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Original Article

Evaluation of In-vivo Anti-Depressant, Anti-Anxiety and Anti-Convulsant Activity of *Ficus Lacor* Bark

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ABSTRACT

Background and Objectives: *Ficus lacor* is a large deciduous, rapidly growing closely foliaceous tree about 20 m in height with a fine shaped crown. It is widely distributed in tropical and subtropical regions of the world. This plant is mentioned in different ayurvedic texts with different therapeutic values and literatures. *Ficus lacor* is used for its anti-inflammatory, anti-arthritic, antioxidant and anti-diabetic activities from time era. The present study was conducted to evaluate Antidepressant, Anxiolytic and Anticonvulsant activity of *Ficus lacor* bark using various animal models.

Materials and Methods: Wistar rats of either sex weighing 150-250 gram were used in this study. Extract was studied for acute toxicity study. Ethanolic extract of low dose (250 mg/kg) and high dose (500 mg/kg) were performed for anti-depressant, anxiolytic and anti-convulsant activity. Antidepressant and anxiolytic activity were performed by Rota-rod and elevated plus maze. Anti-convulsant activity was studied in isoniazid induced convulsion in Wistar rat.

Results: Extract treated 500 mg/kg showed more effective and significant ($P<0.05$) anti-depressant

activity. Extract treated group with 500 mg/kg and 250 mg/kg showed significant anxiolytic activity in both No. of open arm entries and time spent in open arm but have no significant anti-convulsant activity.

Conclusion: The study concludes that the ethanolic extract possesses anti-depressant and anxiolytic activity of *Ficus lacor* bark but have no significant anti-convulsant activity in isoniazid induced convulsion. Our study will aware the community people for the preservation of this medicinal plant and will reduce the wastage of drug by proper utilization of the crude drug.

Keywords: Antidepressant, Anxiolytic, Anti-convulsant, *Ficus lacor*

INTRODUCTION

The *Ficus lacor* belonging to family moraceae it is found in Asian countries like India, Nepal, Srilanka, Bangladeh etc. various types of bioactive constituents which include alkaloids, flavonoids, terpenoids, tannins and proteins. According to the ethnomedicinal uses it is extensively used in Diabetes Mellitus, mental illness, respiratory infection and diarrhea, etc. There is need to continue identifying and quantifying the active principle and determine the mechanism of underlying the properties of Ficus *lacor* [1]. The anxiety and depression are the most common illnesses in the community. The depressive patient has features of the anxiety disorder. Anxiety is the primary condition occurring in childhood. The anxiety and depression mostly genetically vulnerability for both disorders are being an epiphenomenon of the other [2].

According to W.H.O the global and regional estimation of prevalence of anxiety and depression they concluded that depression is more common among females (5.1%) than males (3.6%). The total number of depression patients in the world is 322 million. According to age group, 7.5% females and 5.5% males are 55-75 years old. At global level, overall 264 million peoples are suffering from anxiety. Among them 4.6% females and 2.6% males are suffering from anxiety [3].

Depression is a severe illness according to the study the lifetime prevalence of their illness about 10-20%. In the chronic type of depression, the suicidal thought is the major symptoms shown in 15% of depressed patients according to WHO suicidal thought is the most important cause of disability in the world by the year 2020 [4]. Depression is caused by the decreasing the level of neurotransmitter and depression should be decreased if increase the level of neurotransmitter especially excitatory neurotransmitter is a decrease in presynaptic and postsynaptic junction result decrease the depolarization and repolarization process [5].

Herbal therapies could be considered as alternative or complementary medicines. In the search for new molecules useful for the treatment of neurological disorders, worldwide medicinal plant research has continued to progress, demonstrating the pharmacological effectiveness of different plant species in a variety of animal's models [6]. Therefore, the objective of the present work was to analyze the possible anti-depressant, anxiolytic and anti-convulsant effect of *Ficus lacor* bark in rats using the Rota-rod test for antidepressant, elevated plus-maze test for anxiolytic activity and isoniazid induced convulsion as animal models for convulsion.

MATERIALS AND METHODS

This study was carried out from August 2017 to March 2018 at the department of pharmacology, Universal College of Medical Sciences and Teaching Hospital, Bhairahawa, Nepal. After taking approval from Institutional Review Committee IRC No. (UCMS/IRC/084/17), UCMS, Bhairahawa, Nepal.

Collection and authentication of plant: The plant materials were collected from Mayadevi-6, Rupandehi, Nepal. Herbarium was prepared with fresh plant material and was submitted for its identification and certification of plant at Department of Soil and Environment Science, Institute of Agriculture and Animal Science, Paklihawa Campus.

Extract preparation: *Ficus lacor* bark was dried for four weeks under shade. The dried bark was then grinded to coarse powder. The powder was extracted with ethanol by Soxhlet extractor. The extract was then concentrated to viscous semisolid mass under reduced pressure by Rotary evaporator at 40 ^{0C} and dryness at 32 ± 3 ^{0C} in hot air oven. The dried extract was stored in a refrigerator at 4$^{\circ C}$ [7].

Animals: In this investigation, 150–250 gm Wistar rats of either sex were employed. Under regular circumstances (25 °C, 55% relative humidity, and 12-hour light/dark cycles), the animals were housed in clean propylene cages. The rats were exposed to laboratory settings for a week before behavioral testing and given free access to double-distilled water and regular laboratory rat diet [8].

Acute Toxicity Study: The extract's toxicity was investigated in accordance with OECD recommendation no. 423. First, a limit test will be carried out on female Wistar rats weighing 150–250 grams. As instructed in the guideline, three rats received doses of extract made in 2% between 80 in distilled water in increments of 500 mg/kg, 2000 mg/kg, and 5000 mg/kg. Following each dose administration, each animal was monitored every hour. Following then, toxicity and mortality up to 14 days were monitored daily [9].

Experimental design: The experimental animals were branched into 6 groups (N=6 Rats in each group) as follows:
Group I: Normal Control- Animals received 2% tween 80 in distilled water.
Group II: Isoniazid induced convulsion- Animals received Isoniazid 250 mg/kg body weight.
Group III: Negative control- Animals received Diazepam 1 mg/kg body weight.
Group IV: Standard- Animals received Imipramine 15 mg/kg body weight.
Group V: High dose- Animals received suspension of *Ficus lacor* 500 mg/kg body weight.
Group VI: Low dose- Animals received suspension of *Ficus lacor* 500 mg/kg body weight [10, 11, 12].

Rota-rod Apparatus: Depression was produced by the intraperitoneal dose of 1 mg/kg of diazepam 15 minutes before to treatment with imipramine and extract. The animals were then set in the rotating bar's

four paws, which are 2.5 cm in diameter, rubber-coated, connected to a motor with speed, and 25 cm above the ground. Five minutes were spent watching the animals.

An indicator of CNS depression was the difference between the rat's fall-off time before and after therapy. Only those animals were utilized in the test that had previously shown they could fall off 5 times in a row while being spun at 20 rpm for 5 minutes. The test was conducted 30, 60, and 90 minutes following the administration of medications and vehicles [13].

Elevated plus maze (EPM): The open arms had a 0.25 cm elevated edge that gave the animals more grip, and testing was done in a darkly lit environment to further promote open arm activity. Rats were acclimated to the lab environment for at least an hour before to testing to help them adjust to their new habitat.

An animal was positioned on the maze's central platform, facing an open arm, to begin the trial. The maze was meticulously cleaned in between subjects and a standard 5-min test period was applied. The trials were conducted in front of a spectator who was uninformed of how the rats inside the room were being treated. The frequency and length of arm visits, independently for open and closed arms, were typically measured in this test. When a rat's four paws are on an arm, it is deemed to have entered that arm [14].

Isoniazid induced convulsion: Four groups of six rats each were created from a total of 24 rats. After 30 minutes, several groups received treatment; convulsion was brought on by injecting 250 mg/kg of isoniazid. The latency of the first chronic convulsion and the length of the convulsion were measured throughout the following 60 minutes. Animals were regarded as safe if they did not convulse within 30 minutes [10].

Statistical Analysis

Graph Pad Prism software was used to perform a one-way analysis of variance (ANOVA), followed by Dunnett's multiple and Neuman-Kauls multiple comparison tests between various groups (*P < 0.05, **P < 0.01, ***P < 0.0001) and represent all the data in the tables and figures as mean standard error of mean of each group (n=6) (version 5. 01). P values of 0.05 or below were regarded as statistically significant.

RESULTS

Acute toxicity study

Throughout the 14-day trial, the groups were watched for signs and symptoms of mortality as well as behavioral changes. This shows that the extracts were deemed safe at the dose ranges examined. The LD-50 of the extract was noted more than 5000 mg/kg body weight because all the animals were able to live at a dose of 5000 mg/kg.

According to table 1, no significant behavioral changes were noticed over the study period. About 500 mg/kg of the extract were administered therapeutically, whereas 250 mg/kg was the lowest dose.

Table 1: Change in Behavioural Profile of Extract Administered Rat in Acute Toxicity Study

Behavior type	Extract			2% tween 80 in distilled water
	500 mg/kg	2000 mg/kg	5000 mg/kg	
Alertness	+++	+++	++	+++
Awareness	+++	+++	++	+++
Sound Response	+++	+++	++	+++
Touch Response	+++	+++	++	+++
+++ = Present, ++ = Behavioural Response with Mild sedation				

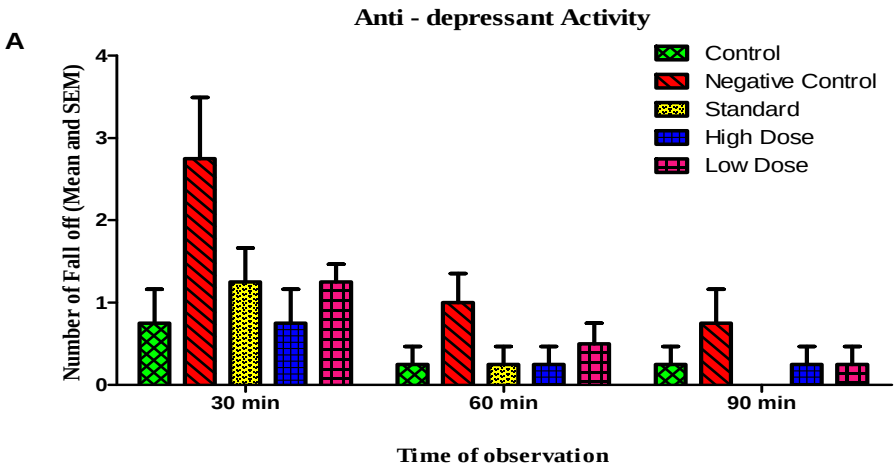

Figure 1: Number of fall off from rotating bar of rota-rod

Anti-Depressant Activity

Number of fall off from rotating bar of rota-rod: Animals treated with 1 mg/kg diazepam had increased the number of falling off when compared with control group, which indicates the induction of depression. The standard drug (imipramine) showed the decrease in number falling off than the negative control, which indicates the effective antidepressant effect. Extract treated with 500 mg/kg showed decrease in number of falling when compared with negative control. There was decreased in number of falling off ongoing from 30 min, 60 min and 90 min after the administration of drug as shown in figure 1, this may be due to elimination of drug or/and increase in ability of rat to perform due to multiple practice. There was a significant decrease no. of falling, showed dose antidepressant activity of *Ficus lacor* bark in dose dependent manner.

Ability of rat to remain on the bar of rota-rod: Standard drug showed improved time of latency of negative control to approximately equal to control group (**P<0.05) when compared with negative control which denotes the anti- depressant effect of standard drug. Group treated with 500 mg/kg of extract showed an effective anti-depressant as of standard (Imipramine 15 mg/kg) when compared with negative control. Group treated with 250 mg/kg of extract showed slightly increase time of latency of falling off when compared with 500 mg/kg dose of extract. There was a significant decreased in latency of falling off [**P<0.05 & **P<0.05] in high dose and low dose of extract when compared with negative control group showing dose dependent

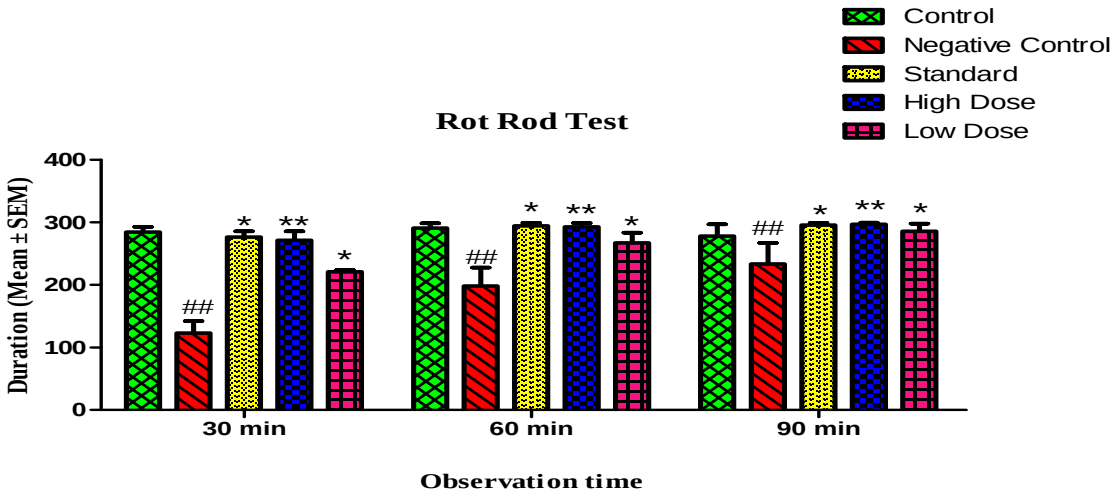

Figure 2: Ability of rat to remain on the bar of rota-rod

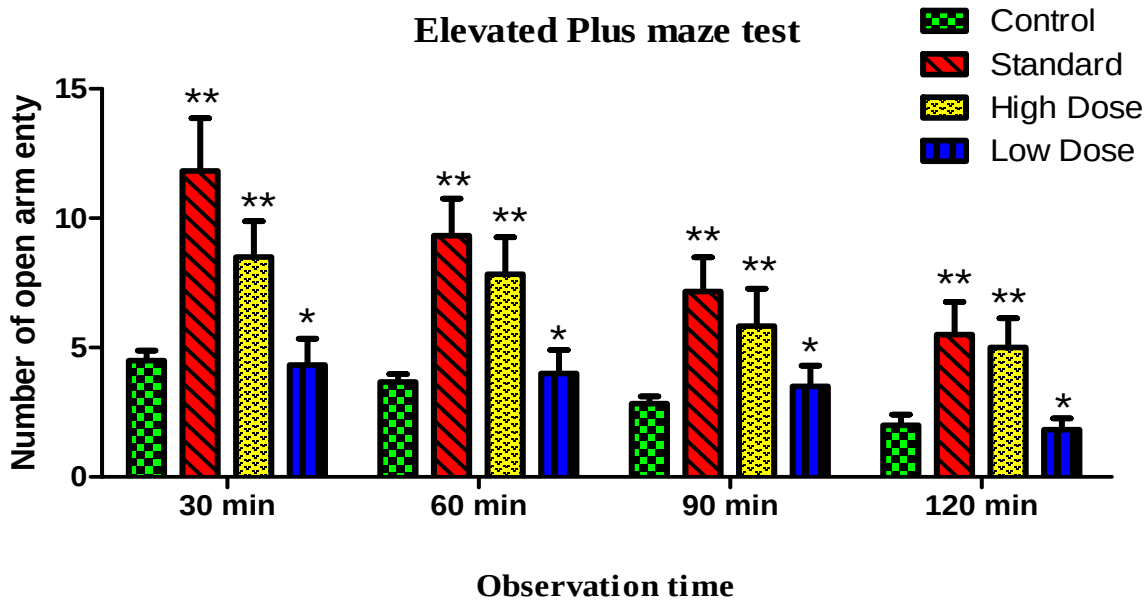

Figure 3: Showed number of open arm entry in elevated plus maze

antidepressant activity of *Ficus lacor* bark. Ongoing from 30 min to 60 min and from 60 min to 90 min time spent on bar of Rota-rod showed the decrease in time spent indicating the elimination of drug or/ and increase in ability of rat to remain on bar of Rota-rod due to multiple practice.

Anxiolytic Activity

Number of open arm entry in elevated plus maze: When compared to the control group, the standard (Diazepam 1 mg/kg)

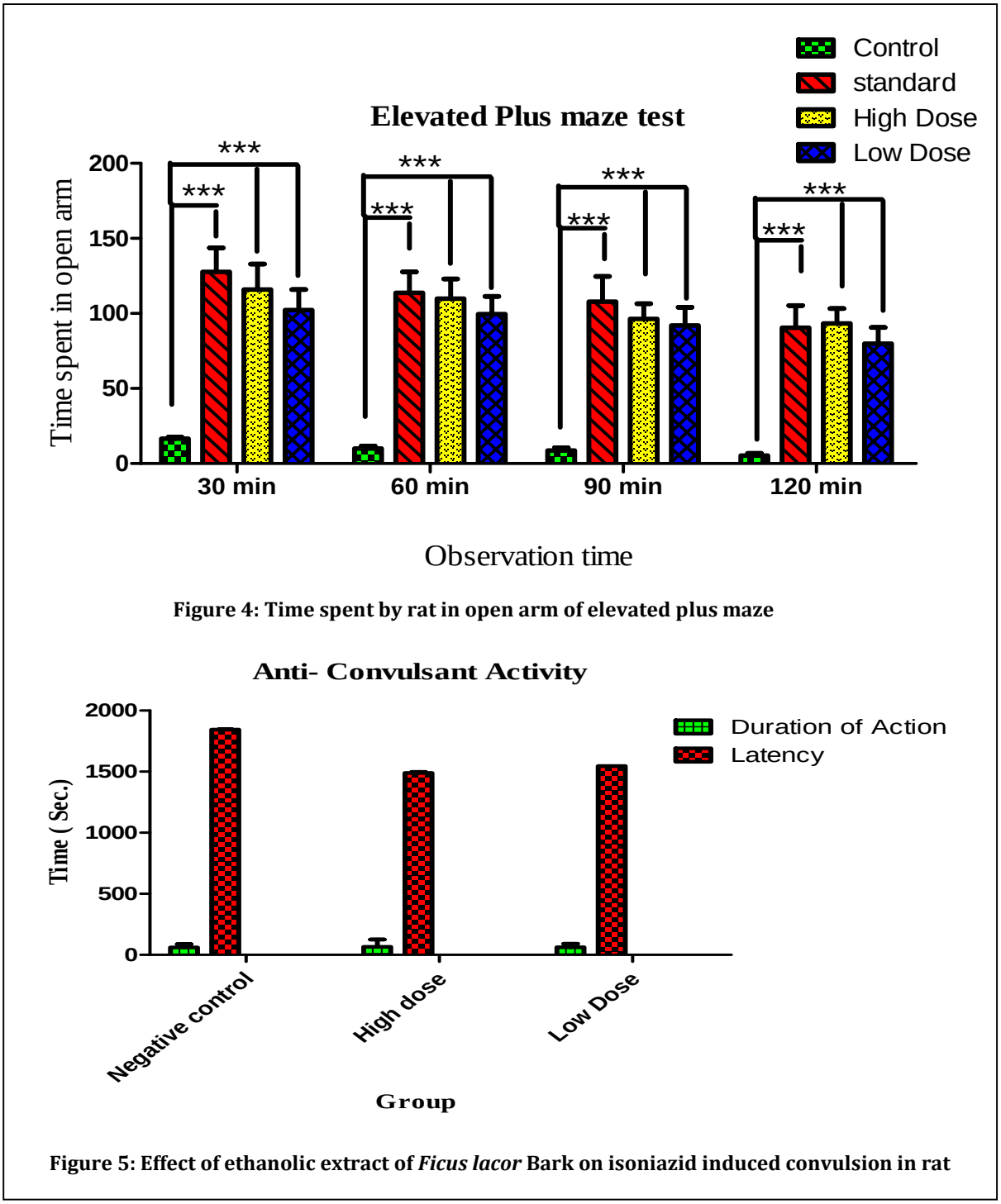

Figure 4: Time spent by rat in open arm of elevated plus maze

Figure 5: Effect of ethanolic extract of *Ficus lacor* Bark on isoniazid induced convulsion in rat

treated group significantly increased the number of entries in the open arm (**P < 0.01), indicating a decrease in anxiety. With extract doses of 500 mg/kg and 250 mg/kg treated group compared to the control group, there were more entries in the open arm.

The results showed that the high dose of extract (500 mg/kg) was more effective than the low dose (250 mg/kg) and less effective than the standard group (*P <0.05). Following medication treatment, the number of open arm entries fell from 30 to 60, 60 to 90, and 90 to 120 minutes, which may be related to the rat's induction of fear as a result of repeated exposure to the Elevated plus Maze.

Time spent by rat in open arm of elevated plus maze: The standard drug, diazepam, considerably increased the duration spent in the open arm (*** P < 0.05), which denoted anxiolytic activity of diazepam, in the determination of the anxiolytic impact of Diazepam (1 mg/kg, i.p) and extract (500 mg/kg and 250 mg/kg, p.o) on the EPM. The time spent in the open arm was significantly lengthened by extract at doses of 500 mg/kg and 250 mg/kg. Effect of time spent in open arm decreased for duration of action ongoing from 30 min, 60 min, 90 min, and 120 min after the supplied dose.

Anti- Convulsant Activity: The usual anti-epileptic medicine, Diazepam (1 mg/kg), stopped the clonic convulsions in latency and duration of seizure characteristics in all of the animals tested when isoniazid (250 mg/kg, i.p.) caused convulsions. The onset and duration of convulsions were not significantly altered by extract treatments at 500 mg/kg and 250 mg/kg.

DISCUSSION

Herbal therapies could be viewed as complementary or equivalent medicines. Wide-ranging medicinal plant research has continued to advance in the hunt for new molecules beneficial for the treatment of neurological illnesses, proving the pharmacological value of several plant species in a number of animal models [15]. The present study has confirmed that the ethanolic extract of *Ficus lacor* bark holds antidepressant and anxiolytic effects. The extract improves the time spent on bar of Rota-rod and decreasing the number of fall off from the bar, suggesting the anti-depressant effect. However, in contrast to extract, DZP decreased the ability of rat to spent time on bar and increased the number of fall off from the bar of Rota-rod significantly (**P<0.05), suggesting the induction of depression.

Data in literature suggests that benzodiazepines, such as diazepam, act as anxiolytics and anticonvulsants at low doses, producing also a myorelaxant and sedative effect at higher doses [16, 17]. This is therefore not surprising as diazepam produced a myorelaxant effect at the dose used. Standard drug (imipramine) showed increased time spent on bar (**P<0.01). Extract of low dose showed less effective anti-depressant action than the high dose when compared with negative control (*P<0.05). Extract of high dose showed more effective anti-depressant than the low dose of extract in comparison with imipramine. The anti-depressant effect of ethanolic extract of *Ficus*

lacor barks may be due to presence of flavonoids & sterols which have been involved in CNS inhibitory and neuromodulatory effects [18]. By increasing the serotonin and/or nor-epinephrine concentration in synapses of CNS, blocking of GABA and α-2 adrenergic receptors Extract of the *Ficus lacor* bark possesses anxiolytic effect comparable with that of diazepam in pharmacologically validated models of anxiety.

In the current investigation, the extract increased the percentage of entrances and time spent in the open arms, which suggests an anxiolytic effect. It also lowered avoidance to open arms. These outcomes mirrored those seen following the administration of the reference anxiolytic medication diazepam. These findings are consistent with those of past research in which anxiolytic diazepam increased the proportion of time spent in open arms and open arm entries in the EPM [19]. When compared to the control group, the extract-treated group with 500 mg/kg demonstrated significantly increased time spent in the open arm and the frequency of open arm entries (***P < 0.0001, *P < 0.05, respectively). High dose of extract exhibited more potent anxiolytic than the low dose of extract. The ethanolic extract of *Ficus lacor* bark may have anxiolytic properties since it contains alkaloids, flavonoids, terpenoids, phenolics, and phytosterols. It's conceivable that the binding of any of these phytochemicals to the GABAA-BZD complex is the mechanism by which the extract of *Ficus lacor* relieves anxiety. This is corroborated by the discovery that berberine alkaloids bind to the GABAA receptor's BZD site with high affinity [20].

The INH test is the acute chemical experimental model that is most widely used in the hunt for novel AEDs. Isoniazid causes convulsions by preventing the synthesis of GABA [21]. It prevents glutamic acid decarboxylase (GAD) activity; an enzyme involved in the production of GABA, which lowers GABA levels [22]. INH administrations reduce pyridoxine activity to increase clinical pyridoxine decrease. This occurs in one of two ways: first, INH inhibits pyridoxine phosphokinase, an enzyme that changes pyridoxine into its active form and the second, the interaction of pyridoxal phosphate with INH results in the formation of an inactive hydrazine complex that is excreted in the urine [23]. Results of the present study showed that ethanolic extract of *Ficus lacor* bark have no significant anticonvulsant activity in isoniazid induced convulsion which is a pharmacologically validated experimental animal model for anti-convulsant activity. This may be due development of tolerance against the extract due to multiple exposure of extract to rat or/and there may be some chemical constituent that inhibit the action of *Ficus lacor* in anti-convulsant activity. Due to a lack of tools and plants with a varied range of chemical contents, the active molecule responsible for anti-depressant and anxiolytic effects was not isolated and identified. As a result, we are unable to link our findings to the precise phytochemicals that are behind their anti-depressant and anxiolytic effects.

CONCLUSION

The study concluded that the bark of *Ficus lacor* exhibits anti-depressant and anxiolytic properties. It does not exhibit any discernible anti-convulsant properties in isoniazid-

induced convulsion. These findings imply that *Ficus lacor* may have promise as an alternative therapeutic strategy for depression and anxiety treatment that complements traditional pharmaceuticals. Additional in-depth research in various models of depression, anxiety, and convulsion is required, and it is preferable to isolate the particular chemical component that is responsible for the aforementioned action.

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Conflict of interest:

None

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Author's contribution:

*Concept and design, data analysis, interpretation of results, literature review, and revision of the manuscript-**RKM**; literature review, Statistical analysis, revision of the manuscript-**AS**; Revision and editing the manuscript-**ST**; Revision and editing the manuscript-**MKC**; Data analysis, Revision and editing the manuscript-**VKM**. All authors read and approved the final version of the manuscript.*

REFERENCES

1. Chawla A, Kaur R, Sharma KA. Ficus carica Linn. A Review on its Pharmacognostic, Phytochemical and Pharmacological Aspects. International Journal of Pharmaceutical and Phytopharmacological Research 2012; 1(4): 215-232.
2. Gorman JM. Comorbid depression and Anxiety spectrum disorder. Journal of Depression and Anxiety 1996; 4: 160-168.
3. Gonçalves DC, Pachana NA, Byrne GJ. Prevalence and correlates of generalized anxiety disorder among older adults in the Australian National Survey of Mental Health and Well-Being. J Affect Disord. 2011; 132: 223-30
4. Andersson D, Magnusson H, Carstensen J, Borgquist L. Co-morbidity and health care utilisation five years prior to diagnosis for depression. A register-based study in a Swedish population. BMC Public Health 2011; 11: 552.
5. Papakostas GI and Ionescu DF. Towards New Mechanisms: An Update on Therapeutics for Treatment-Resistant Major Depressive Disorder. Molecular Psychiatry 2015; 20(10):1142-50.
6. Galdino PM, Oliveira DRD, Florentino IF, et al. Involvement of the monoamine system in antidepressant-like properties of 4-(1-phenyl-1h-pyrazol-4-ylmethyl)-piperazine-1-carboxylic acid ethyl ester. Life Sci 2015;143:187-93.
7. Gahlot KMA, Sharma A. Pharmacological Evaluation of Gelsemium sempervirens roots for CNS Depressant Activity. International Journal of PharmTech Research 2011; 3(2): 693-697.
8. Mahendaran G, Thamodharan G, Sengottuvelu S, Bai NV, et al. Evaluation of anticonvulsant, sedative, anxiolytic and phytochemical profile of the methanol extract from the aerial parts of swertiacorymbosa (Griseb.) Wight ex C.B. Clarke. BioMed Research International 2014; 2: 1-9.
9. Walum E. Acute oral toxicity. Environ Health Perspect 1998; 106: 497-503.
10. Basavaraj P, Shivakumar B, Shivakumar H, Manjunath VJ. Evaluation of anticonvulsant activity of tabernaemontana divaricata (linn) R. BR. Flower extract. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3(3):310-315.
11. Sirohi B, Raj V, Pande A. Qualitative and quantitative estimation of euphorbia thymifolia linn.

Journal of Medical Pharmaceutical and Allied Science 2019; 8(1): 2069-2078.

12. Biesdorf C, Cortez DAG, Audi EA. Assessment of anxiolytic and panicolytic effects of dichloromethane fraction from stems of Kielmeyera coriacea. Journal of Phytomedicine 2012; 19:374-377.
13. Mahendaran G, Thamodharan G, Sengottuvelu S, Bai NV et al. Evaluation of anticonvulsant, sedative, anxiolytic, and phytochemical profile of the methanol extract from the aerial parts of swertiacorymbosa (Griseb.) Wight ex C.B. Clarke. BioMed Research International 2014; 2: 1-9.
14. Kumar D, Bhat ZA, Kumar V, Khan NA, et al. Effects of Stachystibetica essential oil in anxiety. European Journal of Integrative Medicine 2012; 4: 169-176.
15. Rodgers RJ, Johnson NJ. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze. Pharmacology Biochemistry Behaviour 1995; 52 : 297-303.
16. Galdino PM, Nascimento MV, Sampaio BL, Ferreira RN, Paula JR, Costa EA. Antidepressant-like effect of Lafoensia pacari A. St.-Hil. Ethanolic extract and fractions in mice. J Ethnopharmacol 2009; 124(3): 581-585.
17. Onaivi ES, Maguire PA, Tsai NF, Davies MF, Loew GH. Comparison of behavioral and central BDZ binding profile in three rat lines. Pharmacol Biochem Behav 1992; 43(3): 825-831.
18. Wolffgramm J, Mikolaiczyk C, Coper H. Acute and subchronic benzodiazepine-barbiturate-interactions on behaviour and physiological responses of the mouse. Naunyn Schmiedebergs Arch Pharmacol 1994; 349(3): 279-286.
19. Bhutada P, Mundhada Y, Bansod K, et al. Ameliorative Effect of Quercetin on Memory Dysfunction in Streptozotocin-Induced Diabetic Rats. Neurobiology of Learning and Memory 2010;94: 293-302.
20. Adeyemi OO, Yemitan OK, Taiwo AE. Neurosedative and muscle relaxant activities of ethylacetate extract of Baphia nitida AFZEL. Journal of Ethnopharmacology 2006; 106(3): 312-316.
21. Costa E, Guidotti A, Toffano G. Molecular mechanisms mediating the action of diazepam on GABA receptors. Br J Psychiatry. 1978; 133(3): 239-248.
22. Raygude KS, Kandhare AD, Ghosh P, Bodhankar SL. anticonvulsant effect of fisetin by modulation of endogenous biomarkers. Journal of Biomedicine and preventive nutrition 2012; 2(3): 215-222.
23. Vergnes M, Boehrer A, Reibel S, Simler S, Marescaux C. Selective susceptibility to inhibitors of GABA synthesis and antagonists of GABA (A) receptor in rats with genetic absence epilepsy. Experimental Neurolog 2000; 161(2):714-723.