

Original Article

Analysis of anxiolytic and anti-depressant potential of *Bombyx mori* in *in vivo* mouse mode

Abdul Hayee, PhD^{1*}, Hafiz Muhammad Asif, PhD¹, Memoona, PhD², Abdullah Khilji, M.Phil³, Munazza Khilji, Bachelors², Muhammad Subhan Nazar, MS⁴, Muhammad Rahil Aslam, PhD¹, Waseem Abbas, M.Phil⁵, Shahbaz Ahmad Zakki, PhD⁴

¹Department of Eastern Medicine, University College of Conventional Medicine, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Bahawalpur, ²Institute of Physiotherapy and Rehabilitation Sciences, Peoples University of Medical and Health Sciences for Women, Nawabshah (SBA), ³Department of Anatomy, Khairpur Medical College, Khairpur Mir's, ⁴Department of Public Health and Nutrition, The University of Haripur, Haripur, Pakistan, ⁵Department of Molecular and Medical Pharmacology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan.

*Corresponding author:

Abdul Hayee,
University College of
Conventional Medicine, Faculty
of Pharmacy and Alternative
Medicine, The Islamia
University of Bahawalpur,
Bahawalpur, Pakistan.
khiljia.hayee@gmail.com

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ABSTRACT

Objectives: Anxiety and depression are common mental health problems that affect people's quality of life all around the world, and medicinal plants have been utilized for centuries. The anxiolytic and depressive activities of a hydro-alcoholic extract of *Bombyx mori* L. (Silkworm) were investigated using animal models in this study.

Methods: Anxiolytic activity was measured using an elevated plus maze model (EPMM) and open field test (OFT). The antidepressant activity was evaluated using the tail suspension test and forced swim test (FST). The EPMM recorded the amount of time spent in both closed and open arms. A period of unchanging status was detected for every animal for around 300 s in FST. One-way analysis of variance was used to analyze results, and values were considered significant where $P < 0.05$.

Results: Anxiolytic action of *B. mori* through OFT revealed dose-dependent increases in the frequency of line crossings and rearings at the dose of 200 mg/kg b.w. comparable with that of Diazepam. In EPMM, extract-treated groups spent more time in open arms and less time in closed arms compared to the control group. *B. mori* increased mobility time while decreasing immobility time in both experimental animals in a dose-dependent manner. *B. mori* was well-tolerated and showed no signs of toxicity or mortality up to a dose of 5 g/kg.

Conclusion: *B. mori* displayed anxiolytic and antidepressant effects, indicating its promise as a natural treatment. The extract was well tolerated at tested levels, suggesting its safety for further research and possible medicinal applications.

Keywords: Antidepressant action, Anxiolytic activity, *Bombyx mori*, Elevated plus maze model, Forced swim model, Open field test, Tail suspension model

INTRODUCTION

Anxiety and depression are among the most prevalent psychiatric disorders worldwide, with lifetime prevalence rates of 14% and 12%, respectively.^[1] Such conditions present a significant financial burden due to the high cost of medical care. Among the most prevalent mental illnesses

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are anxiety and depression. Due to their strong comorbidity, these conditions are collectively referred to as internalizing mental disorders.^[2] Psychological health is a crucial aspect of overall health, and it is just as vital in later life as it is in any other age.^[3] The exact etiology of both disorders is not clearly known; however, some genetic, biological, environmental, and psychological factors are considered to be the major factors. Both anxiety and depression are characterized by increased concentrations of corticotropin-releasing factors in the cerebrospinal fluid. However, the release of other hormones or peptides of the hypothalamic-pituitary-adrenal (HPA) axis is regulated in the opposite manner in both disorders. Hypocortisolemia in anxiety leads to supersuppression after dexamethasone and increased numbers of glucocorticoid receptors, while hypercortisolemia non-suppression after dexamethasone leads to decreased numbers of glucocorticoid receptors in depression has been reported.^[4] Brain-derived neurotrophic factors, inflammatory cytokines, endogenous metabolites, and some gut microbiomes have been considered to exhibit significant roles in the pathogenesis of these depressive disorders.^[5,6] The major clinical features of these depressive disorders are sadness, loss of concentration and interest, loss of happiness, feelings of guilt or low self-worth, disturbed sleep, loss of appetite, and feelings of tiredness.^[7] Targeting specific neurotransmitters in the brain is frequently used in psychiatric disorders that are successful in treating anxiety and depression.^[8] Key neurotransmitters associated with these conditions include serotonin, gamma-aminobutyric acid (GABA), noradrenaline (norepinephrine), and dopamine.^[9] Serotonin is a primary focus in various medications like selective serotonin reuptake inhibitors, which elevate serotonin levels to alleviate mood disorders.^[10] GABA, an inhibitory neurotransmitter, is crucial for reducing anxiety, and drugs that enhance GABA function are used in anxiety management.^[11] Noradrenaline and dopamine are associated with motivation and pleasure and are targeted by some medications to address symptoms of depression.^[12] Efficient drug therapy for depression will mainly decrease anxiety disorders too. For various disorders of anxiety, such as obsessive compulsive disorder, increased quantities of antidepressants are needed than for depression.^[13] Small quantities of unusual antipsychotic agents can decrease anxiety. Anti-depressants are the most important choices for enduring pharmacological management of anxiety and its related disorders.^[14] The naturally occurring silk cocoon scientifically termed *Bombyx mori* L. (*Lepidoptera*, *Bombycidae*) silkworms is one of the most well-known medicinal substances with a variety of therapeutic uses.^[15] Due to the presence of chemo-factors (morin and β -sitosterol) and antioxidants (flavonoids, anthocyanin, and alkaloids), mulberry leaves (*Morus alba* L.) are considered useful plants rich in nutrients and nutraceuticals. These leaves are also used as a traditional diet for silkworm

larvae.^[16] Due to its comparatively low resilience to illness and stress, *B. mori* is a potential biological model system for research on the toxicity of environmental pollutants, antibiotic resistance, and drug screening.^[17] Due to the widespread perception of herbal products' safety, their usage for medicinal and therapeutic evidence has increased worldwide.^[18] When given orally to healthy individuals, silk fibroin hydrolysate had a notable neuroprotective effect as well as enhanced verbal and visual memory. When patients with mixed anxiety-depressive conditions were given a therapeutic syrup containing an aqueous extract of a silkworm cocoon, the mean levels of both anxiety and depression were dramatically decreased.^[19] *B. mori* has been reported as a source of protein fibroin and sericin, flavonoids, quercetin and kaempferol, alkaloids, coumarin derivatives, and phenolic acids. Several pharmacological investigations of *B. mori* and its phytochemical constituents have revealed antioxidant, antihyperlipidemic, cardioprotective, gastroprotective, anticancer, and antidiabetic properties.^[20] The present study aimed to evaluate the dose-dependent effects of hydro-alcoholic extract of *B. mori* for the management of depression and anxiety at various doses in well-established animal models.

MATERIALS AND METHODS

Insect material and preparation of crude extract

B. mori was purchased from the local market of Bahawalpur, Pakistan. It was identified and authenticated by Prof. Dr. Ghazala H. Rizwani, Director Research, Hamdard University Karachi, Pakistan and was given the reference number A141 for use in the future. After cleaning the fabric in which it was contained, the insect was dried in the shade. The crushed material was soaked in a watery ethanol solvent (30:70), which was stirred periodically at room temperature. Next, the immersed material was separated using Whatman #1 filter paper and muslin cloth. There were two more times that the soaking and filtering procedure was repeated. After discarding the remaining insect material, the collected filtrate was concentrated by first dissolving it under a rotating evaporator and then, after a little oven time, achieving a thick, semisolid extract structure akin to glue. After that, the botanical crude extract was given a name and an estimate of its yield in percentage, and it was placed in a cooler for later use.^[21]

Drugs

One hour before the activity, hydro-alcoholic extract of *B. mori* at doses of 50, 100, and 200 mg/kg was administered separately. Diazepam (DZP; 1 mg/kg) was used as a standard anxiolytic. Fluoxetine (FXT) (10 mg/kg, Sigma-Aldrich, USA) was used as a conventional antidepressant medication. The control groups were managed using normal saline (NS) (10 mL/kg).^[22]

Experimental animals

For this investigation, male Swiss albino mice weighing between 22 and 25 g were purchased from the Faculty of Pharmacy and Alternative Medicine's animal house. Every animal was kept in reserve at the pharmacology research laboratory's animal stock experimental area at the Islamia University of Bahawalpur's Faculty of Pharmacy and Alternative Medicine. The critters were housed in conventional research center settings, with 12-h light/dim cycles at 22 + 2°C, and were fed standard food and drink on an as-needed basis for one week before the start of the experiment.

Experimental groups

All animals were divided into five different groups for management, with five animals in each group. The first group, which was labeled as control, had only normal saline with a concentration of 0.9% medium sodium chloride. The second group was called the standard group and was given DZP 1 mg/kg for anxiolytic effects and 10 mg of FXT for antidepressant effects. *B. mori* extract was administered in different quantities (50, 100, and 200 mg/kg b.w.) to the third to fifth groups. The oral intake of each medication was done 1 h before the experiment.

Evaluation of anxiolytic activity of *B. mori*

Open field test (OFT)

The OFT model was made of white compacted wood and had a length of (32 cm × 32 cm) with partitions 18 cm apart. Animals could be seen in the model since one wall was constructed of plain Plexiglas. Using an indication, blue stripes were painted on the base that was visible from the first to the last of the visible Plexiglas floor. The foundation was split into 16 (12 cm × 12 cm) figures with four sides each by the stripes. A (12 cm × 12 cm) focus square was positioned in the direction of the open field's center. The center square is used because some mouse strains have fine motor abilities and travel over the stripes of the assessment compartment several times during a test session. In a similar vein, the core square has enough room all around it to emphasize the focal point as being unique before the exterior sections. The maze was lighted with a 60-watt red light meant for foundation lighting in a test room measuring (1.84.6 m). Between mice, the open field maze was cleaned with 70% ethyl alcohol. The critters' exploratory mobility was evaluated in the open field. The number of rearings and the number of crossing lines (with four paws) were the monitored metrics.^[23]

Elevated plus maze test

When it comes to measuring anxiety in animal models, the elevated plus maze test (EPMT) is the most widely used and widely acknowledged approach. Four arms made up the

mechanical connection; two of them remained open while the other two were closed. Open arms (35 cm × 5 cm) were intersected with closed arms (35 cm × 5 cm × 20 cm) at an inside point (5 cm × 5 cm). From the first stage, EPM was raised to a height of 50 cm and put in a room with dim lighting. After a ½ h, the creatures were separated into management groups and placed in different groups to handle one of the closed arms on elevated plus maze devices at the center. Three hundred seconds were recorded as the amount of time that each animal spent with its arms open and closed.^[24]

Assessment of antidepressant activity of *B. mori*

Forced swimming test

It is the most widely accepted and utilized *in vivo* method for evaluating the antidepressant effect. The apparatus was a simple Plexiglass box measuring 20 cm × 12 cm. It was filled with water (24°C + 1°C) to a depth of 15 cm, and all the animals were given a 15-min swim each day leading up to the particular test session as a pretest. Animals were treated with a specific collection of medicines after the pretest session, 6 h before the exact session of investigation, and ½ h before the final session of investigation. Half an hour after a specific section or one day after the pretest session, every experimental animal was separately put in a Plexiglass chamber for the final session of swimming (5 min each). A period of unchanging status was detected for every animal for around 300 s. Creatures were observed as unchanging when no attempts were made to escape, apart from developments significant to keep their heads out of water.^[25]

Tail suspension test (TST)

Mechanical assembly was built consisting of a 70-cm-high wooden chamber for the TST. Between the load's side separators, a bar was placed 60 cm above the floor or 10 cm above the top of the mechanical assembly. One inch of sticky tape was put one inch from the tip of the creature's tail to hang it from the pole. Before their daily test session, animals had a 15-min pretest each day. After the pretest, 3 h before the final test, and 30 min before the test itself, the animals were given distinct collection medications. Every creature was individually hung from a pole for 300 s after a specific segment or one day after the pretest session. Each organism's stability was measured for 300 s. The mouse was regarded steady when it clung still to the bar with no move to flee.^[26]

Acute toxicity study of *B. mori*

To conduct acute lethality tests, the Organization for Economic Cooperation and Development's test guideline 425 was followed. All experimental animals were divided into five groups and every animal's typical behavioral characteristics were recorded. While fasting through the

night, the experimental animals had unlimited access to water. *B. mori* extract was given to four groups of mice at dosages of 1, 3, 5, and 10 g/kg orally, whereas normal saline (10 mL/kg) was given to one group. After 30 min, 1, 2, 4, 6, 12, 24, and 48 h, behavioral changes were observed and recorded. Hyperactivity, alertness, convulsions, grooming, lacrimation, sweating, urine, corneal reflex, righting reflex, pain response, touch reaction, gripping quality, and mortality are examples of reaction parameters.^[27]

Ethical consideration

The research was conducted under guidelines approved by the Islamia University of Bahawalpur's Institutional Animal Ethics Committee. The Ethics Board of the University College of Conventional Medicine of the Faculty of Pharmacy and Alternative Medicine, Islamia University of Bahawalpur provided funding for the study, wide study/project no. 7117/Pharm.

Statistical analysis

Descriptive statistics was applied to calculate the mean and standard deviation. The study of the results was totaled by revealing the information to quantifiable investigation, making

use of one-way analysis of variance. All of the results were examined using GraphPad Prism programming version 5. Impacts were found to be significant, where $P < 0.05$.

RESULTS

Anxiolytic activity of *B. mori*

Findings of the anxiolytic effect of *B. mori* using an OFT

The OFT was used to investigate the botanical extract's effect on impulsive motor activity. In general, no line crossings or rearings into OFTs were seen by the experimental animals from the control group throughout a 5-min exercise. The inspection was done 1 h after the medicine was administered and the results were recorded. *B. mori* showed a dose-dependent increase in the number of line crossings and the number of rearings [Table 1]. The consequences of OFTs certainly illustrate the anxiolytic action of *B. mori* as it increased the number of line crossings and rearings in a dosage-dependent design. The number of line crossings and rearings by *B. mori* at the dose 50 mg/kg was 90.01 ± 3.9 and 35 ± 9 , by 100 mg/kg was 98.23 ± 4.5 and 44.50 ± 8.8 , and by 200 mg/kg was 119.90 ± 8.7 and 48 ± 4.2 , respectively [Table 1 and Figure 1]. The results

Table 1: Effects of the *B. mori* crude extracts on the number of line crossings and rearings.

Group	Open field test		Elevated plus maze model	
	No of line crossing	No of rearing	Time spent in open arms (S)	Time spent in closed arm (S)
Control (N/S 10 mL/kg)	87.16 ± 7.0^{ns}	34.12 ± 13^{ns}	32.14 ± 3.05^{ns}	262.19 ± 3.11^{ns}
Standard (DZP 1 mg/kg)	$133.83 \pm 9.10^{***}$	$59.02 \pm 10^{***}$	$104 \pm 8.14^{***}$	$191.5 \pm 8.99^{***}$
<i>B. mori</i> (50 mg/kg)	90.01 ± 3.9^{ns}	35 ± 9^{ns}	49.5 ± 3.76^{ns}	245.14 ± 3.92^{ns}
<i>B. mori</i> (100 mg/kg)	$98.23 \pm 4.5^*$	$44.50 \pm 8.8^*$	$76.44 \pm 4.41^*$	$218.0 \pm 4.01^*$
<i>B. mori</i> (200 mg/kg)	$119 \pm 8.7^{**}$	$48 \pm 4.2^{**}$	$94.05 \pm 5.11^{**}$	$200.45 \pm 4.92^{**}$

N/S: Normal saline, DZP: Diazepam, SEM: Standard error of the mean, ns: Not significant, (*) Progressively significant (**) if $P < 0.01$, and Highly significant (***) if $P < 0.001$

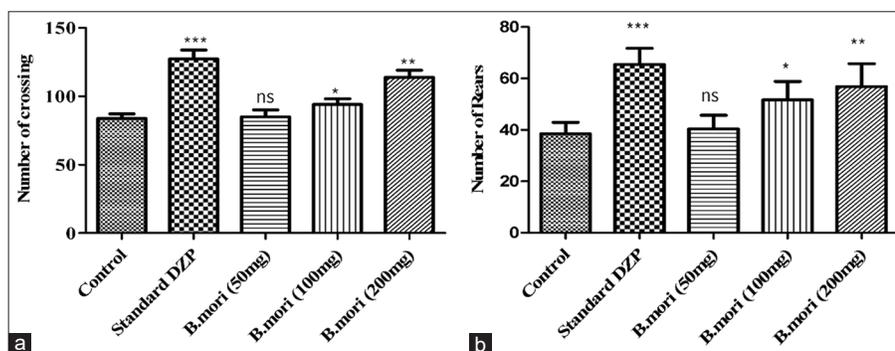


Figure 1: Open field test data. (a) The figures show the groups' means and standard deviations based on how many crossings occurred over 5 min. (b) The figures show the groups' means and standard deviations concerning the total number of rearings during the 5-min duration. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns: Non-significant. DZP: Diazepam

revealed that a dose of 200 mg/kg of *B. mori* extract had a comparable effect to DZP.

Results of the anxiolytic effect of *B. mori* utilizing elevated plus maze model (EPMM)

The time invested in open and shut arms by animals from the standard and management groups in a 5-min interval was compared with that of control group. After 1 h of management, an inspection was conducted and the results were recorded. Time used up in open arms by animals managed with *B. mori* 50, 100, and 200 mg/kg was examined to be 50.49 ± 5 , 74.5 ± 5.43 , and 91.5 ± 6.41 s individually. Time spent in close arms by *B. mori* 50, 100, and

200 mg/kg was 243.8 ± 8.1 , 220.0 ± 5.79 , and 203.5 ± 6.24 s independently [Figure 2].

Antidepressant activity of the Crude Extracts of *B. mori*

Results of antidepressant activity of *B. mori* using forced swim model

The animals in the standard and experimental groups were observed to exhibit varying degrees of mobility and immobility during three hundred-second swimming sessions. The results were compared to those of the standard control group. In a dosage-dependent manner, *B. mori* shortened the period of immobility and lengthened the period of movement [Figure 3 and Table 2].

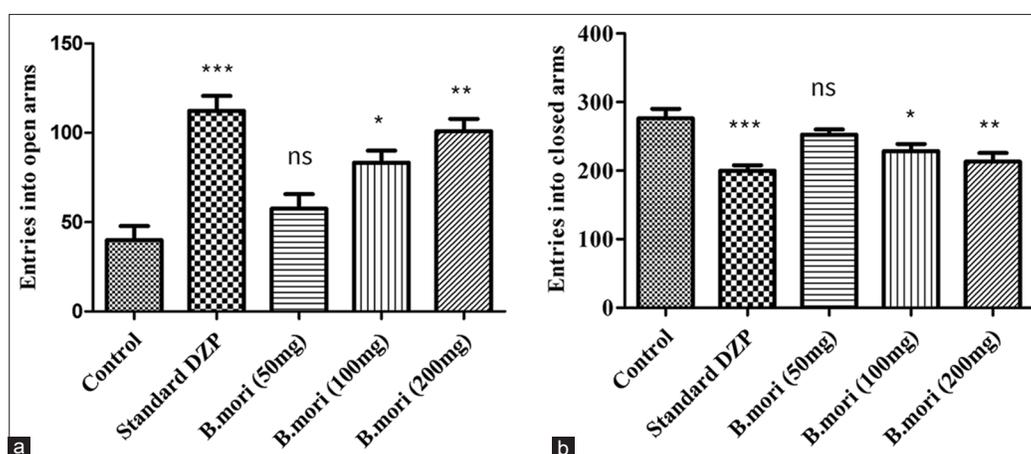


Figure 2: Anxiety-like behavior in the elevated plus maze (EPM). Bar graphs show the results of the control (normal saline) and standard DZP with *B. mori* at different doses. (a) Entries into the open arms. (b) Entries into the closed arms. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns means non-significant. DZP: Diazepam

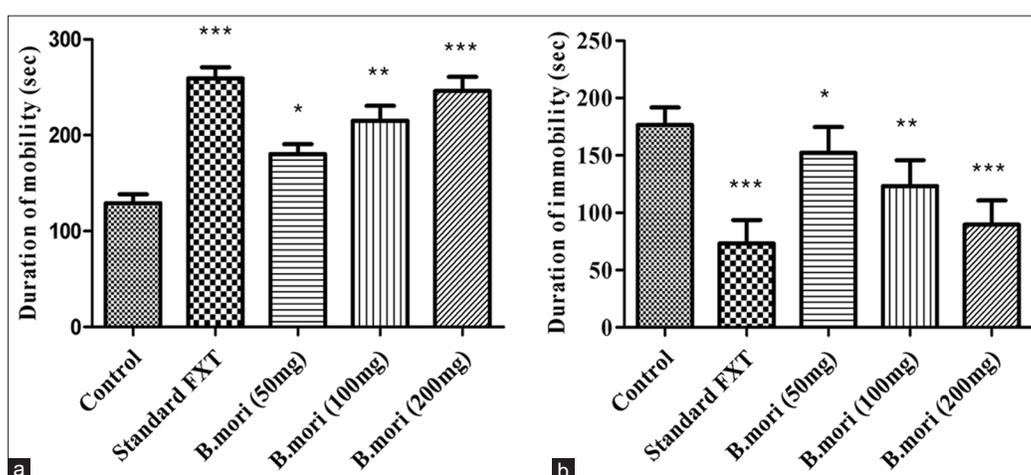


Figure 3: Depression-like behavior in the forced-swim test. (a) Duration of mobility in (sec) compared with control and standard FXT with different doses of *B. mori*. (b) Duration of immobility in (sec) with control and standard FXT with different doses of *B. mori*. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. *B. mori*: *Bombyx mori*, FXT: Fluoxetine.

Result of antidepressant activity of *B. mori* using tail suspension model

The length of the animals' mobility and immobility in the conventional control group and the cure groups were compared. In a similar manner, and a dosage-dependent manner, *B. mori* increased the duration of mobility and decreased immobility [Figure 4 and Table 2].

Acute toxicity studies of *B. mori*

The mice were given the unprocessed concentrate of *B. mori* intraperitoneal at dosage rates of 1, 3, and 5 mg/kg. No signs of toxic quality and fatality were seen up to 5 mg/kg dosage.

DISCUSSION

The preliminary phytochemical constituents that have been reported in *B. mori* are carbohydrates, flavonoids, phenols, proteins, etc. Furthermore, flavonoids contained in *B. mori* have been proven to reduce anxiety and depression.^[28] Studies of plant chemicals have revealed that the Silk Shelter consists of 12-16% total protein, 1.2-1.8% carbohydrate,

and 11-20% fat. Alanine, glycine, and serine represent about 82% of the amino acids found in the cocoon. The remaining amino acids present in the cocoon are cystine, histidine, aspartic acid, lysine, arginine, threonine, valine, proline, glutamic acid, tyrosine, methionine, tryptophan, phenylalanine, leucine, and isoleucine. As stated in several researches, the amino acids phenylalanine, tryptophan, methionine, and tyrosine are a lot useful in managing numerous mood disorders, including depression.^[29] Studies have specified that everyday supplements of essential nutrients are very helpful in minimizing the symptoms of anxiety and depression in patients. Supplements holding amino acids have also been identified to minimize the symptoms, as they are changed to neurotransmitters, which in turn, ease depression and other mental health issues. These may be suitable for managing and to some point, preventing anxiety, depression, and a lot of other mental disorders,^[30] while the content of fat comprises alpha-linolenic acid (ALA) - the indispensable fatty acids (FAs) together with palmitic acid. There are five flavanol glycosides recognized in a cocoon of silk that are responsible for the powerful anti-oxidant activity of *B. mori*.^[31]

These *in vivo* assays signify that this hydro-alcoholic extract

Table 2: Effect of *B. mori* crude extracts on the length of time spent moving and being immobile in tail suspension and forced swim models.

Group	Forced swim model		Tail suspension model	
	Duration of mobility (S)	Duration of immobility (S)	Duration of mobility (S)	Duration of immobility (S)
Control (N/S 10 mL/kg)	138.4±8.6 ^{ns}	161.6±8.6 ^{ns}	66.50±7.14 ^{ns}	233.5±7.14 ^{ns}
FXT (1 mg/kg)	247.1±5.90 ^{***}	52.9±5.90 ^{***}	223.4±3.45 ^{***}	76.6±3.45 ^{***}
<i>B. mori</i> (50 mg/kg)	170.2±4.11 [*]	129.8±4.11 [*]	101.2±8.0 ^{ns}	198.8±8.0 ^{ns}
<i>B. mori</i> (100 mg/kg)	199.3±4.32 ^{**}	100.7±4.32 ^{**}	149.1±6.73 [*]	150.9±6.73 [*]
<i>B. mori</i> (200 mg/kg)	231.4±3.92 ^{***}	68.6±3.92 ^{***}	165.0±5.99 ^{**}	135.0±5.99 ^{**}

B. mori: *Bombyx mori*, FXT: Fluoxetine, N/S: Normal saline, ns: Not significant. If the results are $P < 0.05$, they are considered notable (*), $P < 0.01$ indicates more significant (**), and $P < 0.001$ indicates extraordinarily significant (***)

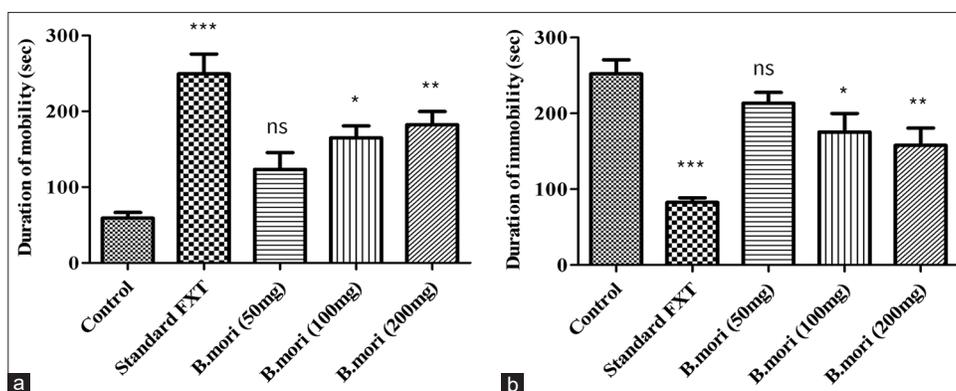


Figure 4: Tail suspension model. (a) Duration of mobility in (sec) compared with control and standard FXT with different doses of *B. mori*. (b) Duration of immobility in (sec) with control and standard FXT with different doses of *B. mori*. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns means non-significant. *B. mori*: *Bombyx mori*, FXT: Fluoxetine.

of *B. mori* is a considerable source of natural antioxidants that can reduce anxiety. In the present research, hydro-alcoholic extract of *B. mori* was shown to have an anxiolytic effect in the EPMT and OFT. Several plants that are utilized in folk medicine to reduce anxiety are accounted to create a rise in the investigation of the open-arms in the EPMT, while the locomotor activity of the mice was examined using the OFT.^[32] Raised T maze pharmacological research verifies that the avoidance of open-arm and escape behaviors are expressions of emotional situations similar to the comprehensive panic and anxiety disorder, correspondingly.^[33] In the OFT, *B. mori* amplifies the number of lines crossing in addition to several rearing in dose-reliant design. The effects of a 200 mg/kg dose of *B. mori* extract were comparable to those of DZP. In the elevated plus maze test, *B. mori* enhanced the time spent in open arms while decreasing the time spent in closed arms in a dosage-dependent way.

According to the EPM Model, *B. mori* (200 mg/kg) exhibited anxiolytic properties similar to DZP. DZP as a standard considerably augmented the number of line crossings in open fields along with the number of rearing ($P < 0.001$). The researched plant was ascertained to be comparably competent and better than placebo in reducing the symptoms. Statistically, considerable improvement was detected in the experimental group ($P < 0.001$) in anxiety. However, little considerable progress was identified in the anxiety of the placebo group. In this study, the potentiated effect of *B. mori* extract on anxiety and depression was studied using the forced swim model and tail suspension models. The results showed that *B. mori* reduced immobility time and increased mobility time in a dose-dependent manner. A dose of 200 mg/kg of *B. mori* had a strong antidepressant effect ($P < 0.001$) equivalent to that of FXT. The forced swim and tail suspension model results showed *B. mori* possess considerable antidepressant potential. The antidepressant effect of the extracts may be due to an inhibitory action on monoamine oxidase-A. As a result of selective serotonin reuptake inhibition, FXT demonstrated substantial antidepressant potential. In the tail suspension model, 200mg/kg of *B. mori* extract was significantly more significant ($P < 0.01$) than FXT. Our findings are comparable with a previous study reported by Xu *et al.*, in which antidepressant potential of curcumin supplement (1.25-10 mg/kg, po for 14 days) exhibited significant improvement in the open field essays. In addition, immobility time was decreased by curcumin treatment in the forced swimming test by improving the noradrenaline, bilateral olfactory bulbectomy-induced alteration of 5-HT, and dopamine in the hippocampus.^[34] Hyperactivation of the HPA axis in mice model reported by Bhutada *et al.*, in a pre-clinical study by the induction of quercetin (20-40 mg/kg, po), resulting the prevention of depression-like behaviors comparing with FXT (10-20 mg/kg, ip) also

demonstrated the significant importance of polyphenolic flavonoids for the management of depressive disorders.^[35] Our results are comparable with another previous report in which fruit of *Nelumbo nucifera* (Nelumbinis semen) was prescribed in a rat model exhibiting antidepressant potential through reversing a decrease in 5-HT1A receptor binding. Rearing number, start latency, and grooming time was significantly improved while visiting counts were decreased by chronic mild stress.^[36]

In a previous report, the neuroprotective activity was restored by silk fibroin in a traumatic brain injury model of rats by reverting the neurological status up to 25% after 4 days of injection indicating the growth of primary neuronal cells and astrocytes.^[37] Tyrosinases inhibition has been reported by silk sericin which may leads to protection of several psychological disorders such as in Parkinson's disease, hydroxylation is caused by overexpression of tyrosinases, resulting in the reduction in dopamine content. It further causes the neuronal cell death by increasing the oxidative stress.^[38] Dopaminergic neurons have also been reported to be preserved by silk fibroin peptides in a Parkinson's disease model by the effects of 6-hydroxydopamine neurotoxicity.^[39] Aqueous extract of silkworm cocoon syrup was prescribed in a previous trial to the patients suffering from anxiety and depressive disorder exhibiting a reduction in anxiety and depression scores in weeks 6 and week 12 comparable with standard group ($P < 0.001$).^[40] In a nutshell, our current research revealed that *B. mori* possess significant anxiolytic and anti-depressant activities. Further pharmacological and chemical investigations of the extract are required to segregate and differentiate the active constituents responsible for these effects of *B. mori*.

Limitations and future directions

Hydroalcoholic extract of *B. mori* showed possible anxiolytic and depressive effects using the EPMM and the OFT but it is needed to be further validate these effects by more diverse methods to analyze the anxiolytic and depressive activities. Furthermore, the mechanism of action of *B. mori* and biochemical estimations of specific neurotransmitters related to anxiety and depressant effects are needed to be evaluated in future studies to utilize it in the management of anxiety and depression.

CONCLUSION

The hydroalcoholic extract of *B. mori* showed promising anxiolytic activity using the EPMT and the OFT, while the antidepressant activity was confirmed using the TST and the forced swim test. Overall, this study demonstrates the therapeutic potential of *B. mori* extract in the management of anxiety and depression, providing a promising route for

further research and potential clinical applications.

Authors' contributions: AH and MRA: Designed the study, performed experiments, and prepared initial draft of manuscript; M, AK, and MK: Organized and interpreted data; SAZ, MSN, and WA: Helped performed data analysis; HMA: Supervised, reviewed, and approved final manuscript.

Ethical approval: The research was conducted under guidelines approved by the Islamia University of Bahawalpur's Institutional Animal Ethics Committee.

Declaration of patient consent: Patient's consent is not required as patients identity is not disclosed or compromised.

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Conflicts of interest: There are no conflicts of interest.

Availability of data and material: All data and material are presented in this article and nothing submitted to any other directory and will be provided on request to corresponding author.

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