Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E) 2320-3862
ISSN (P) 2394-0530
NAAS: 3.53
JMPS 2017; 5(1): 181-186

Received: 04-11-2016
Accepted: 30-12-2016

Kailash Sharma
PhD Research Scholar
Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar-125001 Haryana, India

Milind Parle
Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar-125001 Haryana, India

Correspondence
Kailash Sharma
PhD Research Scholar
Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar-125001 Haryana, India

Methanol extract of *Artocarpus heterophyllus* attenuates pentylenetetrazole induced anxiety like behaviours in mice

Kailash Sharma and Milind Parle

Abstract
Plant-derived medications have augmented in the management of psychiatric disorders. Therefore, the aim of the present study was to investigate the protective effects of methanol extract of *Artocarpus heterophyllus* against anxiety. The effects of methanol extract of *Artocarpus heterophyllus* (MEAH) on brain GABA and serotonin levels were also determined. We used the light-dark model, elevated plus maze and marble burying behavior model, standard animal models of anxiety, to evaluate the effects of sub-acute (10 days) administration of MEAH at doses of 250 and 500 mg/kg, (p.o.) against pentylenetetrazole (PTZ; 20 mg/kg, i.p.) induced anxiety in mice, followed by biochemical estimations. A significant increase in the time spent in the open arms, light compartment and decrease in marble burying, were observed in MEAH treated mice. MEAH increased the levels of GABA and serotonin in brain. These findings suggest the anxiolytic effects of MEAH, and modifications in brain GABA and serotonin levels could have contributed to the current results.

Keywords: Anxiety, *Artocarpus heterophyllus*, GABA, serotonin

1. Introduction
Anxiety is generally defined as a state of unwarranted worry or feeling of fear, often accompanied by tension, restlessness, irritability, distraction, and sleep disturbance. These disproportionate responses can hyperactivate the autonomic nervous system and hypothalamic pituitary adrenal axis leading to somatic manifestations of anxiety disorders [1]. The GABAergic, serotonergic and noradrenergic neurotransmitter systems are also involved in the development of anxiety. The treatment of anxiety depends on the type of anxiety disorder. Antidepressants, antianxiety, first generation antihistamines and β-blockers are usually prescribed for the treatment of different type of anxiety [2]. As these drugs have a narrow safety profile and unwanted side effects including sleep disturbances, sexual dysfunction, paradoxical effects and weight gain, it has prompted research in order to investigate new drugs with fewer side effects. Gamma-aminobutyric acid (GABA), primary inhibitory neurotransmission, and various phytoconstituents are found in some plants having GABA-receptor agonistic effect, may prove to convey anxiolytic effects [3]. Herbal drugs have been used for the treatment of psychiatric disorders with the virtue of their negligible side effects. Although, the exact mechanisms of action of these drugs have yet to be determined, but some of them have been shown to exert antioxidant, anti-inflammatory, neuroprotective effects [4,5]. *Artocarpus heterophyllus* (Moraceae), commonly known as Jackfruit is a power house of therapeutic phytoconstituents and also reputed to have numerous medicinal properties such as anti-inflammatory, antioxidant, antiepileptic, neuroprotective and antidepressant [6,7,8,9], But so far there are no studies reporting their antianxiety effect of jackfruit. Thus, we have hypothesized that *Artocarpus heterophyllus* may be beneficial in the treatment of anxiety and allied disorders. Thus, the aim of present study was to elucidate the anxiolytic effect of *Artocarpus heterophyllus* using various animal models and also studied its effect on biochemical changes in animal brain.

2. Materials and methods
2.1 Plant material
The heart wood of *Artocarpus heterophyllus* was collected from horticulture garden of Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar -Haryana (India), and got authenticated by National Institute of Science Communication and Information

Resources (NISCAIR), Raw Material Herbarium and Museum (RHMD), New Delhi (Ref. No. NISCAIR/RHMD/Consult/2016/3002-29-2).

2.2 Extraction
The heart wood of *Artocarpus heterophyllus* (500 gm) were crushed to coarse powder and extracted with methanol using soxhlet extraction method. Then, the extract was evaporated on water bath to obtain dried extract and kept in desiccators. Dried extract was used for further study.

2.3 Experimental Animals
Swiss male albino mice, weighing 25-30g were procured from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar, Haryana (India). Since, estrogens are the female sex hormone, found to have neuroprotective effect [10], therefore, we have excluded female mice and used only male mice for the present study. The animals were acclimatized for seven days before the start of experiments. The animals had free access to feed and water except the duration of experiment. They were housed in a natural (12h each) light-dark cycle. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC) and the care of animals were taken as per the guidelines of CPCSEA, Ministry of Environment and Forests, Government of India, New Delhi, India (Registration No. 0436).

2.4 Drug protocol
Pentylenetetrazole (Sigma, USA), Diazepam (Calmpose inj. Ranbaxy, India) were used in this study. Drugs were dissolved in normal saline and injected intra-peritoneal.

2.5 Experimental Design
Animals were divided into 15 groups with 6 mice in each. Pentylenetetrazole (PTZ) (20mg/kg, i.p.) were used to induce anxiety in small laboratory animals. Methenol extract of *Artocarpus heterophyllus* (MEAH) heart wood was administered (250mg/kg and 500mg/kg, p.o.) for 10 sucessive days and on the10th day PTZ was injected after one hour of test drug administration.

Group 1: Control mice received Saline only.
Group 2: Negative control mice received PTZ (20mg/kg, i.p) only.
Group 3: Positive control, mice received diazepam (2mg/kg, i.p), after 30 minutes PTZ (20mg/kg, i.p) was injected.
Group 4 and 5: Test drug, mice received MEAH (250mg/kg, p.o.) and MEAH (500mg/kg, p.o.) respectively, after 30 minutes PTZ (20mg/kg, i.p) was injected.
Animals for group 1 to 5 were used for Light-Dark Model and animals in group 6 to 10 were treated as above used for Elevated-Plus Maze and animal in group 11 to 15 were treated as above used for Marble burying behavior model.

2.5.1 Light-Dark Model
Light and dark model (LDM), commonly employed model to screen antianxiety effect of new compounds. The model is consisted of two boxes connected together. One box was made dark by covering its top with plywood, while other box was illuminated with 40 W lamp. The source of light was located 25 cm above the open box. The test drug was administrated to mice 30 minute before being placed in the light box. The time spent in light box was recorded for 10 minute [11].

2.5.2 Elevated-Plus Maze
The Elevated Plus-Maze (EPM) was used as the exteroceptive behavioral model. This model was consisted of two enclosed arms and two open arms; the maze was elevated at height of 25 cm from the floor. Animal was placed individually at the central platform of maze by head facing towards an open arm and record total time spent and number of entries into open arm for 5 minute [12].

2.5.3 Marble burying behavior model
In this model, mouse was placed in a plastic cage containing 5 cm thick sawdust bedding, 20 clean glass marbles with diameter of 10 mm arranged evenly on the bedding. After the administered of test drug, mouse was exposed for 30 minute to the marbles, mouse were removed, and unburied marbles were counted. A marble was considered as buried, if the marble 2/3rd size were covered with saw dust. The total number of marbles buried was considered as an indicator of anxiety behavior [13].

2.6 Biochemical estimations
2.6.1 Isolation of brain
After behavioral tests, animals were sacrificed by decapitation and the brain was isolated, washed with isotonic saline and then weighed. The isolated brains were used for the estimation of serotonin level and GABA level. The brains of animals in 1 to 5 were homogenized in 3 ml HCl-Butanol (0.1 M HCl in butanol) in ice cool environment and used for estimation of serotonin level. The brains of animals in groups 6 to 10 were homogenized in 5 ml of 0.01M hydrochloric acid and used for the estimation of GABA level.

2.6.2 Estimation of brain serotonin levels
The above homogenate was centrifuged at 2000 rpm for 10 minute. 0.8 ml of obtained supernatant phase was removed and added into an Eppendorf reagent tube containing 2 ml of heptane and 0.25 ml 0.1 M HCl. After 10 minute of vigorous shaking the tube, centrifuged to separate 2 phases. The aqueous phase was used for serotonin method and upper organic phase was discarded. 1.25 ml of o-phthaldialdehyde reagent was added to 1 ml of the aqueous phase. The fluorophore was developed with heating for 10 minute at 100 ˚C. After the samples reached to equilibrium with the ambient temperature, fluorescence at 360-470 nm were taken in systronic photofluorometer (Model 152, Ahmedabad, Gujarat). Compared the tissue values (fluorescence of tissue extract minus fluorescence of tissue blank) with an internal reagent standard (fluorescence of internal reagent standard minus fluorescence of internal reagent blank). For serotonin tissue blank, 0.025 concentrations HCl without o-phthaldialdehyde were added. Internal standard was obtained by adding 500 ng of serotonin creatinine sulfate monohydrate in 0.125 ml distilled water and 2.5 ml HCl-Butanol, which was then carried through the entire extraction procedure. For the internal reagent blank, 0.125 ml distilled water was added to 2.5 ml HCl-Butanol [14].

2.6.3 Estimation of brain GABA levels
Lowe method was used for the estimation of the brain amino butyric acid (GABA). 0.1ml of homogenate was added into 0.2ml of 0.14 M ninhydrin solution in 0.5M carbonate bicarbonate buffer (pH 9.95) and kept for on water bath for 30

minute at 60˚C then, cooled and added 5ml of copper tartrate reagent (0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid). After 10 minute, resultant samples were read at 377/455nm using spectofluorimeter [15].

2.6.4 Statistical analysis

Results were expressed as mean ± SEM. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests using Graph Pad Instat. Differences between data sets were considered as significant when $p < 0.05$.

3. Results

3.1 Effect of MEAH on PTZ induced anxiety using light-dark model

PTZ (20mg/kg, i.p.) were significantly decreased the time spent in light box of light-dark model on the 10th day of treatment as compared to saline treated group. MEAH (250 and 500mg/kg, p.o) were significantly ($p<0.001$) increased the time spent in light box as compared to PTZ treated group (Figure 1).

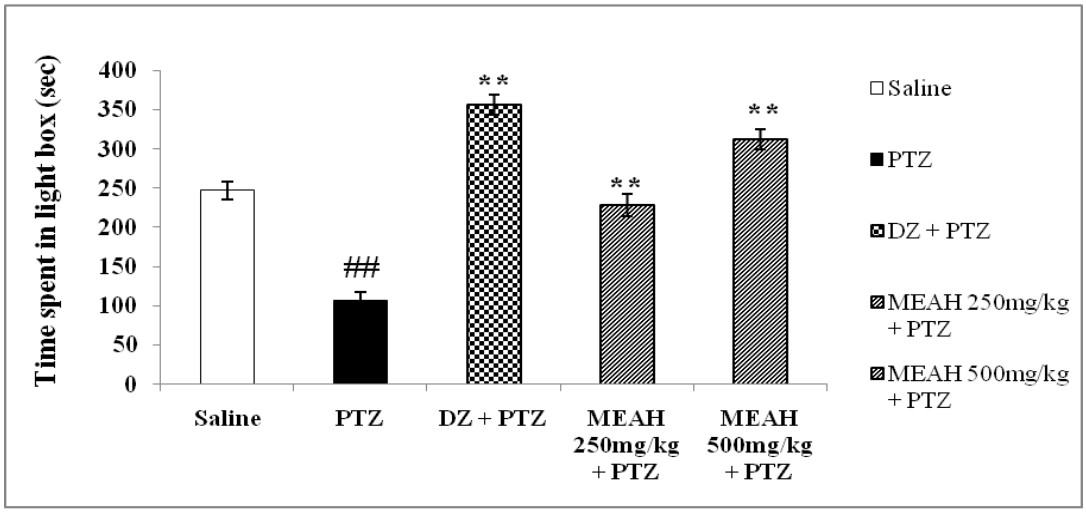

Fig 1: Effect of MEAH on PTZ induced anxiety in light-dark model of mice

Values are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by Tukey's multiple comparison test. ## denotes ($p<0.01$) as compared to Saline group. ** denotes ($p<0.01$) as compared to PTZ group.

3.2 Effect of MEAH on PTZ induced anxiety in Elevated-Plus Maze model of mice

PTZ (20mg/kg, i.p.) were significantly decreased the time spent in open arms in Elevated -Plus Maze model on the 10th day of treatment as compared to saline treated group. MEAH (250 and 500mg/kg, p.o) were significantly ($p<0.001$) reversed the time spent in open arms in Elevated-Plus Maze model as compared to PTZ treated group (Figure 2).

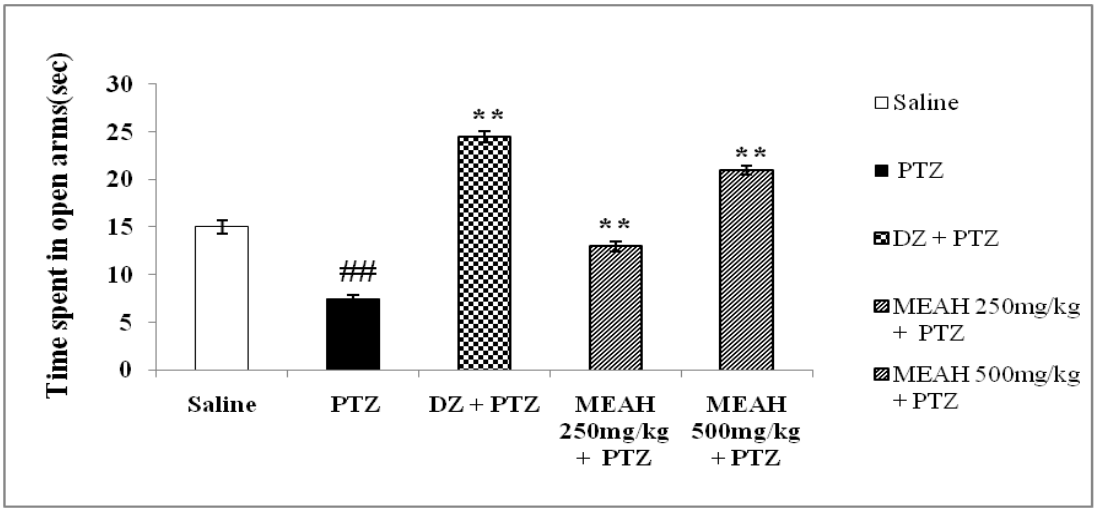

Fig 2: Effect of MEAH on PTZ induced anxiety in Elevated-Plus Maze model of mice

Values are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by Tukey's multiple comparison test. ## denotes ($p<0.01$) as compared to Saline group. ** denotes ($p<0.01$) as compared to PTZ group

3.3 Effect of MEAH on PTZ induced anxiety in Elevated-Plus Maze model of mice

PTZ (20mg/kg, i.p.) were decreased the number of entries in open arms in Elevated-Plus Maze model on the 10th day of treatment as compared to saline treated group. MEAH at dose (500mg/kg, p.o) were significantly ($p<0.001$) increased the number of entries in open arms in Elevated-Plus Maze model as compared to PTZ treated group. Lower dose (250 mg/kg, p.o.) of MEAH remarkably ($p<0.005$) were decreased the number of entries in open arms in Elevated-Plus Maze model as compared to PTZ treated group (Figure 3).

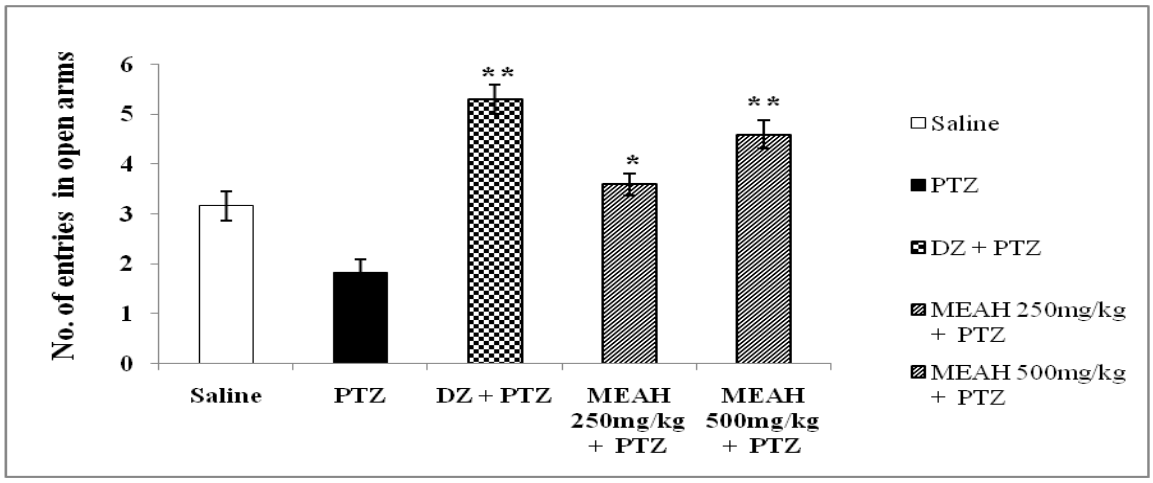

Fig.3: Effect of MEAH on PTZ induced anxiety in Elevated-Plus Maze model of mice

Values are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by Tukey's multiple comparison test. ## denotes ($p<0.01$) as compared to Saline group. ** denotes ($p<0.01$) as compared to PTZ group

3.4 Effect of MEAH on PTZ induced anxiety in Marble burying behavior model of mice

PTZ (20mg/kg, i.p.) were increased the number of Marble burying behavior of mice on the 10th day of treatment as compared to saline treated group. MEAH at dose (500mg/kg, p.o) were significantly ($p<0.001$) decreased the number of Marble burying as compared to PTZ treated group. (Figure 3)

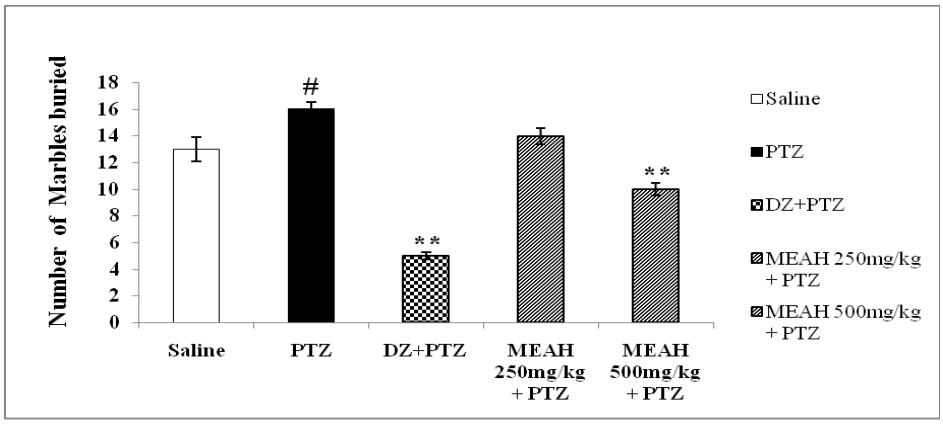

Fig.4: Effect of MEAH on PTZ induced anxiety in Marble burying behavior model of mice

Values are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by Tukey's multiple comparison test. # denotes ($p<0.05$) as compared to Saline group. ** denotes ($p<0.01$) as compared to PTZ group.

3.5 Effect of MEAH on PTZ induced anxiety on brain GABA levels of mice

PTZ (20mg/kg, i.p.) were significantly decreased brain GABA levels as compared to saline treated control. MEAH (250,500mg/kg, p.o) were significantly ($p<0.001$) enhanced the Brain GABA levels as compared to PTZ treated group (Figure 5).

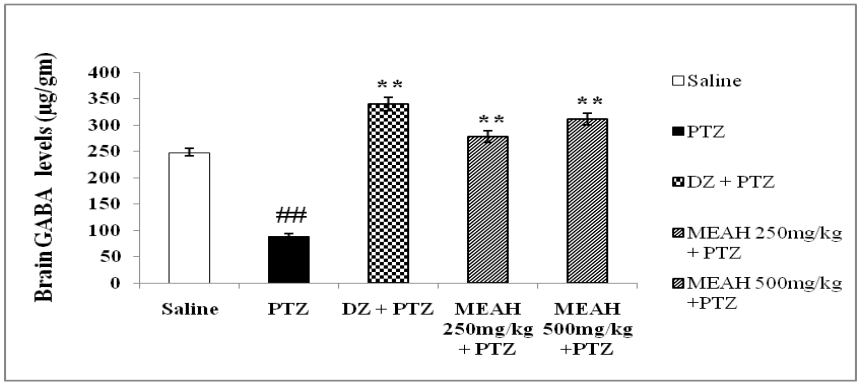

Fig. 5: Effect of MEAH on PTZ induced anxiety on brain GABA levels of mice

Values are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by Tukey's multiple comparison test. ## denotes ($p<0.01$) as compared to Saline group. ** denotes ($p<0.01$) as compared to PTZ group.

3.5 Effect of MEAH on PTZ induced anxiety on brain Serotonin levels of mice

PTZ (20mg/kg, i.p.) were decreased the brain Serotonin levels on the 10th day as compared to saline treated control. MEAH at dose (500mg/kg, p.o) remarkably ($p<0.005$) enhanced Brain Serotonin levels in mice as compared to respective PTZ treated group. Lower dose (250 mg/kg, p.o.) of MEAH did not increase the brain serotonin levels to PTZ treated group (Figure 6).

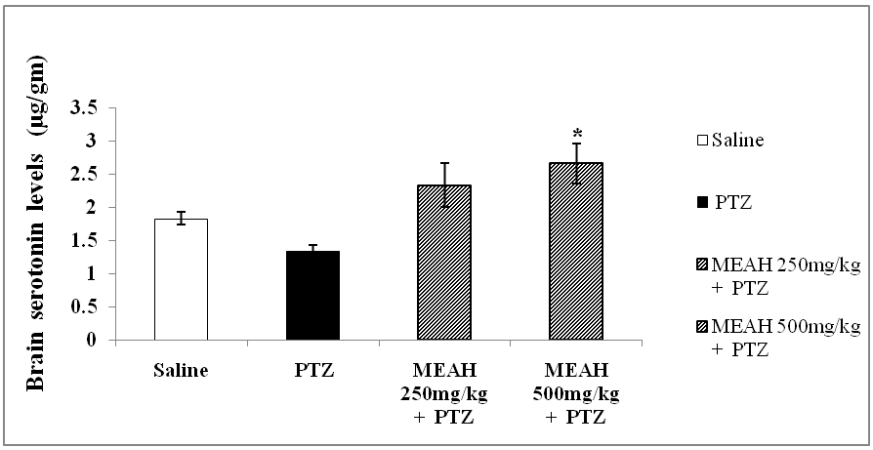

Fig. 6: Effect of MEAH on PTZ induced anxiety on brain Serotonin levels of mice

Values are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by Tukey's multiple comparison test. ## denotes ($p<0.01$) as compared to Saline group. ** denotes ($p<0.01$) as compared to PTZ group.

4. Discussion

Neuroinflammation, oxidative damage, neurotransmitter imbalance, neuronal injury and drug abuse are involve in the development of various psychiatric disorders such as anxiety [2,5]. Anxiety is a disorder that we face in our everyday life. Synthetic drugs unable to reverse the symptoms of anxiety completely and also have some serious side effects. Thus, the intake of natural component in the form of dietary supplements, herbal drugs and phytonutrients may provide favorable effects in anxiety with the virtue of neuroprotective, anti-inflammatory and antioxidant effects [4,5]. Therefore, we have chosen *Artocarpus heterophyllus* as it has excellent phytoconstituents and medicinal uses. The aim of the present study was to evaluate the effect of Methnol extract of *Artocarpus heterophyllus* (MEAH) heart wood against PTZ induced anxiety in mice using animal models of anxiety viz. Light-Dark Model, Elevated Plus Maze and Marble Burying Behavior Model. Biochemical estimations were done to understand the possible mechanism of action of selected plant in anxiety.

In previous studies indicate the role of GABA receptors and GABA neurotransmitter in the pathophysiology of anxiety [16]. PTZ inhibits gamma amino butyric acid (GABA) pathway in CNS and able to produce anxiety like symptoms in small animals [17]. Benzodiazepines are antianxiety drugs, act via GABAergic neurotransmission. Other target for the treatment of anxiety is serotonin. Light-Dark Model, Elevated-Plus Maze and Marble Burying Behavior Model are the most common behavior model used to assess the antianxiety effect of new compounds on animals.

In the present study, MEAH was able to reduce the PTZ induced anxiety in mice by increasing total time spent in light box of light-dark model and total time spent in open arm and number of entries in open arms of Elevated-plus maze test and Marbles buried in Marble burying behaviour model, which shows that suppression of anxiety like behavior in animals. Diazepam, an antianxiety drug, was used as a standard drug in the present study for the comparable test. Diazepam has also shown significant anxiolytic effects in mice. Furthermore, brain GABA and serotonin levels were increased with the treatment of MEAH, which revealed that effect of MEAH might be mediated by GABAnergic and Serotonergic pathways. There were no side effects observed in the mice with the treatment of MEAH, whereas with the diazepam administration sedation was seen in the animals. In literature it has been reported, Jackfruit has antioxidant, neuroprotective, antiepileptic, and antidepressant and also have various therapeutic phytoconstituents [6,7,8,9]. These effects and its phytoconstituents might be responsible for the antianxiety activity too. Furthermore, therapeutic constituent found in this plant act synergistically could also contribute in the resultant effects.

5. Conclusion

The present study shows that methanol extract of *Artocarpus heterophyllus* (MEAH) heart wood was effective against PTZ induced anxiety like symptoms in mice using behavioral models of anxiety. Moreover, MEAH was also effective to increase brain GABA and serotonin levels in biochemical studies. Both behavioral and biochemical findings, when taken together reflect that the MEAH appears to be a promising candidate for reducing anxiety. This study gives us an evidence for the investigation of novel antianxiety agent. Therefore, it would be worthwhile to isolate the phytoconstituents of *Artocarpus heterophyllus* and evaluate their therapeutic potential in the management of human anxiety.

6. References

1. Jung JW, Lee S. Anxiolytic Effects of Quercetin: Involvement of GABAergic System. Journal of Life Science 2014; 24(3): 290-296.
2. Preeti, Parle M, Monu, Sharma K. Medicinal plants possessing anxiolytic activity: A brief review. Der Pharmacia Sinica 2015; 6(5):1-7.
3. Scheepens A, Bisson JF, Skinner M. p-Coumaric acid activates the GABAA receptor *in vitro* and is orally anxiolytic *in vivo*. Phytotherepy Research 2014; 28(2):207-211.
4. Monika, Parle M, Sharma K, Yadav M. Antioxidant Effect of Some Medicinal Plants: A Review. Inventi Rapid: Planta

Activa 2016; 1: 1-8.
5. Yadav M, Parle M, Sharma N, Ghimire K, Khare N. Role of Bioactive Phytoconstituents from Several Traditional Herbs as Natural Neuroprotective Agents. Inventi Rapid: Planta Activa 2016; 4: 1-4.
6. Fang SC, Hsu CL, Yen GC. Anti-inflammatory Effects of Phenolic Compounds Isolated from the Fruits of *Artocarpus heterophyllus.* Journal of Agricultural and Food Chemistry 2008; 56: 4463-4468.
7. Rawat S, Sati OP, Tomar A. Evaluation of antioxidant effect of *Artocarpus heterophyllus* leaves extract. World journal of pharmacy and pharmaceutical sciences 2015; (4) 7: 626-631.
8. Jain AP , M.V.Tote, N.B.Mahire, V.R.Undale, A.V.Bhosale. Evaluation of Anticonvulsant activity of methanolic extract of *Artocarpus heterophyllus lam*. (Moraceae) in mice. Journal of Pharmacy Research 2009; 2(6): 1004-1007.
9. Porwal M, Kumar A. Neuroprotective effect of *Annona squamosa* & (-) Anonaine in decreased GABA receptor of epileptic rats. Journal of Applied Pharmaceutical Science 2015; (5) 1: 018-023.
10.Gordon, M E, Philpot, JW, Burt, R E, Thompson, C A, Spiller, W E. Commitment to the union Development of a measure and an examination of its correlates. Journal of Applied Psychology 1980; 65: 479-499.
11. Bourin M, Hascoet M. The mouse light/Dark box test. European Journal of Pharmacology 2003; 463: 55-65.
12. Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 1987; 92: 180-185.
13. Njung'e K, Handley SL. Evaluation of Marble-burying behavior as a model of anxiety. Pharmacology Biochemistry and Behavior 1991; 38: 63-67.
14. Schlumpf M, Lichtensteiger W, Langemann H, Waser GP and Hefti F. A fluorometric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue. Biochemistry and Pharmacology 1974; 23:2437-2446.
15. Lowe IP, Robins E, Eyerman GS. The fluorimetric measurement of glutamic decarboxylase and its distribution in brain. Journal of Neurochemistry 1958; 3: 8-16.
16. Gilhotra N, Dhingra D. Thymoquinone produced antianxiety-like effects in mice through modulation of GABA and NO levels. Pharmacological Reports 2011; 63: 660-669.
17. Chimakurthy J, Talasila M. Effects of curcumin on pentylenetetrazole-induced anxiety-like behaviors and associated changes in cognition and monoamine levels. Psychology & Neuroscience 2010;(3) :239-244.