EVALUATION OF THE ANTIDEPRESSANT ACTIVITY OF TRIBULUS TERRISTRIS IN DIABETIC DEPRESSION IN RAT MODEL

Rupasri Dutt-Roy*1, E. Kayalvizhi 2 and M. Chandrasekhar 2

Department of Physiology 1, Sathyabama University Dental College and Hospital, Chennai - 600119, Tamil Nadu, India.
Department of Physiology 2, Meenakshi Medical College and Research Institute, Enathur, Kanchipuram - 631552, Tamil Nadu, India.

ABSTRACT: Tribulus terristris is a well known medicinal plant as anti-inflammatory, antimicrobial and nephroprotective etc. It is a very good drug for urinogenital problems, hepatic problems, oxidative stress etc. The aim of the study is to compare the depression induced by PCPA and STZ treated diabetic depression in mice and which could be restored by the ethanolic exact of fruits of Tribulus terristris in 21 days. Method: Depression was created in animals by PCPA (100 mg/k.b.w i.p. for 4 days) and STZ (25 mg/k.b.w i.p for 4 days) induced diabetic depression. Then that depression was restored by ethanolic extract of fruits of Tribulus terristris in a dose of 400 mg/k.b.w/day for 21 days. Fluoxietin kept as standard (5 mg/k.b.w) and continued for 21 days. Depression accessed by forced swimming test and measured the selected antioxidants level in brain homogenate and blood serum. Result: Tribulus terristris (TT) showed significant decrease in immobility in forced swim test and restored the antioxidant levels in animals as fluoxietin does, at the dose of 400 mg/k.b.w. Conclusion: Hence the result proposed that Tribulus terristris has antidepressant and antioxidant properties in chronic treatment of 400mg /k.b.w.

INTRODUCTION: Depression is a neuropsychiatric disorder with altered mood and persistent anxiety 1 which interfere everyday life. The important symptoms are feeling of emptiness, guilt, loss of confidence and lack of interest in favourite activities. Depression leads to various health problems 2. Its Symptoms might be loss of appetite or overeating, insomnia or too much sleep and decision making problems etc. It is a very common but serious illness. In elderly people most of the times it leads to diabetes 3. Diabetes and depression are linked to each other. Diabetes Mellitus is steadily increasing everywhere in the world. According to WHO (2014) 422 million adults were living with diabetes compared to 108 million in 1980. Diabetes Mellitus, the endocrine disorder was the seventh leading cause of death 4. Analyzed the death certificates of death due to diabetes from 2002 to 2012 in Songjiang District of Shanghai because China is one of the leading diabetic countries likek India.

Among four people, at least 1or 2 people are suffering from clinically significant depression in type II diabetes 5. This diabetes often leads to depression due to neurotransmitter imbalance which increases the risk of morbidity, mortality and suicidal tendency in depressed population 6. According to pharmacological reports, hyperglycemia increases oxidative stress which leads to depression 7, 8. Tribulus terristris is a familiar medicinal plant of Zygophyllaceae family.
It is being used as a single component and part of combination like Rasayana Ghana. All parts are having medicinal property but thorny and stout fruits are more promising. It is used since Vedic period in Ayurved, Unani and Homeopathy as diuretic, hepatoprotective and nephroprotective etc. The study was conducted to evaluate the antidepressant activity in diabetic depression and depression induced by PCPA in animal model through forced swimming test and by measuring the anti-oxidant levels in blood serum and brain homogenate.

**MATERIAL AND METHODS:**

Site of Experiments: The experiment were carried out in the department of Physiology at Meenakshi Medical College and Research Institute, Kanchipuram with due ethical committee clearance (IAEC NO. 009/2015) on male Swiss albino rat 120 -200 g of body weight and caged in a healthy environment. (22 ± 1 °C, 12 h day and night cycle) were fed with pellet and water *ad libitum* by using male wister albino rats.

Collection of Plant Material: The dry medicinal plant was collected from, Mr. Netai pal (Thirty years experience in Herbal trading) Herbal trading Co. Ltd. College Street, Kolkata, West Bengal, India and authenticated by Dr. D. Aravind, MD (S), M.Sc of National Institute of Siddha, Ministry of AYUSH (Government of India). The authentication certificate no.: NISMB2952017.

Preparation of Plant Extract: The plants were made into powder using a normal mixy grinder. The fine powder got after using iron net and then 250 g powder was mixed with 2500 ml of 70% ethanol which is more capable than pure ethanol to extract alkaloids and polyphenols from plant. It was kept for 48 hours on electrical gyratory shaker under constant shaking. Then filtered the solution and got golden brown fluid. The residue was re-extracted once again with 2000 ml of ethanol and the combined extracts were concentrated under vacuum and dried using a rotary flash evaporator. The dry weight of the plant was 6.7 %. Dry plant mixed with distilled water freshly every day.

Drug’s Information: The analytical grade of PCPA (Para-chlorophenyl-alanine) and STZ (streptozotocin) bought from Sigma Chemical Company Inc. St Louis, Mo, USA. Prozac, the brand name and the generic name is fluoxetine, purchased from the Pharmacy of the Meenakshi Medical College.

Solvent of PCPA and STZ: PCPA dissolved in tween 80 is miscible in water (0.1 ml/ml) and produced a hazy light yellow colour solution. Appropriate concentration of STZ was made *i.e.* 7mg/ml in citrate buffer with pH 4.5. Always STZ should mix at the time of injection to prevent STZ’s degradation in citrate buffer. TT mixed with distilled water in requisite amount every day for drug delivery by orally.

Animals Grouping and Dosing: Animals were allowed to acclimatize in new peer groups. Standard environment maintained in the cages. All the rats were divided into 7 groups with 6 animals in each cage. 3 groups were treated by PCPA (100 mg/k. b.w. i.p) and 3 groups for STZ (25 mg/k. b.w. i.p) administered once daily for 4 days. Chronic low dose was chosen for 4 days to prevent death of STZ administration from previous experiences. 4 hours fasting in animals is enough to create diabetes in animals.

First group kept for normal control treated with distilled water. On 4th day glucose level was measured by glucometer through pricking needle at 1 cm away from the cloaca on ventral side of the tail of the animals. Among 3 PCPA groups, 1 group was kept for PCPA control and other 2 groups were treated with standard antidepressant drug fluoxetine (3 mg/k.w) for 21 days and TT (400 mg/k. b.w) for 21 days respectively. Among STZ groups one group kept for diabetic control, another 2 groups further treated by TT (400 mg/k. b.w, once daily for 21 days.) and standard antidepressant drug fluoxetine (3mg/k. b.w, once daily for 21 days).

Chronic Administration of *Tibullus terestris* and Fluoxetine: *Tibullus terestris* 400 mg/k. b.w and Fluox-5 mg/k. b.w once daily p.o. for 21 days.

**Study design:** Order

1. Control: (6 animals) Distilled water (10 ml/k. b.w)
2. PCPA Control: (6 animals) 100 mg/k. b.w i.p.
3. Diabetic Control: (6 animals) 25 mg/kg. b.w. i.p
4. PCPA + Fluoxetine: (6 animals) 5 mg/k. b.w. p.o.*21 days
5. **PCPA + TT**: (6 animals) 400/k. b.w for p.o.*21 days

6. **STZ + Fluoxetine**: (6 animals) 5mg/k. b.w p.o. *21 days

7. **STZ + TT**: (6 animals) 5mg/k. b.w p.o.*21 days

**Force Swimming Test Selected for Accessing the Depression**: The test discovered by Porsolt RD 1997 15 selected in this study. All groups were subjected to do forced swimming test in a glass cylinder of 18 cm diameter, 40 cm height filled with water height of 15 cm. Maintained the water temp of 25 ± 2 °C. Measured the immobility time the last 4 mins of 6 mins duration 16 of swimming. In each test fresh water was provided 17.

**Forced Swimming Test Performed on 4th day and Repeated on 21st day of Drug Treatment**: Forced swimming test performed on 4th day, 1 hour after the last dose of PCPA and STZ, again repeated 1 hour after the last dose on 21st days of treatment by TT and standard fluoxetin.

**Biochemical Analysis**

**Glucose Estimation**: Fasting blood glucose level was measured on 4th day of treatment by PCPA and STZ in animals by using glucometer from the ventral side of the tail, 1cm away from the cloacae. 2 % lidocain was used for every pricking to reduce the pain 18.

**Antioxidants Estimation**: Antioxidants like CAT assayed by the method of Sinha (1972) 19 while SOD used by the method of Kakkar et al., 1984 20 and MDA determined by the method of Satoh K, 1978 21 in blood serum and brain homogenate. The absorbance of catalase was measured at 530 nm. The result was expressed by the consumption of H₂O₂ (μM /min/mg protein). In SOD the butanol layer was measured at 560 nm in spectrophotometer. The enzyme activity was expressed as units/mg protein. At the end for MDA estimation, the supernatant was measured at 540 nm in spectrophotometer. The level of lipid peroxides was expressed as n moles of MDA formed/mg protein.

**Brains were removed from sacrificed animals (isoflurane) by keeping on ice slab, then immediately put into nitrogen refrigerator for 10 min after that, prefrontal area and hippocampus separated from each brain.**

The cut area approximately should have the same weight (w/v). Tissue was homogenized with phosphate buffer (0.1M, with pH 7.4). The clear supernatant were considered after centrifugation for due estimation. Homogenate was centrifuged in 3000 rpm for 15 min.

**Statistical Analysis**: All the values were expressed as mean ± S.E.M and analyzed by one-way ANOVA followed by Dunnet’s multiple comparison test were P< 0.05 was considered to be statistically significant.

**RESULTS**:

**Contribution of Phytochemical in Depression, Diabetes and Oxidative Stress**: Fig. 1, compared the depression level between PCPA and STZ treated animals through forced swimming test. Each group is compared with normal control (6 animals) which is p<.001in. Performed Dennett’s test values are displayed as mean ± SEM, each treated group contains 18 animals. Hence P values are statistically significant.

**Fig. 1**: COMPARED THE DEPRESSION LEVEL BETWEEN PCPA AND STZ TREATED ANIMALS THROUGH FORCED SWIMMING TEST

**Fig. 2**, is showing the effect of PCPA and STZ on blood glucose level.

PCPA did not showed any alteration in blood glucose level, whereas streptozotocin treated groups showed increased fasting blood sugar level of 290 mg/dl in animals considered to be diabetic. Values are expressed in standard error of mean (± SEM) of 18 animals in each treated group. Performed Dunnet- t-test, showed P<.001which is significant.
FIG. 2: IS SHOWING THE EFFECT OF PCPA AND STZ ON BLOOD GLUCOSE LEVEL

**Fig. 3**, is showing effect of antidepressant activity of TT on forced swimming test.

Chronic administration of TT at the dose of 400/mg. b.w for 21 days showed significant reduction in immobility in PCPA and STZ treated diabetic groups compared with standard antidepressant fluoxetine (5 mg/k. b.w)and normal control. Values are expressed as mean (± SEM). Computed one-way Anova followed by Dunnett’s test showed very much significant (p<.001).

**FIG. 3**: IS SHOWING EFFECT OF ANTIDEPRESSANT ACTIVITY OF TT ON FORCED SWIMMING TEST

**Effect of Antioxidants**: Fig. 4 and Fig. 5 chronically treated groups by TT (400 mg) and fluoxetine in Fig. 4 and Fig. 5 the catalase and superoxide dismutase antioxidants level increased significantly (p<.05) in blood serum and brain homogenate compared to positive controls of PCPA and STZ. In each case performed Dunnet’s test.

**Effect of MDA in Experiment**: Fig. 6 MDA concentration is significantly higher in blood serum and brain homogenate of depressed group created by PCPA and STZ, this concentration went down as normal after treating with TT (400 mg) for 21 days. This restoration is significant (p<.001). Performed two ways Anova followed by Dunnett’s test.

**FIG. 4**: EFFECT OF TREATMENT ON CATALASE

**FIG. 5**: EFFECT OF TREATMENT ON SOD

**FIG. 6**: COMPARISON OF MDA IN DIFFERENT GROUP

**DISCUSSION**: Depression is a kind of mood disorder with persistent feelings of sadness. It affects our feelings, decision making capabilities and certain behaviours. Gradually they became a burden of society. The available treatment of...
depression is not satisfactory. Hence, herbal medicines are gaining popularity in every field because of short comings in synthetic antidepressant medicines like delayed onset of activity and side effects. TT is a renowned medicinal plant since long back due to its potential pharmacological activities. Diabetes is an endocrine disorder with high blood sugar level with other associate symptoms. The abnormal high blood sugar level is due to malfunction of either insulin secretion or insulin action in the body. Scientists have already explored that one of the co-morbid symptoms of diabetes is depression. In comparison to non diabetic community, depression is 2-3 times more in diabetic world population. Mostly untreated diabetes leads to depression. It is an established fact that diabetes significantly increases the possibility of depression.

The underlying pathophysiology of depression is error in neurotransmitters. Mostly serotonin, noradrenalin and small amounts of dopamine are directly involved in depression. We know that serotonin (5-hydroxytryptamine) which is known as happy hormone controls all kinds of human behaviour like mood, memory, attention, focus, sexuality, anger and aggression. To study the experiment, force swimming test was selected which provides the speedy and dependable screening test for antidepressant activity of medicinal plants. Immobility is the core symptom of depression which was considered in forced swimming test for assessment of depression.

In depression, the approved antidepressants are classified as selective serotonin reuptake inhibitors (SSRI), MAO-inhibitors or miscellaneous antidepressants. But each drug has 40 % failure rate. In this experiment, depression induced in animals by PCPA and STZ was evidenced by forced swimming test in Fig. 1 and antioxidants level. PCPA created depression could be due to inhibition of MAO specifically 5-HTP which is the intermediate precursor of serotonin in synapse due to its selective and irreversible inhibitor of tryptophan hydroxylase. This is the rate-limiting enzyme in the biosynthesis of serotonin. The Graph II indicates no alteration of blood glucose level in case of PCPA treated groups, whereas STZ treated groups increased the fasting blood sugar level and confirmed diabetes.

STZ created diabetes leads to depression in experimental animal. Similar depression found in diabetic and psychiatric patients. Hence, it is proved that depression and diabetes is interlinked. This depression in diabetes might be due to the altered level of 5HT in hippocampus region or due to neural cell death. Fluoxietin was selected for positive control to ensure the antidepressant activity of TT in depression in serotonergic pathway. Fluoxietin is selective serotonin reuptake inhibitor (SSRI) which is a great choice of drug for clinician in case of major depressive disorder and its trade name is Prozac. This is the first line of defence for the treatment of major depressive disorder. In Fig. 3 Chronic administration of TT (400 mg) for 21 days restored depression in PCPA treated groups and diabetic groups by increasing mobility in forced swimming test.

Depression is further confirmed by biochemical analysis of antioxidants in blood serum and brain homogenate in all groups. Recent studies suggested that any kind of stress induced oxidative damage due to decreased level of antioxidants and increased level of reactive oxygen species. The catalase, superoxide dismutase and especially malondealdehyde enzymes are trait markers of depression for oxidative stress. The treated groups of PCPA and STZ showed the significant decrease of catalase and superoxide dismutase and increased level of MDA in blood serum and brain homogenate in Fig. 4, Fig. 5 and Fig. 6. Hence the oxidant and antioxidant defence systems are getting deranged in depression and diabetes.

Clinical depression is associated with a 30 % increase of inflammation in the brain, according to a new study published in JAMA Psychiatry. A study conducted by researchers in patients experienced cumulative depressive episodes with high level of c-reactive proteins which is the indicator of inflammation at Duke University Medical Center in 2012. The inflammation is an integral part of the stress response. Inflammatory cells induced oxidative stress by secreting some hydrolyzing enzymes and ROS (hydrogen peroxide, superoxide, hydroxyl radical etc). This creates imbalance between oxidant and antioxidant enzyme system in the animal. Hence neuro-inflammation are involved in the pathophysiology of depression and anxiety.
In diabetes, several studies have shown that elevated extra and intra cellular glucose concentrations directly increase the reactive oxygen species generation in the cell. This causes membrane lipid peroxidation. As a result, it may alter neurotransmission, neuronal function and overall brain activity. Hence oxidative stress might have contribution in the pathogenesis of depression.

In the study, the depressed groups when treated by TT and fluoxetine chronically for 21 days. TT showed similar results like fluoxetine in restoration of antioxidants like catalase, superoxide dismutase and malondialdehyde. This indicates that the medicinal plants could work like synthetic drug fluoxetine due to having duel and triple therapeutic actions in animals. It was studied that steroids, triterpenoid etc have potential role in alloxan-induced diabetic rats. Similarly methanol and aqueous extract of its fruit pulp have neuroprotective activities in-vivo. Claimed that Phenolic, flavonoids in Phaseolus vulgaris are helpful in preventing depression as well as having the ability to remove ROS.

The secondary metabolites reach the brain tissues via blood brain barrier to work on central nervous system as potential neuroprotectants. Earlier reports showed that herbs containing flavonoids, tannins and phenolic compounds were found to possess antioxidant property and attenuated cell death induced by oxidative stress Schroeter H2000. It has observed that glycosides possess both antioxidant and antidiabetic effects. The diverse activity of same phytochemical is studied by various scientists in various diseases. The isolated alkaloids showed antidiabetic and antioxidant potential in-vitro study. Sadia Perviz et al., 2016 reviewed the antidepressant activity of β caroline alkaloid, a monoamine oxidase inhibitor in plants.

This theory was supported by Deole YS et al., 2011. They claimed that the anti-depressant and anxiolytic activity of Rasayana Ghana Tablet in ayurved is due to presence of β caroline alkaloid of TT which is the main contributor among 3 medicinal plants of Rasayana Tablet. It is observed that steroid phytochemical is responsible for hypoglycaemic activity in diabetic rats in C. papaya leaves.

This is further explained by Isela E. Juarez-Rojopa et al., 2014 that steroids hamper the glucose absorption by inhibiting the hydrolyzing enzymes and that helped in hypoglycaemic activity. Saponins of bitter melon also showed hypoglycemic activity in alloxan-induced hyperglycemic mice which increased the mass of β cells in the pancreas and insulin production studied by scientists.

According to certain researchers, phenol are highly reactive as antioxidant. This scavenging effect is carried out by the O-dihydroxy group of phenolic components of radical. Phenols are not only antioxidants they are anti-diabetic too. Pradeep 2013 reported that the anti-diabetic activity of the ethanolic extracts of the leaves of Strobilanthes asperrimus plant is possible due to the presence of tannins, flavonoids, and alkaloids. Rajasree et al., 2016 revealed that the presence of flavonoids and phenolic compounds have multiple biological effects in Central nervous system disorders. The different pharmacological activities of bioactive components of TT were explored by various scientists. In 2011, A. D Sathisha et al., examined the antioxidant quality of TT through DPPH radical scavenging activity in-vitro. The antidiabetic activity of saponin of TT was explored by Chu S et al., in 2003. The depression was restored when the experimental groups were chronically treated by TT (400 mg/ k.b.w, once daily orally). It is concluded that photochemical of medicinal plants having tremendous therapeutic potential in curing various disorders and diseases compared to the synthetic drugs with minimum side effects.

CONCLUSION: The restoration of depression and diabetic depression might be due to synergistic effect of various secondary metabolites like alkaloids, steroids, glycosides, saponins, flavonoids, phenols, tannins due to their antioxidant, anti-diabetic and antidepressant activities.

ACKNOWLEDGEMENT: Nil

CONFLICTS OF INTEREST: Nil

REFERENCES:


