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Original Article

Evaluation of Analgesic, and CNS Depressant Activities of Ethanolic Extract of Roots of *Heritiera fomes* Buch.- Ham.

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Abstract: The purpose of the study was to examine analgesic and neuropharmacological effects of ethanolic extract of root of *Heritiera fomes* Buch.-Ham (RHF). All the tests were conducted on Swiss albino mice in three distinct doses (100, 200, 300 mg/kg body weight). The peripheral and central analgesic actions in rodents were investigated using the acetic acid-induced writhing test and the tail immersion test, respectively. The extract significantly ($p < 0.05$, $p < 0.01$) prolonged the latency period to the thermal stimuli in the tail immersion method in a dose-dependent manner which was comparable to the reference standard Morphine. The extract (300 mg/kg) produced a maximum of 68.08% inhibition ($p < 0.01$) of the writhing reflex in the acetic acid-induced writhing test, compared to the reference medicine Diclofenac-Na (10 mg/kg) (76.45%). Hole cross, open field, and thiopental sodium-induced sleeping time tests were carried out to assess its CNS depressant action. According to statistical analyses, the extract makes mice sleep longer when they are exposed to thiopental sodium. RHF showed a significant ($P<0.05$, $P<0.01$) diminution in locomotion of rodents in both open field and hole cross methods at utmost dose in comparison with control group. Taken together, these findings imply that *H. fomes* root extract has strong analgesic and central nervous system depressive qualities. Advanced investigations are required for extensively phytochemical screening and establishing precise mechanisms of action.

Keywords: *Heritiera fomes* Buch.-Ham; analgesics; CNS depressant; thiopental sodium-induced sleeping time test, hole cross, open field

1. Introduction

Since the dawn of human civilization, medicinal plants that contain bioactive compounds have served as a crucial source of therapeutic drugs. Some estimates claim that about 80% of modern medications are either directly or indirectly derived from medicinal herbs. They act as significant natural resources for the production of traditional and modern healing agents [1, 2, 3]. Due to their antidiabetic, anti-inflammatory, antimicrobial, anti-carcinogen, sedative, depressive, anxiolytic, anticonvulsant, antispasmodic, antimalarial, antidiarrheal and

anti-HIV characteristics, medicinal plants with a high concentration of bioactive phytochemicals are still popular as remedies in developing countries [4].

Mangroves are halophile plants with important economic and environmental ecosystem [5]. Scientists have become interested in them over time due to their unique morphology, ecology, and applications. The local traditional healers and the rural residents who live in these areas frequently employ them for the treatment of leprosy, elephantiasis, tuberculosis, malaria, dysentery, ulcers and some skin diseases. The numerous ways that various mangrove plant species have been used to relieve human suffering provide persuasive evidence of their medicinal value and urge further study. However, there is a dearth of information on mangroves because of the rarity of these species as well as the difficulties in collecting and identifying them. Additionally, there is a lack of relevant knowledge regarding their ethnomedicinal usage [6, 7].

Heritiera fomes Buch. Ham. is an imperative moderate-sized mangrove plant that grows mainly in the world's single largest mangrove forest, Sundarbans, which extends across Bangladesh and West Bengal of India. It is also noticed in coastal regions of India, Myanmar, Thailand, and Northern Malaysia. Over the years traditional medicine practitioners extensively used these plant parts to treat a variety of ailments including diarrhea, dysentery, constipation, indigestion, diabetes, cancer, skin disorders like dermatitis, rash, eczema, boils, itch, scabies, sores, and infections, as well as hepatic disorders like jaundice and hepatitis. Additionally, the plant showed anti-inflammatory, anti-nociceptive, antimicrobial, wound-healing, antioxidant, and insect-repelling properties in previous investigations [8, 9, 10, 11, 12]. The earlier studies revealed the presence of valuable bioactive components in *H. fomes*, such as alkaloids, glycosides, flavonoids, saponins, polysaccharides, phenols, gums, sterols, and procyanidins. trimeric, pentameric, hexameric procyanidins, β-Sitosterol, stigmasterol and stigmast-4-en-3-one partially of which were further confirmed in our phytochemical screening [13, 14, 15].

Despite the high concentration of bioactive components, the literature study showed that the root of *H. fomes* has received very little scientific attention. This inspired us to conduct a thorough investigation into the ethanolic extractive and analgesic and neuropharmacological qualities of the aforementioned plant's root, bearing in mind the worldwide need for natural mineral supplements and bioactive substances derived from roots.

2. Materials and Methods

2.1 Plant sample collection, identification, and processing technique

Fresh root of *H. fomes* were collected from Sundarbans, Bagerhat district, Bangladesh in 2023. They were kept in our lab for future reference after being identified and verified by a taxonomist from the Bangladesh National Herbarium in Mirpur, Dhaka (DACB Accession No: 50664). To eliminate dirt, the collected plant roots were carefully washed in running tap water, followed by a rinsing in distilled water. Then the roots were cut into small pieces, sorted, and dried in the shade for 14 days prior to getting pulverized into a coarse powder in a lab electric grinder. 700 g root powder of the plant was taken into a clean glass container and submerged in 2.0 liters of ethanol for two weeks with frequent shaking and agitation. The liquid was then filtered into a different clean beaker with the number 1Whatman filter paper [16]. For future assessment, the powdered plant material was kept apart in airtight containers in a cool, dark, and dry environment.

2.2 Chemicals and reagents

We obtained the following drugs from Incepta Pharmaceuticals Ltd. in Bangladesh: morphine, diclofenac sodium, thiopental sodium, and diazepam. All chemicals and reagents, including acetic acid, and tween 80, were of analytical grade (Merck, Darmstadt, Germany).

2.3 Phytochemical Analysis

The freshly prepared crude extract was qualitatively analyzed for the existence of bioactive phytochemicals to relate the activities of the *in vivo* pharmacological tests by using standard techniques [17].

2.4 Animals

Swiss-albino healthy adult male mice (weighing 35–45 g and aged 4-5 weeks) were used for the *in vivo* studies. They were bought from the Animal Resource Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR, B). Because of the high vulnerability of the animals to environmental divergence, rodents were accommodated in plastic cages with free access to pelleted food and tap water under typical animal house conditions (temperature: 28 to 31°C, photoperiod: approximately 12h of natural light per day, and relative humidity: 55–60%) for at least 3 - 4 days for acclimation. The study was conducted in conformity with State University of Bangladesh's Institutional Ethical Review Committee's (SUB-IERC) norms and procedures, as well as generally accepted worldwide standards for the use and care of animals. All surgical interventions were accomplished under ether anesthesia via aseptic techniques [2, 18].

2.5 Animal experimental design

Rodents were divided into the following groups each with six animals. The vehicle (1% tween 80 in normal saline, 10 mL/kg) was administered to Group I as the negative control, whilst the reference drug for the specific test was administered to Group II as the positive control. Groups III–V received RHF (100, 200, and 300 mg/kg, respectively).

2.6 Analgesic activity

2.6.1 Peripheral analgesic activity

The peripheral analgesic activity of roots of *H. fomes* was estimated by acetic acid-induced writhing test [2, 19]. Similar to the Central analgesic test design, both standard diclofenac sodium and tested plant sample were administered to the experimental mice by oral route (Group I- Group V). After 45 minutes, 0.7% (v/v) acetic acid was intraperitoneally injected to each rodent at a dose of 10 mL/kg body weight. Fifteen minutes after the administration of acetic acid, the number of writhing (contraction of the abdomen) responses was counted for each animal during a 5-min period. Besides, the mean abdominal writhing for each group was calculated. The percent inhibition of writhing was computed with the following formula:

$$\% \text{ inhibition of writhing} = \frac{N_{Control} - N_{Test}}{N_{Control}} \times 100$$

In the above formula, N denotes the mean number of abdominal writhing for each group.

2.6.2 Central analgesic activity

Central analgesic activity was evaluated by following the previous protocol [19]. A total of 36 mice were used in this investigation; each group consisted of six animals and included the control group (a vehicle), reference group (morphine, 2 mg/kg body weight), and test group (RHF at 100, 200, and 300 mg/kg body weight). Here, the thermal stimulation caused by dipping the tip of the tail in warm water-induced painful reactions in rodents. Mice were grouped and treated as described earlier. Morphine was used as the reference drug. After the treatment of each group, the basal response time was measured by submerging the last 1-2 cm of the mice's tail tips of the mice in hot water of (55 ± 1) °C. The flick reaction of mice, i.e., time taken (in second) to remove it from the hot water source was calculated and outcomes were compared with the control group. To avoid rodents agitation a fifteen-second latency period was chosen as the cut-off threshold. The latent period of the tail-flick response was recorded at 0 min and after 30, 60 and 90 min of drug and extract administration.

2.7 Evaluation of neuropharmacological activity

Neuropharmacological activity of plant extract was evaluated by open-field, hole-cross and thiopental sodium induced sleeping time methods.

2.7.1. Open field method

The open field behavioral test is widely used to assess the emotional state and locomotor activity of rodents [20]. The test was conducted using the approach described in Sultana et al., 2018, with a few minor adjustments. Three test groups, each with five mice, were formed from the animals, along with a control and a positive control. Oral extract was administered to the test groups at doses of 100, 200, and 300 mg/kg body weight, whereas distilled water (0.1 mL/mouse, p.o.) was given to the control group. Using i.p., diazepam (1 mg/kg) served as the positive control group. There were squares all throughout the vast field. Black and white are applied separately to each square. The equipment featured a 40 cm-tall wall. At intervals of 0, 30, 60, 90, and 120 minutes, the animals were brought into the squares, and the number of squares visited was counted for three minutes.

2.7.2. Hole cross method

The strongest alterations in behavior come from a strong emotional reaction to a new environment. The prior technique was followed in order to conduct the hole cross test [20] a 30 cm by 20 cm by 14 cm cage with a partition in the center. The device is made up of hardwood planks. At a height of 7.5 cm, a hole with a diameter of 3 cm was drilled in the middle of the cage. A mouse was positioned in the center of the cage, and oral treatments (vehicle, extracts, and standard) were given. At 0, 30, 60, 90, and 120 minutes, the number of mice that passed through the aperture connecting one chamber to the next was counted for three minutes.

2.7.3. Thiopental sodium induced sleeping time test

The animals in this experiment were divided into five groups, each with five mice. When the control group was given distilled water (0.1 ml/mouse, p.o.), the test groups anticipated the extract at doses of 100, 200, and 300 mg/kg. As a positive control, conventional medication diazepam (1 mg/kg, intraperitoneally) was employed. Thirty minutes later, thiopental sodium (40 mg/kg, i.p.) was administered to each mouse in order to put them to sleep. The rodents were kept under observation by positioning them in separate chambers throughout the duration of their sleep and the latent period, which is the interval between the loss and recovery of the righting reflex after administering thiopental sodium [20].

2.8 Statistical analysis

The results were expressed as mean ± SEM (Standard Error of Mean). For statistical comparisons, we used one-way analysis of variance (ANOVA) followed by Dunnett's t-test. $P< 0.05$ was considered as the threshold for statistical significance in all tests, which were conducted using SPSS (Statistical Package for Social Sciences) version 28.0.

3. Results

3.1. Phytochemical screening

The experimented extract of root of *H. fomes* revealed the tested positive for alkaloid, carbohydrate, tannins, flavonoid, saponin, glycoside, steroid, phenol and resin (**fig 1**).

Table 1. Screening of bioactive phytocompounds in different extracts of *H. fomes*

Phytocompounds	RHF
Carbohydrate	+
Glycoside	+
Tannin	+
Alkaloid	+
Saponin	+
Resin	+
Phenol	+

Phytocompounds	RHF
Flavonoid	+
Steroid	+
Fixed oil	+

Here, RHF= ethanolic extract of root of *H. fomes*, respectively; + = present; - = negative

3.2 Analgesic Tests

3.2.1 Peripheral Analgesic Activity

According to **Table 2**, the oral administration of the ethanolic root extract of *H. fomes* (100–300 mg/kg) significantly inhibited (P <0.05) the nociception brought on by acetic acid. The extract produced maximal protection at a dose of 300 mg/kg (65.56% writhing inhibition) compared to standard (76.45% writhing inhibition). Furthermore, the findings showed that lower dosages have modest to moderate peripheral analgesic activity.

Table 2. Peripheral analgesic effect of *H. fomes* extract on mice by writhing test

Group	Treatment	Number of writhing	% of Inhibition
Control (I)	Tween 80 solution	19.83 ± 1.47	---
Standard (II)	Diclofenac sodium 10 mg/kg	4.67 ± 1.51**	76.45
III	RHF 100 mg/kg	12.33 ± 1.03	37.82
IV	RHF 200 mg/kg	9.00 ± 0.89*	54.61
V	RHF 300 mg/kg	6.83 ± 0.41*	65.56

Each value represents mean ± SEM for n = 6, ** p < 0.01, and * p < 0.05 vs. control.

3.2.2.Central Analgesic Activity

Table 3 displays the outcomes of the central analgesic effect of *H. fomes* extract in the tail immersion method. In comparison to the reference drug, diclofenac sodium, all extracts significantly (p<0.05) exhibited a strong escalation in pain reaction time (PRT) in a dose-dependent manner. At 90 minutes after loading plant extracts, root extract demonstrated an increased pain reaction time of 9.17±0.90 at the largest dose in comparison to the standard 18.25±0.33.

Table 3. Central analgesic effect of *H. fomes* extract on mice by tail immersion method.

Group	Treatment	Average Time of Tail Immersion of Mice			
		Time (in Sec) after Loading the Plant extracts/Drug Standard			
		0 min	30 min	60 min	90 min
Control	Tween 80 solution	2.63 ± 0.22	2.55 ± 0.26	2.98 ± 0.54	3.39 ± 0.24
Standard (II)	Morphine 2 mg/kg	1.65 ± 0.04*	6.41 ± 0.23***	10.73 ± 0.15**	18.25 ± 0.33***
III	RHF 100 mg/kg	2.31 ± 0.10	4.42 ± 0.16*	5.48 ± 0.94	8.16 ± 0.69*
IV	RHF 200 mg/kg	2.06 ± 0.8*	3.40 ± 0.19*	5.88 ± 0.54*	7.27 ± 0.82
V	RHF 300 mg/kg	1.88 ± 0.08*	5.31 ± 0.19	6.39 ± 0.12*	9.17 ± 0.90*

Each value represents mean ± SEM for n = 6, *** p < 0.001, ** p < 0.01, and * p < 0.05 vs. control.

3.3 Neuropharmacological activity

3.3.1 Open Field Method

At 100, 200, and 300 mg/kg body weight, the extract dramatically reduced the locomotor activity in mice ($p<0.05$; $p < 0.01$), and this effect was visible from 60 to 120 minutes (**Table 4**). As anticipated, mice given 1 mg/kg, i.p. of diazepam demonstrated a discernible reduction in movement from 30 to 120 minutes of observation.

Table 4. Neuropharmacological effect of *H. fomes* root extract on mice in Open Field method

Groups	**Treatment**	**Number of movements**				
		0 min	**30 min**	**60min**	**90 min**	**120 min**
Negative Control (I)	Tween 80 solution	158.80 ± 0.83	155.00 ± 0.63	155.20 ± 0.74	150.40 ± 0.66	152.60 ± 0.33
Positive control (Standard) (II)	Diazepam 1 mg/kg,i.p.	142.00 ± 0.84*	98.20 ± 0.56**	70.20 ± 2.3**	60.20 ± 0.88**	9.20 ± 0.56**
III	RHF 100 mg/kg	141.33 ± 1.92	130.88 ± 0.54*	93.00 ± 1.64*	86.33 ± 0.89**	70.62 ± 1.58*
IV	RHF 200 mg/kg	128.00 ± 1.53*	87.2 ± 2.86**	71.4 ± 1.14**	60.2 ± 1.65*	48.0 ± 1.88**
V	RHF 300 mg/kg	110.66 ± 0.89*	76.8 ± 0.83	62.20 ± 1.33	56.88 ± 1.58**	39.0 ± 0.56*

The values are revealed as mean±SD (n=5); One-Way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, **P<0.01 significant compared to the negative control.

3.3.2. Hole cross method

At doses of 100, 200, and 300 mg/kg body weight, the RHF extract demonstrated a substantial decrease in movement from its initial value at 0 min to 120 min ($p < 0.05$; $p < 0.01$). Mice given the usual medication diazepam (1 mg/kg, i.p.) had fewer holes spanned between chambers after 0 to 120 minutes (**Table 5**). The extract exhibited dose-dependent action, with the fifth observation period showing the greatest depressive effect.

Table 5. Neuropharmacological effect of *H. fomes* root extract on mice in hole cross test.

Groups	**Treatment**	**Number of movements**				
		0 min	**30 min**	**60min**	**90 min**	**120 min**
Negative Control (I)	Tween 80 solution	30.00 ± 1.64	29.60 ± 0.88	29.00 ± 0.75	29.00 ± 0.56	29.80 ± 0.58
Positive control (Standard) (II)	Diazepam 1 mg/kg,i.p.	24.80 ± 0.75*	18.00 ± 1.94*	9.80 ± 0.65**	3.00 ± 0.00**	1.00 ± 0.00**
III	RHF 100 mg/kg	28.40 ± 0.49*	25.2 ± 0.52*	23.2 ± 1.89*	18.60 ± 0.86	16.00 ± 0.89*
IV	RHF 200 mg/kg	23.20 ± 1.02*	17 ± 1.66*	14.0 ± 0.97*	11.40 ± 0.49	9.40 ± 0.80**
V	RHF 300 mg/kg	20.00 ± 0.38*	12.63 ± 0.53*	10.80 ± 0.48*	8.00 ± 0.88**	4.0 ± 0.63*

The values are revealed as mean±SD (n=5); One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. *P<0.05, **P<0.01 significant compared to the negative control.

3.3.3 Thiopental induced hypnosis test

The extract demonstrated a significant reduction in the time of onset of sleep and an increase in sleep duration in the thiopental-induced hypnosis test at dosages of 100, 200, and 300 mg/kg. The findings were deemed statistically significant ($p< 0.05$). In comparison to controls, all doses of the extract prolonged the test animals' thiopental sodium-induced sleep period. The dose-dependent activity of RHF was clear from the results of dosages between 100 and 300 mg/kg. The effects of diazepam were most pronounced ($p<0.001$) during onset of sleep and during the peak of sleep (**Table 6**).

Table 6. Effects of *H. fomes* extract on thiopental sodium induced sleeping time test in mice.

Groups	Treatment	Thiopental sodium induced sleeping time test	
		Onset of sleep (Min)	**Duration of sleep (Min)**
Control (I)	Tween 80 solution	27 ± 1.79	57.67 ± 6.31
Standard (II)	Diazepam 1 mg/kg,i.p.	11.50 ± 1.05**	249.33 ± 3.4**
III	RHF 100 mg/kg	14.83 ± 1.47	70.33 ± 1.41
IV	RHF 200 mg/kg	11.33 ± 1.20*	109.67 ± 1.57
V	RHF 300 mg/kg	10.33 ± 1.03*	200.67 ± 1.03*

Each value represents mean ± SEM for n = 6, ** $p < 0.001$, and * $p < 0.05$ vs. control.

4. Discussion

The use of medicinal plants has emerged as a fascinating avenue for the development of traditional and modern medications, and research has demonstrated the true medical benefits of herbal medicines [21, 22, 23]. Our current study's objective was to look into *in vivo* analgesic and CNS depressant bioactivities of *H. fomes* root. Biological effectiveness of medicinal plants is largely dependent on their phytochemical content. A key factor in the discovery of novel, uncommon, and active chemicals is phytochemical analysis. The existence of secondary metabolites in plants is associated with their biological significance [22, 24]. The crude root extract of the experimented plant exhibited the presence of numerous valuable secondary metabolites such as alkaloids, glycosides, tannins, reducing sugars, steroids, fixed oil, terpenoids, flavonoids, and phenols (**Table 1**). *H. fomes* plant parts are already reported as an ailment for various diseases in the traditional system [13, 14]. The seed of the plant is reported to have a diverse nature of compounds including anthocyanins, complex polyphenols, leucoanthocyanidin, catechin, ellagic acid, geraniin, corilagin etc. [14, 15].

The central pain mechanism heavily relies on the brain and spinal cord. A variety of inhibitory pain-targeting biomolecules, including prostaglandins, somatostatins, bradykinins, and others, are abundant in the dorsal portion of the spinal cord. Pain, or algesia, is invariably an unpleasant experience. Usually, it is brought on by irritating stimuli, either internal or external. These often cause the phospholipids in the afflicted tissues to release arachidonic acid. Consequently, numerous intracellular components start to secrete. The sense of pain has been attributed to secreted prostacyclin (PGI2), leukotrienes, cytokines, and PGE2, PGF2α [24]. The acetic acid-induced writhing response technique is a widely used method to evaluate the peripheral analgesic activity of any plant component, as acetic acid is the major inducer of pain in an animal model [25]. Because of its sensitivity and ability to detect antinociceptive effects of natural products and test compounds at dose levels that are inert for other procedures, the acetic acid-induced writhing test is a valuable paradigm for assessing the peripheral analgesic potentials of test compounds [26]. A variety of endogenous inflammatory mediators, such as histamine, serotonin, bradykinin substance P, and PGs, are produced and released when acetic acid is injected intraperitoneally because it stimulates and irritates the peritoneal cavity. The body lengthens and the forelimbs extend while the abdominal muscles contract as a result of the chemically induced visceral discomfort caused by these various endogenous inflammatory mediators. Moreover, this model has been connected to increased PGE and PGF2α levels. By activating primary afferent nociceptors and expanding capillary permeability, raising PG levels in the peritoneal cavity intensifies inflammatory pain [26, 27, 28]. All three dosages of RHF extract showed substantial ($p < 0.05$ and $p < 0.01$) peripheral analgesic effects by reducing the number of writhing (**Table 2**),

with respective values of 37.82%, 54.61%, and 65.56%, when compared to the negative control. In animal models where thermal stimuli are utilized to generate pain, the tail immersion method is frequently used to assess central analgesic action. These techniques highlight the alterations above the level of the spinal cord, which provide a useful example of centrally mediated anti-nociceptive responses. Due to its great selectivity for analgesics derived from opioids, the approach is preferred [25]. Using the tail immersion method, the RHF extract demonstrated a strong and dose-dependent anti-nociceptive effect (**Table 3**). A potential suppression or alteration of pain induction via a spinal reflex is indicated by the inhibition of nociceptive activity. Since peripherally acting drugs are ineffective in response to certain kinds of heat stimulation, this technique is specific to central analgesia [29]. Spinal reflexes, which may be assessed by the tail immersion method, are a means by which the μ2, κ1, and δ2 opioid receptors contribute to nociception [25, 30, 31]. This approach also shown an increase in anti-nociceptive activity that was dose-dependent. The results of both techniques point to the anti-nociceptive properties of *H. fomes* root extract being mediated by spinal and supraspinal receptors. The combined analgesic effects of RHF extracts are thought to be caused by the reduction of prostaglandin synthesis and its impact on the central and peripheral analgesic mechanisms. Plant extracts might have a stronger analgesic effect.

Three neuropharmacological models- the open field, hole cross, and thiopental sodium induced sleeping time tests- were used to investigate the CNS depressive impact of *H. fomes* root extract. These models are commonly employed classical models for the purpose of neuropharmacological activity screening [20]. Locomotor activity serves as a gauge for the central nervous system's excitability, and a decline in this activity may be specifically linked to sedation brought on by central nervous system depression [32]. The plant extract's CNS depressive action may be the cause of this drop in spontaneous motor activity. The primary inhibitory neurotransmitter in the central nervous system is gamma amino butyric acid [20, 32, 33]. It has a role in physiological processes associated with neurological and psychological illnesses include epilepsy, depression, Parkinson's disease syndrome, and Alzheimer's disease [33]. A variety of medications have the ability to alter the GABA system at the level of its manufacture by increasing GABA-mediated postsynaptic inhibition via altering the allosteric properties of GABA receptors. It either directly increases chloride conductance or indirectly, similar to barbiturates, by potentiating GABA-induced chloride conductance while concurrently depressing voltage-activated Ca^{2+} channel [20]. Hence, it is foreseeable that the extract may function by directly activating GABA receptors or by potentiating GABAergic inhibition in the central nervous system via membrane hyperpolarization, which lowers the firing rate of important brain neurons. A longer GABA-gated channel opening duration or increased affinity for GABA could possibly be the cause [20, 33]. According to prior phytochemical research, neuroactive steroids and flavonoids bind to GABA-A receptors in the central nervous system, suggesting that they have benzodiazepine-like properties [20, 32, 33]. Furthermore, several flavonoids have been shown to bind to the GABA-A receptor's benzodiazepine region with great affinity.

Thiopental sodium is a barbiturate that puts rodents and people to sleep. The sedative-hypnotic medications were examined using the thiopental sodium induced sleeping time test in mice. This test is a traditional method used in behavioral pharmacology to look into the hypnotic and sedative effects of extract from medicinal plants [34, 35]. The drug binds to the GABA receptor complex and causes postsynaptic neurons to become hyperpolarized by GABA [35]. By extending the time of the chloride channel opening, it increases GABA activity and allows chloride to enter the neuron. However, thiopental has the ability to inhibit glutamate receptors that are excitatory. In the current investigation, varying concentrations of the extract markedly reduced the latency to induce sleep while lengthening the thiopental sodium-induced hypnosis duration (**Table 4**). Our findings indicate a possible correlation between the CNS depressive action of roots of *H. fomes* and diazepam in this test. Thus, the phytoconstituents in the RHF extract may be the cause of the CNS depressive action. It has been observed that triterpenoids and saponins exhibit agonistic actions at the GABA-A receptor complex [32, 35, 36]. These phytoconstituents might have played a role in the mice's CNS depressive effects. The precise compounds causing the CNS depressing effects are not well demonstrated.

5. Conclusions

Traditional herbal medicines have been used to treat and prevent a wide range of illnesses throughout human history. By considering the medicinal properties of herbs, researchers are allegedly evaluating the development of plant-based pharmaceuticals as a key and demanding topic of focus. Large amounts of glycosides, alkaloids, tannins, flavonoids, terpenoids, resin, and other secondary bioactive metabolites present in *H. fomes* Buch root have been demonstrated to have potent analgesic and CNS depressant properties. Every experimental dosage examined results in an immediate, pervasive, and statistically significant effect. Our findings suggest that pharmaceutical companies could be able to lower the cost of healthcare by using the roots of *H. fomes* to generate new, safer, more effective, and less toxic candidate medications. Further research will be conducted to determine the precise molecular pathways and identify the bioactive compound(s) in order to establish a safe and effective dosage and validate the possibility of using it for the prevention and treatment of different illnesses.

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