice although rats and gerbils have been used after initial escape behavior, develop an immobile position when subjected to inescapable depress situation. It has been recommended that acute antidepress drugs decreases the immobility time in TST and, FST thus, using immobility

time as an indicator of a state of lowered mood or hopelessness in animals.

CONCLUSION

This study herbal extract of TEF showed important antidepressant activity as evidenced by the TST and FST that decreases immobility time. Further, This indicates that TEF extract has a potential clinical application for the management of stress disorders. However, isolation, characterization and identification of neuropharmacological disorders and active phytoconstituents for antidepressant activity need to be investigated.

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