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Neuropharmacological Evaluation of the Mangrove Plant *Xylocarpus granatum* J. Koenig Fruit Extract: Anxiolytic-Like and Sedative Profile in Murine Models

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Abstract: The fruit extract of *Xylocarpus granatum* J. Koenig (Meliaceae) is rich in bioactive phytoconstituents with documented pharmacological activities, but its precise role in modulating neurological disorders remains insufficiently understood. The present study was designed to evaluate the anxiolytic-like and sedative potentials of *X. granatum* fruit extract (XGFE) using established *in vivo* experimental models. To assess behavioral, locomotor, and exploratory parameters associated with anxiolytic-like activity, Swiss albino mice were divided into experimental groups and treated with diazepam (1 mg/kg), flumazenil (0.1 mg/kg), and XGFE (250 and 500 mg/kg) across four distinct behavioral paradigms (the open-field, hole-cross, swing, and light-dark tests). Sedative activity was subsequently assessed using the thiopental sodium-induced sleeping test to measure sleep latency and duration. Treatment with XGFE (250 and 500 mg/kg) elicited significant ($p < 0.05$) decreases in locomotor and exploratory measures, thereby indicating an anxiolytic-like calming effect. In addition, XGFE (500 mg/kg) reduced sleep latency to 19.2 min (NC: 30.4 min) and prolonged sleep duration to 147.2 min (NC: 47.2 min), indicating a potential sedative effect in mice. Moreover, co-administration of XGFE alongside diazepam (DZP) at 3 mg/kg prolonged the duration of CNS-depressant activity (177 min). Thus, the present findings indicate that XGFE exhibits anxiolytic-like and sedative effects, suggesting its possible utility as a naturally derived therapeutic candidate for neurological management.

Keywords: *Xylocarpus granatum*; anxiolytic; sedative; $GABA_A$

1. Introduction

Insomnia ranks among the most common sleep disorders, marked by ongoing trouble falling asleep, staying asleep, or waking unrefreshed, and it is usually treated with behavioral approaches or sedative medications [1] Around the world, sleep problems impact as much as 45% of people, and conditions such as insomnia and sleep apnea play a major role in systemic health issues, among them cardiovascular disease, diabetes, and depression, while also creating heavy socioeconomic costs through reduced productivity and accidents [2,3]. Additionally, anxiety, an enduring and disproportionate emotional reaction to stress that interferes with everyday functioning, is

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tightly bound up with disrupted sleep [4,5]. Roughly 301 million people across the globe live with anxiety disorders, a figure that surged by 55% from 1990 to 2019, and women are affected more often [6].

From a clinical standpoint, the two conditions share an intricate two-way relationship: anxiety intensifies sleep problems, while ongoing sleep loss aggravates anxious states [7]. Although standard medical sedation and anti-anxiety treatments, including benzodiazepines, barbiturates, and assorted psychotropic drugs, are commonly prescribed, their extended use tends to be constrained by adverse effects, tolerance, and dependence [8]. Moreover, a range of physiological influences, metabolic disturbances, and environmental stressors can set off or imitate both conditions [9–11]. Such drawbacks highlight a pressing and expanding need to investigate natural remedies drawn from botanical sources that are safe, effective, and well tolerated for managing anxiety and sleep disorders.

Herbal medicine has been an integral component of the health care system of the world for hundreds of years. It includes a vast range of plant materials containing numerous different bioactive phytochemicals, which are effective as therapeutics. Herbal medicines are selected because of their variable safety profiles compared to synthetic drugs. The Sundarbans is one of the world's largest mangrove forests and it has a diverse number of medicinal plants. Due to the Sundarbans' unique and inhospitable environment, the plants of this region acquire various morphological and physiological adaptations that may explain the presence of diverse array of bioactive compounds [12]. The plants may respond to multiple abiotic factors, stresses, and the attacks from biotic factors by producing different new metabolites, such as phenolics, polyketides, alkaloids, terpenes, steroids, fatty acids, and carbohydrate derivatives with protective and medicinal functions. The defensive chemicals present in the plants are responsible for their curative potential against various pathogenic events including pain, inflammation, diarrhea, diabetes, infections, neurodegeneration, hepatic disorders, and cancer [13–16].

One of the Sundarbans' plant species is *Xylocarpus granatum* (locally known as "Dhundal" in Bengali, family: Meliaceae), which is used traditionally to treat inflammatory, infectious diseases and immunological disorders. *X. granatum* grows abundantly in the saline and muddy intertidal flats of the Sundarbans mangrove forest. This harsh environment may lead to the production of secondary metabolites such as flavonoids, glycosides, fatty acids, alkaloids, terpenoids, and phenolics by *X. granatum* that are responsible for its diverse pharmacological potential [17]. However, the medicinal properties of *X. granatum* against various stress- related diseases such as anxiety, insomnia and other related mental disorders have not been investigated yet. Given the limited scientific studies on *X. granatum*, particularly regarding its potential anxiolytic-like and sedative effects, this study highlights the need for more focused research on various stress- related mental disorders and other associated ailments.

2. Materials and Methods

2.1. Collection of Plant Material

In March 2023, fresh and healthy fruits of the *X. granatum* J. Koenig plant were gathered from Karamjal Eco-Tourism Center, Bangladesh, a location recognized as one of the entry points to the Sundarbans, the world's largest mangrove wildlife sanctuary. Moreover, experts at the Bangladesh National Herbarium in Dhaka carried out the authentication of the plant material and provided a voucher specimen (DACB 12789) for reference.

2.2. Preparation of Plant Extract

Once the collected pericarps of the fruits had been washed thoroughly with fresh water, they were set out to shade-dry for several days. The desiccated pericarps were first cut into smaller bits, and then milled into a coarse powder with the help of a specialized laboratory grinding apparatus. In an amber glass reagent bottle, 400 g of the pulverized fruits was steeped in 800 mL of ethanol over 15 days, with manual agitation and stirring. The mixture was first passed through clean, white cotton for coarse filtration, and then filtered through Whatman filter paper. The filtrate was evaporated under reduced pressure at 50 °C in a rotary evaporator, yielding a dark brown semisolid extract (XGFE). The extract was stored in an airtight vial to await further analysis. The obtained XGFE yield value was 15.66%.

2.3. Chemical and Reagent Sources

Ethanol at a concentration of 95% was procured from Loba Chemie Pvt. Ltd. (Mumbai, India). Furthermore, diazepam (DZP) was supplied by Opsonin Pharmaceuticals Limited, Bangladesh, whereas flumazenil (FLU) came from Akshar Pharma, Surat, India. Additionally, thiopental sodium was procured from Incepta Pharmaceuticals Limited, Bangladesh.

2.4. Dose Selection and Preparation

The doses of XGFE (250 and 500 mg/kg, p.o.) were selected based on previous pharmacological studies of *X. granatum*. One study reported pharmacological activity of the fruit extract at 200, 400, and 800 mg/kg [18]. Additionally, another study reported no observable toxicity or mortality following oral administration of *X. granatum* bark extract at doses up to 1000 mg/kg in mice [19]. These findings provided a preliminary basis for dose selection. Before administration, XGFE was suspended in the vehicle (0.05% Tween 80 in 0.9% NaCl solution). The suspensions were prepared according to individual body weights and administered orally at 250 or 500 mg/kg, 30 min before the experiments. The reference drugs, diazepam (DZP) and flumazenil (FLU), were prepared accordingly by suspending them in the same vehicle (0.05% Tween 80 + 0.9% NaCl solution) prior to administration.

2.5. Animals Used in the Experiment

Male Swiss albino mice, each weighing 25 to 30 g, were procured from the animal house of Khulna University and housed in the Pharmacology Laboratory of the Pharmacy Department, Khulna University, Khulna 9208, Bangladesh. The animals were given unrestricted access to food and water and were maintained under standard laboratory conditions. Environmental conditions were sustained at 27 ± 2 °C under a 12-h light/dark cycle.

2.6. In Vivo Anxiolytic-Like Study

Before testing to evaluate their anxiolytic-like activity, the animals used in this experiment were subjected to a 6-h fasting period. Afterwards, the test animals were randomly assigned to different treatment groups, with five animals in each group. The rodents were split into eight groups, which were designated Gr.-I through Gr.-VIII (Table 1). The doses of the plant extracts and control medications were modified in line with the weight of each mouse. Stock suspensions of XGFE, DZP-1, and FLU-0.1 were suspended in the vehicle with the assistance of Tween 80. The control group (NC) received a dose of 10 mL/kg of the vehicle (Gr.-I). The second experimental group (Gr.-II) received a dose of 1 mg/kg of DZP administered intraperitoneally. The third experimental group (Gr.-III) received a dose of 0.1 mg/kg of FLU administered intraperitoneally. Next, the following two groups (Gr.-IV & Gr.-V) received XGFE at the doses of 250 and 500 mg/kg, respectively. All the treatments were given to the animals 30 min prior to the studies. Furthermore, XGFE at the dose of 500 mg/kg was combined with DZP to administer to Gr.-VI while flumazenil (FLU) was administered with XGFE-500 in Gr.-VII. Additionally, flumazenil (FLU) was administered in Gr.-VIII with diazepam (DZP) and XGFE at a dose of 500 mg/kg.

Table 1. Treatment protocol for anxiolytic-like activity.

Treatment Groups	Description	Administration Design
NC	Vehicle (0.05% Tween 80 + 0.9% NaCl solution) at 10 mL/kg	At a time
DZP-1	Diazepam (positive allosteric modulator of $GABA_A$ receptors) at 1 mg/kg (i.p.)	
FLU-0.1	Flumazenil (benzodiazepine-site antagonist) at 0.1 mg/kg (i.p.)	
XGFE-250	XGFE (test sample) at 250 mg/kg (p.o.)	
XGFE-500	XGFE (test sample) at 500 mg/kg (p.o.)	
XGFE-500 + DZP	XGFE at 500 mg/kg (p.o.) + Diazepam at 1 mg/kg (i.p.)	One followed by another
XGFE-500 + FLU	XGFE at 500 mg/kg (p.o.) + Flumazenil at 0.1 mg/kg (i.p.)	
XGFE-500 + FLU + DZP	XGFE at 500 mg/kg (p.o.) + Flumazenil at 0.1 mg/kg (i.p.) + Diazepam at 1 mg/kg (i.p.)	

NC: Negative control (Vehicle; (0.05% Tween 80 + 0.9% NaCl solution)); DZP: Diazepam; FLU: Flumazenil; i.p.: Intraperitoneal; p.o.: Per oral.

2.6.1. Open-Field Test

To assess the animals' exploratory and locomotor activity across a five-minute period, a wooden apparatus featuring a square floor painted in black and white (30 × 30 × 30 cm) was employed in the open-field test [20]. For every mouse, the grooming activity, rearing activity, and number of square crossed were recorded. After each session, the apparatus was thoroughly cleansed with 95% ethanol.

2.6.2. Hole cross Test

For the hole cross test, a wooden box (30 × 20 × 14 cm) divided by a partition containing a 3-cm-diameter hole in its lower center was utilized. After 3 min of the open-field test, each mouse was placed on one side of the apparatus, and the number of crossings through the hole was recorded over a five-minute period. Following each experiment, the equipment was thoroughly cleaned with 95% ethanol [21].

2.6.3. Swing Test

The swing test was performed following the procedure reported by previous study [20]. After 3 min of Hole cross test, each mouse was placed into the swing apparatus, and the number of swings produced by the mice's spontaneous movements was recorded over five minutes. After each test, the apparatus floor was washed with 95% ethyl alcohol.

2.6.4. Light-Dark Test

As reported by previous study, the light-dark apparatus consisted of two equally sized (27 × 18 × 29 cm) wooden compartments joined by a small opening [22]. The dark compartment was enclosed and kept dark, whereas the light chamber was brightly illuminated. After three minutes of swing test, each mouse was placed in the light compartment, and a stopwatch was used to measure the time each mouse spent in the illuminated area (referred to as light residence time, or LRT) throughout a 5-min observation session.

2.7. In Vivo Sedative Activity

This investigation was carried out using thiopental sodium-induced sleep time model outlined by previous investigator, with minor adjustments [23]. Five groups (Gr.-I through V) were each assigned five randomly selected test animals, and the corresponding groups then received their previously prepared medications (Table 2). Half an hour after the treatments, sodium thiopental (20 mg/kg, i.p.) was given to every animal in the five groups (Gr.-I to Gr.-V) in order to induce sleep before they were placed in an observation chamber, such as a plastic cage [24]. Once sodium thiopental had been administered, the interval until the righting reflex was lost was recorded as the latent time for sleep induction. Total sleeping time was defined as the duration of sleep, that is, the period between the loss and the recovery of the reflex. Moreover, calculations were made for the modulation (increase or decrease) in latency or sleep duration.

Table 2. Treatment strategies for sedative activity.

Treatment Group	Description
NC	Vehicle (0.05% Tween 80 + 0.9% NaCl solution) at 10 mL/kg
DZP-3	Diazepam (positive allosteric modulator of $GABA_A$ receptors) at 3 mg/kg (i.p.)
XGFE-250	XGFE (test sample) at 250 mg/kg (p.o.)
XGFE-500	XGFE (test sample) at 500 mg/kg (p.o.)
XGFE-500 + DZP	XGFE at 500 mg/kg (p.o.) + Diazepam at 3 mg/kg (i.p.)

NC: Negative control (Vehicle; (0.05% Tween 80 + 0.9% NaCl solution)); DZP: Diazepam; i.p.: Intraperitoneal; p.o.: Per oral.

2.8. Statistical Analysis

Data are presented as mean ± SEM (standard error of the mean). Differences among the experimental groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple-comparison test. Statistical analyses were performed using GraphPad Prism version 10.4.2 (633) (GraphPad Software, LLC, released 30 March 2025). A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Anxiolytic-Like Activity

The results demonstrate that, within the open-field paradigm (Figure 1A–C), the evaluation covers the count of square crossed, grooming bouts, and rearing behaviors in both control and test groups. For the Negative Control (NC) group, the recorded values are 115.20 ± 1.77 for square crossed, 8.40 ± 1.08 for grooming bouts, and 24.40 ± 0.93 for rearing. By contrast, flumazenil (FLU), a benzodiazepine-site antagonist, displays greater measurements: 131.60 ± 1.33 for square crossed, 9.20 ± 0.73 for grooming, and 27.20 ± 0.86 for rearing. Diazepam ($GABA_A$ receptor positive allosteric modulator), on the other hand, yields lower values than both the NC and the flumazenil, measuring 74.60 ± 1.75 for square crossed, 1.40 ± 0.24 for grooming, and 11.40 ± 1.78 for rearing.

The measurements obtained for XGFE-250 and XGFE-500 are greater than those recorded for DZP, yet remain lower than the corresponding values for FLU and NC; specifically, XGFE-250 exhibits 93.20 ± 0.58 square crossed, 4.60 ± 0.24 grooming, and 15 ± 1.22 rearing, whereas XGFE-500 exhibits 91.40 ± 1.96 square crossed, 4.20 ± 0.20 grooming, and 12.40 ± 2.06 rearing. Additionally, the XGFE-500 + DZP condition exhibits 54.00 ± 1.14 square crossed, 1.20 ± 0.20 grooming, and 7.40 ± 1.21 rearing, thereby registering values beneath those of NC, FLU, DZP, and the extract administered alone. Conversely, XGFE-500 + FLU exhibits 126.00 ± 0.71 square

crossed, 5.40 ± 0.24 grooming, and 16.20 ± 1.39 rearing, which suggests it may exert intrinsic behavioral effects in relation to the DZP combination, while the triple combination XGFE-500 + FLU + DZP exhibits 96.00 ± 2.35 square crossed, 3.40 ± 0.24 grooming, and 13.40 ± 2.06 rearing, denoting a modulatory or intermediate behavioral result in which the introduction of flumazenil partially modifies the suppressive effects produced by the diazepam and extract combination.

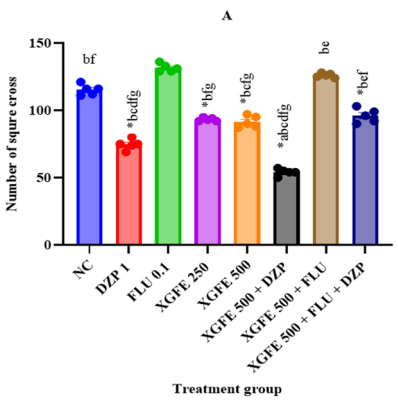
Drawing on the hole cross and swing investigations, the behavior of Swiss mice in response to the standard and test samples points to variation across the different tests. NC displays higher values except relative to FLU, recording 6.40 ± 0.87 for hole cross and 27.00 ± 1.58 swings. Conversely, FLU displays higher values than the rest, registering 8.40 ± 0.24 for hole cross and 34.00 ± 0.71 swings. DZP shows markedly lower values for holes crossed (3.40 ± 0.51) and swings (9.00 ± 1.14) when compared with NC. XGFE-250 records 3.60 ± 1.21 hole cross and 25.20 ± 0.92 swings, whereas XGFE-500 displays a further decrease, with 2.60 ± 1.08 hole cross and 21.60 ± 1.69 swings. In addition to this, the XGFE-500 + DZP combination yields values that are even lower (1.60 ± 1.12 hole cross and 15.80 ± 1.39 swings). Conversely, co-administration of XGFE-500 with flumazenil produced changes in hole-crossing and swinging behaviors (5.00 ± 1.58 and 26.20 ± 1.16, respectively). However, these findings do not establish a definitive reversal of the depressant effect, suggesting that the behavioral interaction between XGFE and flumazenil requires further investigation. Additionally, the triple combination of XGFE-500 + FLU + DZP shows 16.80 ±1.39 for swings and 2.60 ± 1.47 for the number of crossings through the holes (Figure 1E,F).

The DZP group exhibited a significantly longer light residence time (LRT) than the NC group ($p < 0.05$), with values of 144.8 ± 0.66 s and 73 ± 1.73 s, respectively. XGFE-250 and XGFE-500 also significantly increased LRT relative to both the NC and FLU groups ($p < 0.05$), although the effect was less pronounced than that of DZP. FLU alone produced an LRT of 63 ± 6.13 s, whereas XGFE-250 and XGFE-500 yielded 95.6 ± 2.06 s and 120.6 ± 7.93 s, respectively (Figure 1D). In co-administration treatments, XGFE-500 + DZP produced the longest LRT (164 ± 2.07 s), while XGFE-500 + FLU resulted in a shorter LRT of 83.2 ± 1.53 s. Thus, FLU administered alone reduced residence time in the light compartment, whereas co-administration with DZP increased it.

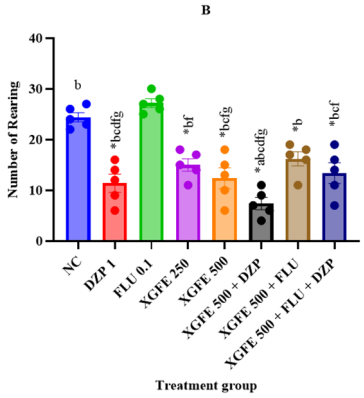
3.2. Sedative Activity

As reported in this study, animals that received the vehicle (NC) exhibited a delayed latency relative to the others, with a latency time of 30.4 ± 1.43 min. Our standard DZP-3 mg/kg produces a significantly ($p < 0.05$) shortened latency time relative to NC, which stands at 11.6 ± 1.07 min. Our two test extracts, XGFE-250 and XGFE-500, significantly ($p < 0.05$) shorten the latency time relative to NC. The latency times recorded for XGFE-250 and XGFE-500 are 24.6 ± 0.92 min and 19.2 ± 1.39 min, respectively. In comparison with the group given DZP alone, the animals receiving the combination (XGFE-500 + DZP) displayed the shortest latency (9.4 ± 0.59 min). Figure 2A provides a graphical representation of the latency times across the various groups.

Moreover, Figure 2B presents the results of a separate investigation into sleeping time, measured in minutes, across the different treatment groups of animals. The vehicle group (NC) exhibits the shortest sleeping time, at 47.2 ± 2.12 min. Standard drug diazepam produced longer sleep duration of 166.8 ± 4.32 min. Both test extracts, XGFE-250 and XGFE-500, produced a significant ($p < 0.05$) prolongation of sleep duration (106.4 ± 1.77 min and 147.2 ± 13.73 min respectively), yet this remains below that of DZP, suggesting that XGFE might act as a sedative agent. Under co-administration therapy, XGFE-500 + DZP demonstrated a significantly ($p < 0.05$) longer sleep duration than the others, with a value of 177 ± 4.38 min.

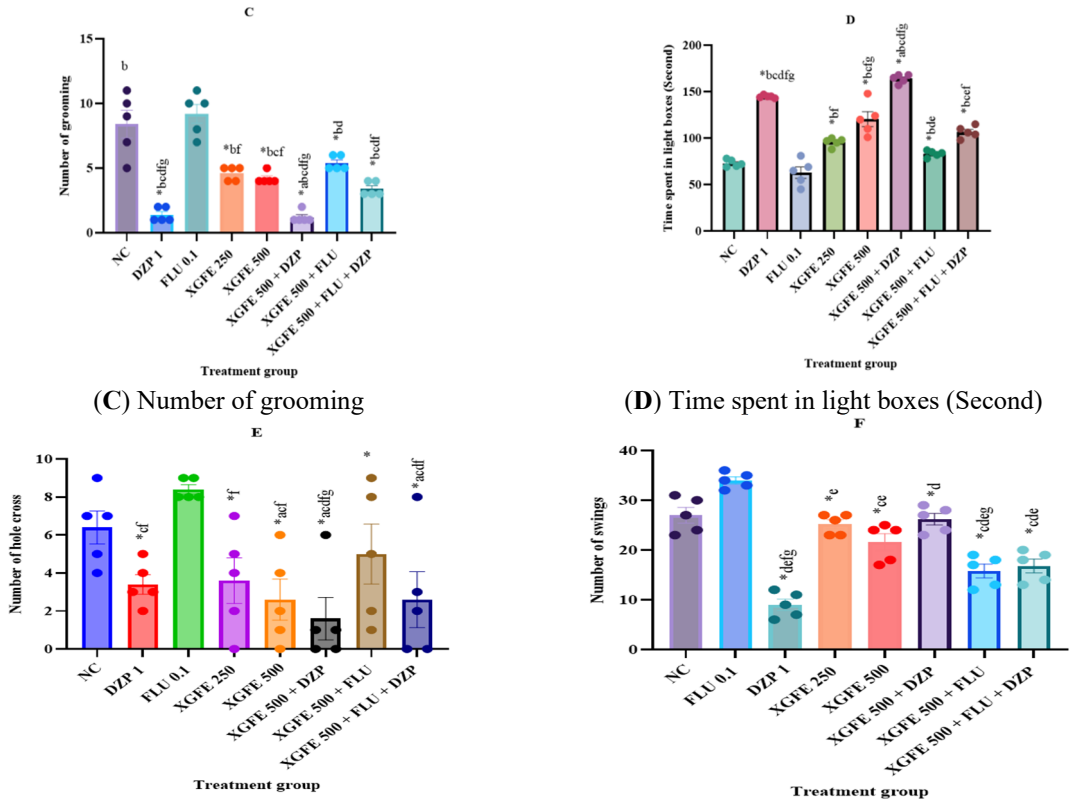


(**A**) Number of square crossed



(**B**) Number of rearing

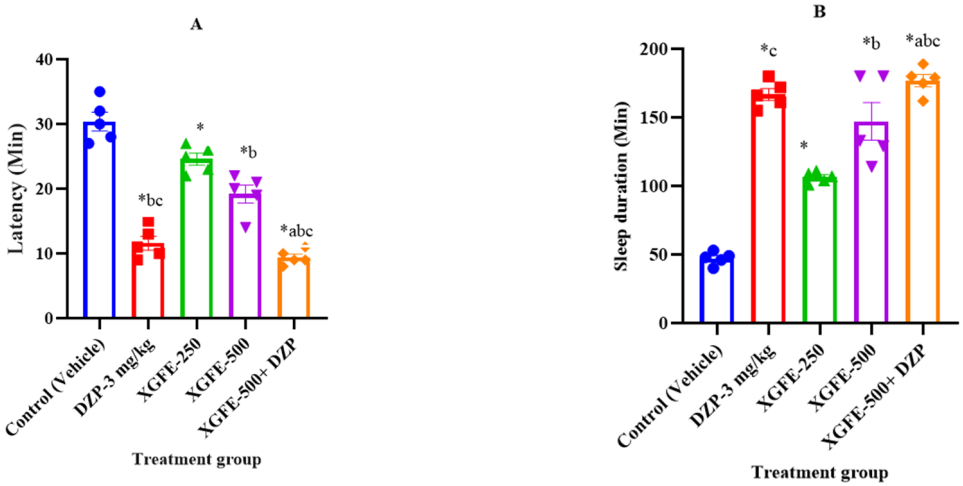
Figure 1. *Cont.*



(**C**) Number of grooming (**D**) Time spent in light boxes (Second)

(**E**) Number of hole cross (**F**) Number of swings

Figure 1. Anxiolytic activity of ethanolic extract of *X. granatum* J. Koenig fruits. [The Mean ± SEM (n = 5), ANOVA one-way and the Tukey post hoc test are the values; $p < 0.05$ in comparison to the * Gr.-I: NC, [a] Gr.-II: DZP (1 mg/kg), [b] Gr.-III: FLU (0.1 mg/kg), [c] Gr.-IV: XGFE-250, [d] Gr.-V: XGFE-500, [e] Gr.-VI: XGFE-500 + DZP, [f] Gr.-VII: XGFE-500 + FLU, [g] Gr-VIII: XGFE-500 + FLU + DZP Where (**A**) Number of square crossed; (**B**) Number of rearing; (**C**) Number of grooming; (**D**) Time spent in light boxes; (**E**) Number of holecross; (**F**) Number of swings; (SEM: Standard Error of the Mean, NC: Negative Control)].



(**A**) Latency (Min) (**B**) Sleep duration (Min)

Figure 2. Sedative activity of ethanolic extract of *X. granatum* J. Koenig fruits (**A**) Latency to sleep onset (time taken to fall asleep, min); (**B**) total sleep duration (min). Values are expressed as Mean ± SEM (n = 5), ANOVA one-way and the Tukey post hoc test are the values; $p < 0.05$ in comparison to the * Gr.-I: NC, [a] Gr.-II: DZP (3 mg/kg), [b] Gr.-III: XGFE-250, [c] Gr.-IV: XGFE-500, [d] Gr.-V: XGFE-500 + DZP, (SEM: Standard Error of the Mean, NC: Negative Control).

4. Discussion

Anxiety disorders are highly prevalent and an estimated 70% of people with an anxiety disorder also have a chronic comorbid condition that makes the burden on health more significant and quality of life and daily functioning poorer [25]. Pharmacological first-line treatments of anxiety (benzodiazepines, SSRIs, serotonin-norepinephrine

reuptake inhibitors (SNRIs)) work well for many people while about 1 in 3 do not respond well [26,27]. Benzodiazepines, especially, have been found to have significant side effects such as fatigue, muscle twitching, cognitive impairment and dependence [28]. Insomnia is another common and significant public health problem with an estimated prevalence of 16.2% in the world's population [29]. Benzodiazepines and Z-drugs are still the most frequently prescribed agents for their sedative-hypnotic effects [30]; however, both classes carry risks of tolerance and dependence. The restrictions highlight the need for safer and effective alternative anxiolytic and sedative agents. This has led to the growing interest in traditional medicines from plants due to their relative lower side-effect profiles and low cost [31].

X. granatum J. Koenig is a mangrove species with a high content of bioactive secondary metabolites and traditional medicinal applications. It contains chemical composition such as limonoids, phragmalins, mexanicolides, protolimonoids, flavonols, lactones and alkaloids [32]. One study isolated and identified ten compounds from the fruit extract: spicatin, xyloccensin K, 6-acetoxycedrodorin, aurantiamide acetate, (+)-catechin, α-tocopherol, abscisic acid, daucosterol, 4-hydroxybenzoic acid, and ethyl 3,4-dihydroxybenzoate [33]. The limonoids found in the fruit have significant neuroprotective properties [34,35]. The fruit possesses at least 17 limonoids, with xylocarpin H and cipadesin A being the two compounds that have been shown to have measurable effects on the central-nervous-system function in mice to date [34,36]. Furthermore, *X. granatum* fruit derived xylocarpin H and cipadesin A, alongside phenolic compounds are plausible candidates for the sedative activity, given that structurally related compounds have demonstrated neuroprotective and CNS-depressant properties in experimental models. According to a different study, using the hole cross and open field test models in mice, the crude extract from the stem bark of *X. granatum* showed mild to moderate central nervous system depressive action [37]. The pneumatophore extract of the same genus, *Xylocarpus moluccensis*, is more effective than the bark extract at depressing the central nervous system [38]. Bark extract from the same family, *Trichilia catigua* (Meliaceae), has demonstrated neuroactive and neuroprotective properties [39]. These findings highlight the pharmaceutical significance of the flora within this family. Even more compellingly, laboratory studies show that the fruit extract of *X. granatum* contains important compounds such as α-tocopherol and (+)-catechin, both of which have been shown to be potential contributors as $GABA_A$ receptors modulator in animals, thereby reducing anxiety [36,40]. Moreover, the effects of phenolic constituents, especially flavonoids, have been shown to alter GABAergic transmission by enhancing activation of $GABA_A$ receptors, which may lead to sedative and anxiolytic-like properties. In combination, these phytochemicals offer a plausible mechanism of action to support the CNS-depressant effects of this study. The current *in vivo* results corroborate the anxiolytic-like potential of XGFE and suggest that this plant can serve as a source of key GABAergic compounds that can be isolated by bioactivity-guided extraction. The outcome is compatible with the recent reports that are reported to be neurological [41–43].

GABA (γ-aminobutyric acid) is the major inhibitory neurotransmitter in the central nervous system. The activation of $GABA_A$ receptors opens up the chloride ion channel, which causes hyperpolarization and decreases the excitability of the neuron. GABAergic enhancement can thus be used as a treatment for sedative and anxiolytic effects, potentially resulting in a reduction of excessive neuronal activity [44,45]. To clarify our rationale, established preclinical and molecular evidence demonstrates that these central nervous system responses operate along a distinct pharmacological window governed by specific $GABA_A$ receptor subtypes. Mechanistically, anxiolysis is primarily mediated by α2 and α3-containing $GABA_A$ receptors concentrated within emotional and limbic circuits, whereas sedation and motor impairment are predominantly driven by α1-containing $GABA_A$ receptors distributed across the cortex and brainstem [46]. Because lower drug concentrations selectively engage these higher-affinity limbic pathways before triggering widespread cortical depression, anxiolytic-like calming effects can be functionally distinguished from generalized sedation. Behaviorally, work by another study confirmed this separation by showing that $GABA_A$ modulators produce clear anxiolytic-like efficacy at lower doses without suppressing baseline locomotor activity or impairing motor output [47]. In our study design, we intentionally included diazepam at 1 mg/kg (DZP 1) to serve as an internal control for anxiolytic activity (engaging α2 and α3 pathways while maintaining normal motor function) and diazepam at 3 mg/kg (DZP 3) as a benchmark for sedative-motor impairment (recruiting broader α1-mediated central depression). This classic dose titration allows us to reliably differentiate genuine anxiolytic-like responses from non-specific sedative or motor-depressant artifacts in test extracts.

Standardized rodent tests of anxiety-like behavior (open field test, hole-cross test, swing test, and light/dark residence-test) measure decreased mobility and dark space avoidance [48]. In the present study, XGFE significantly decreased the number of square crossed, grooming episodes, rearing events, hole crossings, and swing counts as compared to the negative control group and increased the amount of time spent in the light chamber of the LDT. A reduction in exploratory locomotion is seen with diazepam (DZP), a positive allosteric modulator of $GABA_A$ receptors which increases chloride influx, hyperpolarizes neuronal membranes and decreases arousal in

cortical and limbic circuits [49,50]. The 500 mg/kg dose of XGFE demonstrated anxiolytic-like effects, although its effects were not necessarily comparable to those of diazepam (DZP). In addition, co-administration of XGFE-500 with DZP resulted in a potentiation of all behavioral endpoints, including a significantly prolonged residence time in the light chamber (164 s). These effects were attenuated by flumazenil (FLU), an antagonist at the benzodiazepine site of $GABA_A$ receptors, possibly supporting the involvement of the GABAergic pathway, most probably through α2/α3 subunit containing receptors that mediate anxiolysis. When comparing the effects across different treatment groups, XGFE reduced sleep latency and increased total sleep time in the thiopental sodium-induced sleep test compared to the control, likely operating via a GABAergic mechanism. Latency declined to 24.6 min and 19.2 min at 250 and 500 mg/kg, respectively, while sleep duration increased to 106.4 min and 147.2 min. Compared with the control groups, these effects followed a trend similar to that of DZP, which increases chloride conductance through $GABA_A$ receptors [24,51]. The agents that decrease sleep latency and increase sleep time in the thiopental sodium induced sleep model are typically thought to interact with the α1 subunits of $GABA_A$ receptors responsible for sedative-hypnotic effects [52,53]. The combination of XGFE-500 and DZP resulted in the shortest latency (9.4 min) and longest sleep duration (177 min) suggesting a possible potentiation at the $GABA_A$ receptor complex. The present sleep data agree with that obtained in the previous studies using related plant extracts [52,54,55].

Collectively, behavioral and pharmacological data suggest that XGFE has sedative and anxiolytic-like calming properties that feasibly involve at least in part modulation of $GABA_A$ receptors. The extract can thus be considered a natural source for isolation of the active compounds and for the assessment as a safer alternative or adjunct to conventional central nervous system modulators. The *in vivo* sedative findings indicate that XGFE possesses CNS-depressant activity, although the underlying mechanism remains unclear. While GABAergic modulation may be one possible mechanism, further investigations are required to establish its involvement. These findings provide a rationale for future fractionation to isolate and characterize the constituents responsible for the observed anxiolytic-like and sedative effects. A schematic overview of the experimental design and proposed GABAergic mechanism underlying the anxiolytic and sedative effects of XGFE is presented in Figure 3.

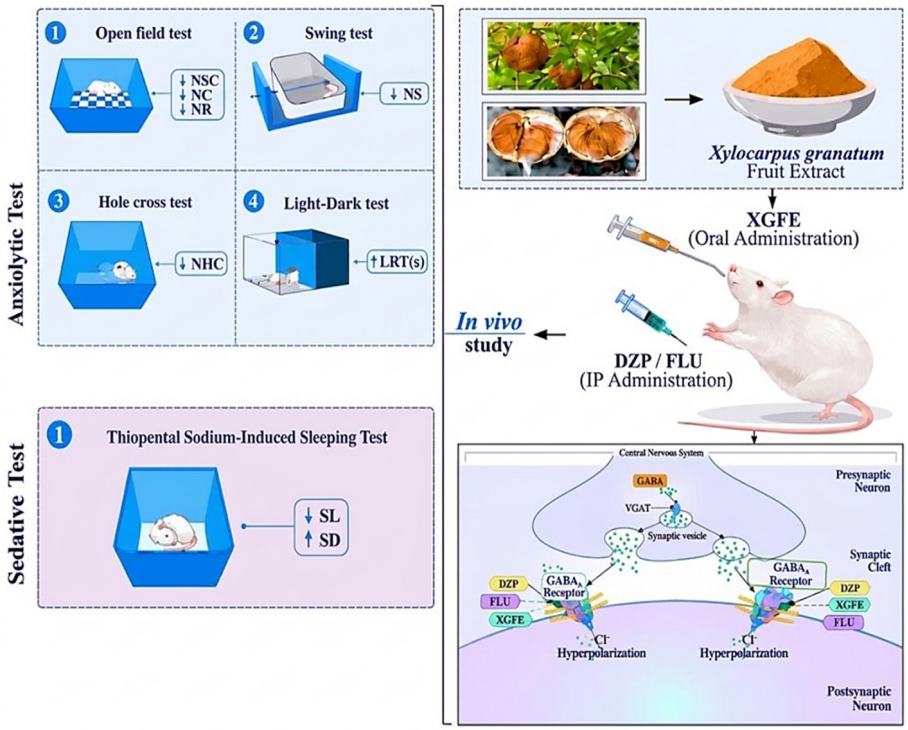


Figure 3. Schematic representation of the experimental design and proposed mechanism of action of *X. granatum* fruit extract (XGFE) in evaluating *in vivo* anxiolytic and sedative activities. Behavioral evaluations included the open field test (number of square crossed (NSC), groomings (NG), and rearings (NR)), swing test (number of swings (NS)), hole cross test (number of hole crossings (NHC)), light-dark test (light residence time (LRT)), and thiopental sodium-induced sleeping test (sleep latency (SL) and sleep duration (SD)). Oral administration of XGFE was evaluated alongside the reference drugs diazepam (DZP) and flumazenil (FLU). The proposed mechanism suggests that XGFE acts through the $GABA_A$ receptor complex, enhancing Cl^- influx and inducing neuronal hyperpolarization to drive its anxiolytic and sedative effects.

This study has some limitations; because it was conducted using the crude extract of XGFE, the specific bioactive phytochemicals responsible for the observed effects were not isolated or identified. Further studies, including chemical standardization, receptor-specific assays (such as flumazenil co-administration in the sedative model), and pharmacological validation are warranted to confirm the exact interaction with the GABAergic system or other neuro-receptors. Furthermore, expanded preclinical studies utilizing larger animal cohorts, broader dose ranges, extension of inter-test resting interval, and complementary behavioral paradigms, such as the elevated plus-maze test and detailed open-field center-zone analysis, are necessary to obtain a more robust dataset and fully elucidate the underlying mechanisms. A comprehensive *in silico* study and ADMET analyses were not performed in this study, which could have provided further insights into the potential toxicological and pharmacokinetic profiles of the extract's constituents. Moreover, a key limitation is that sex-dependent variations were not fully evaluated, which would otherwise strengthen the generalizability, robustness, and translational relevance of the observed anxiolytic-like and sedative effects. Future studies should include both male and female animals to investigate potential sex-dependent effects of XGFE. Furthermore, toxicity studies should be conducted to establish the safety profile of the extract.

5. Conclusions

In conclusion, ethanolic extract of *X. granatum* J. Koenig fruit shows anxiolytic-like and sedative activity in Swiss albino mice. XGFE exhibited anxiolytic-like calming effects, as evidenced by reduced locomotor activity associated with CNS depressant properties and an avoidance of dark spaces, along with changes in square crossed, grooming, rearing, hole crossing, and swinging behaviors. It also reduced sleep onset latency and prolonged sleeping duration in the thiopental sodium-induced sleeping test. Both tests indicate that the observed responses are likely mediated through $GABA_A$ or other neuro-receptor systems. Our results suggest that bioactive phytochemicals in XGFE are likely responsible for mitigating anxiety-like behavior and enhancing sedative action, demonstrating its potential as a candidate for dual-action anxiolytic and sedative applications.

Author Contributions

H.M.S.H.S.: Conceptualization, methodology, formal analysis, investigation, writing—original draft; S.J.U.: Conceptualization, supervision, resources, writing—review & editing, project administration; M.T.I.: Conceptualization, supervision, methodology, formal analysis, project administration; R.R.: Methodology, investigation, validation, writing—review & editing; J.A.S.: Supervision, resources, conceptualization, writing—review & editing; R.I.C.: Conceptualization, investigation, data curation, formal analysis; A.K.S.: Investigation, software, data curation, writing—original draft; B.D.: Investigation, visualization, data curation; T.R.A.: Formal analysis, data curation; M.A.H.: Investigation, validation; S.A.: Investigation, visualization; M.S.H. (Md. Sabbir Hosain): Formal analysis, validation; M.S.H. (Md Shourov Hossen): Investigation, data curation. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The animal study protocol was approved by the Animal Ethics Committee of the Pharmacy Discipline, Khulna University, Khulna 9208, Bangladesh (protocol code: KU/PHARM/AEC/23/07/41).

Informed Consent Statement

Not applicable.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request. The raw data generated and analyzed during the current study can be provided for verification and further scientific evaluation.

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Conflicts of Interest

The authors declare no conflict of interest. Given the role as Editorial Board Member, Jamil A. Shilpi had no involvement in the peer review of this paper and had no access to information regarding its peer-review process. Full responsibility for the editorial process of this paper was delegated to another editor of the journal.

Use of AI and AI-Assisted Technologies

During the preparation of this manuscript, the authors used QuillBot (QuillBot, Inc.) for grammar checking, language refinement, and paraphrasing, and ChatGPT (OpenAI) to assist in drawing the mechanistic schematic/illustration during the final stage of manuscript preparation. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Abbreviations

XGFE = *Xylocarpus granatum* J. Koenig Fruit extract; DZP = Diazepam; FLU = Flumazenil; CNS = Central Nervous System.

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