International Journal of Pharmacy and Pharmaceutical Sciences

Print ISSN: 2656-0097 | Online ISSN: 0975-1491 Vol 12, Issue 11, 2020

Original Article

CNS DEPRESSION POTENTIAL EVALUATION, FORMULATION AND CHARACTERIZATION OF LYOPHILIZED HERBAL ORAL CAKE OF *TERMINALIA CHEBULA* FRUITS

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Received: 05 Jul 2020, Revised and Accepted: 16 Sep 2020

ABSTRACT

Objective: *Terminalia chebula* fruits are used as traditional herbal medicine from the ancient era but still now, the extract has not revealed any research data on CNS depression activity as well as its lyophilized herbal formulation. The present study was designed to evaluate CNS depression activity and develop lyophilized oral cake of *Terminalia chebula* fruit extract.

Methods: CNS depression potential of *Terminalia chebula* fruit was examined using the hole board, hole cross, and thiopental sodium induced sleeping time test. The human equivalent dose was calculated based on US FDA guidelines of dose conversion between animals and humans. A novel lyophilized herbal oral cake of aqueous extract of *Terminalia chebula* fruits with additives was then formulated. The formulated cake was subjected to characterize its physicochemical properties such as appearance, residual humidity, drug content, dissolution, and drug release profile, extract-polymer compatibility by Fourier-transform infrared spectroscopy (FTIR) and stability. The prepared cake was further evaluated with the hole board and hole cross model in mice and compared with control to ensure its CNS depression activity.

Results: Crude extract at the doses of 100, 150, 200, and 400 mg/kg body weight showed significant ($p<0.01$) dose-dependent inhibition of locomotor behavior. Crude extract dose of 200 mg/kg body weight in mice compared with standard diazepam dose (1 mg/kg) was used to calculate the human equivalent dose of 1000 mg/60 kg. The formulation presented a rapid drug release profile while drug content was approximately 99.5%. FTIR spectroscopy of formulation showed no drug-excipient interaction. The oral cake at the dose of 200 mg/kg body weight showed significant ($p<0.01$) CNS depression activity.

Conclusion: CNS depression activity, FTIR, and stability analysis ensure the preservation of active ingredients in the lyophilized oral cake as in the crude extract.

Keywords: *Terminalia chebula*, Lyophilized herbal formulation, Fourier-transform infrared spectroscopy (FTIR), Locomotors movement

DOI: http://dx.doi.org/10.22159/ijpps.2020v12i11.39029. Journal homepage: https://innovareacademics.in/journals/index.php/ijpps.

INTRODUCTION

Plants provide humankind with new remedies when fulfilling the fundamental needs like food and shelter. Thus plants have fabricated the premise of sophisticated traditional systems of medicine for thousands of years [1]. Traditional medicine is used everywhere in the world and its economic importance is growing fast, mainly because the use of medicinal plants has gained a respectable position today, especially in developing countries [2]. About 60-85% of the population in developing countries has to rely on traditional medicine [3]. Traditional medicine is practiced widely in China, India, Japan, Pakistan, Sri Lanka, Thailand, and Korea [4]. *Terminalia chebula* (TC) Retz (Combretaceae) is one of the most revered medicinal plants in the Indian subcontinent.

Tibetans, ayurvedic apothecaries, and other folk medicinal practitioners consider the fruit of *Terminalia chebula* as the "king of medicines" [5]. The fruit of the tree exhibits diverse health benefits and has been used as traditional medicine for a household remedy against various human ailments since antiquity [6].

It is reported that depression and anxiety disorders are the most familiar mental illness in humans. It is life-threatening and causes a greater decrement in health state than major chronic physical illnesses such as angina, arthritis, asthma, and diabetes [7]. These disorders incur a huge economic and social cost to depressed people. Nowadays, there seems to be overdependence on synthetic drugs for alleviating certain emotional disorders. Meanwhile, researches have exposed that many people look for herbal products for the treatment of different kinds of psychiatric disorders [8].

As the global scenario is now changing towards the use of non-toxic edible plant products having traditional medicinal use, we developed oral lyophilized cake of aqueous extract of *Terminalia chebula* (AETC) fruits. The oral lyophilized cake is prepared by enclosing a drug and suitable additives in a water-soluble matrix. Because of the high porosity, oral lyophilized cake disintegrates faster than other systems providing the advantage of rapid onset of action and patient compliance [9].

So far, there is no report demonstrating the sedative-hypnotic activity of *Terminalia chebula* fruits. As a result, we aimed to evaluate the CNS depression activity of this fruit extract on mice. At the same time, we formulated and characterized a novel lyophilized herbal oral cake of AETC fruits for CNS depression.

MATERIALS AND METHODS

Chemicals

Mannitol, sorbitol, lactose, aspartame used in the investigation were obtained from Merck KGaA (Darmstadt, Germany) and the dialysis membrane was purchased from Spectrum Medical Industries Inc, (60916 Terminal Annex, Los Angeles, US). The standard drug diazepam and thiopental sodium were purchased from Square Pharmaceuticals Ltd, Bangladesh, and Advanced Chemical Industries Ltd, Bangladesh, respectively.

Experimental animal

In the present study, young Swiss-albino mice of average 20-30 g aged 4-5 w were used. They were kept at an ambient temperature of

25±1 °C, the relative humidity of 55-65% with 12 h light: 12 h dark cycle in the animal house of Pharmacy Discipline, Khulna University, Bangladesh. The animals were purchased from the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR'B), and acclimatized to laboratory conditions for one week prior to the experiment. The experimental protocol was approved by the Animal Ethics Committee, Life Science School, Khulna University, Bangladesh (Approval number**:** KU/PHARM/AEC/15/006/023).

Extraction and preparation of test sample

Terminalia chebula (Retz.) fruits were collected from the local area of Khulna district, Bangladesh in January 2017 in the daytime. The collected fruits were dried by shade drying for 15-20 d. The separated peels were crushed into fine particles and placed into Soxhlet extractor. Distilled water (heated to reflux) passed through the main chamber and the partially soluble components were slowly transferred to the solvent (Soxhlet extraction-Royal Society of Chemistry). Then the liquid solvent was filtered by using the Whatman filter paper. Finally, the filtrate was allowed to freeze at-20□C and lyophilized at-54 °C with<100mTorrin (Labconco Lyophilizer, China) and then fully dried with a secondary drying process with increasing temperature up to 35 °C. The total yield of the freeze-dried extract was 4.62% (w/w).

Hole board test of crude extract

The hole board test was conducted following the methods of Anisuzzman *et al.* 2017[10]. Mice were divided into 6 groups and each group comprised of 5 mice with 20-30 g in weight. Group I was administered 1% tween-80 orally at 10 ml/kg, Group II (standard) was treated with diazepam at 1 mg/kg body weight dose as an oral suspension and Group III, IV, V, and VI termed as test groups were given AETC fruits orally at the doses of 100, 150, 200, and 400 mg/kg body weight respectively. At the beginning of the test, the mouse was placed on the edge of the board. The number of head dipping into the holes was counted as the measurement for 3 min on 0, 30, 60, 90, 120, and 150 min entire the observation period. The experiment was carried out in a sound-attenuated room.

Hole cross test of crude extract

Mice were divided into 6 groups and each group comprised of 5 mice. Group I was given 1% tween-80 orally at 10 ml/kg, Group II (standard) was treated with diazepam at 1 mg/kg bodyweight oral dose and Group III, IV, V, and VI termed as test groups were given AETC fruits at the oral doses of 100, 150, 200, and 400 mg/kg body weight respectively. Then mice were placed individually in the darker chamber of the box segregated by a wall with a hole into dark and white chambers. The total number of crosses through the hole from one chamber to another by the mouse of each group within 3 min was counted on 0, 30, 60, 90, 120, and 150 min. The experiment was conducted in a sound-attenuated room [11].

Thiopental sodium-induced sleeping time test of crude extract

In this experiment, 30 experimental laboratory mice were arbitrarily chosen and accurately weighed. The mice were divided into six groups termed as a negative control group, positive control (standard) group and test groups (I, II, III, and IV). Five mice in each group were kept in six cages separately. Each mouse of the negative control group received orally 1% Tween-80 in distilled water at the dose of 10 ml/kg body weight. The mice of the positive control group received standard drug diazepam at the dose of 1 mg/kg body weight and each test group received crude extract at the dose of 100, 150, 200, and 400 mg/kg body weight respectively. All doses were given orally with the help of a feeding needle. After 30 min, thiopental sodium (40 mg/kg) was given intraperitoneally to all groups for inducing sleep. The onset time of sleep was noted for all the animals. After induction of sleep, mice were placed in the inverted position and when sedation was over, the mice came to normal posture, and time was noted. The interval between loss and recovery of the righting reflex was used as an index of the hypnotic effect. The time interval between the injection of thiopental sodium and the start of sleep was recorded as latency time [12].

Acute toxicity test

The mice were kept in a fasting condition for 16 h. Then experimental mice were divided into 6 groups of 5 mice per group. After that, the aqueous extract was administered orally at the dose of 800, 1000, 1500, 2000, and 3000 mg/kg body weight in each of 5 groups, while the control group received distilled water. The animals were observed 72 h for measurement of mortality. General signs and allergic symptoms of toxicity were noted for each group according to the Organization for Economic Co-operation and Development (OECD) guide with slight modification [13, 14].

Human equivalent dose calculation based on body surface area

The dose is equally related to bodyweight although it is not the lone factor that influences the scaling for dose calculation. The correction factor (K_m) is estimated by dividing the average body weight (kg) of species to its body surface area (m^2). The K_m factor value of various animal species is used to estimate the Human Equivalent Dose (HED) as:

$$\text{HED in mg/kg} = \text{Animal Dose (mg/kg)} \times (\text{Animal Km/Human Km})$$

If the human body weighs 60 kg and the human body surface area approximates 1.6 m^2, K_m is 37 and for typical adult mice, K_m value is 3. An animal dose of 200 mg/kg body weight produced promising CNS depression effect comparable with that of standard diazepam. Taking the animal dose of 200 mg/kg body weight into consideration, we calculated the human equivalent dose [15].

Lyophilized formulation development

Various quantities (table 1) of mannitol, sorbitol, lactose, and aspartame were dissolved in 5 ml distilled water and stirred with a magnetic stirrer until a clear solution was obtained. To formulate oral lyophilized cake, 1000 mg of aqueous extract (10% w/v) was dispersed in the excipient mixture and 10 ml volume was adjusted with distilled water. The resulting preparation was poured into vials.

Table 1: An oral lyophilized cake of different formulation

Properties	Ingredients	F_1	F_2	F_3	F_4
Active	AETC fruits (% w/v)	10	10	10	10
Bulking agent	Mannitol (% w/v)	3	4	5	6
Bulking agent	Sorbitol (% w/v)	5	4	3	2
Binder	Lactose (% w/v)	2	2	2	2
Sweetener	Aspartame (% w/v)	0.1	0.1	0.1	0.1
Solvent	Water (distilled) up to 10 ml	10 ml	10 ml	10 ml	10 ml

The vials were then transferred to a freeze dryer (Lyofast 3, Italy) and the lyophilization was carried out according to the parameters that are presented in table 2. Then vials were sealed with inert nitrogen gas [16].

Table 2: Parameters of the lyophilization cycle

Parameters	Temperature (°C)	Hold for (min)	Pressure (µ bar)
Freezing step	-45	540	--
Primary drying step	-10	960	250
Secondary drying step	+15	360	200

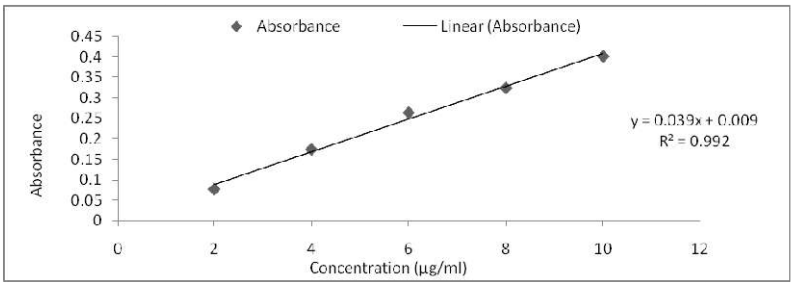

Fig. 1: Standard calibration graph

Appearance and residual humidity analysis

Formulated herbal cakes were inspected visually and also analyzed for residual humidity by Karl Fisher titration using methanol as a sample solvent [17].

The drug content in the oral lyophilized cake

The concentration of AETC fruits was determined spectrophotometrically from the calibration graph using the equation, $x = (y - c)/m$. Where, y = Absorbance, x = concentration of drug (µg/ml), m = gradient and c = intercept. A standard calibration graph of crude extract (fig. 1) was plotted between absorbance and concentration using the computer interface [18].

***In vitro* dissolution studies**

The dissolution profile of crude extract and optimized oral lyophilized cake were determined in a dissolution tester (Veego, India). All tests were conducted in 900 ml of 0.1N HCl and phosphate buffer medium, pH 6.8 maintained at 37±0.5 °C with a paddle rotation speed at 100 rpm. After specified time intervals (10, 20, 30, 40, 50, and 60 min), 5 ml samples from the dissolution medium were withdrawn with the help of a pipette and 5 ml of fresh medium was added quickly again in the dissolution medium. Then, the withdrawn sample was filtered and assayed for drug content by using UV spectrophotometer at 216.9 nm wavelength (Hitachi U-2900 spectrophotometer, Japan). The percentage of drug dissolved from the preparations was calculated using calibration equations [19].

***In vitro* release studies using dialysis bag**

In vitro release of lyophilized cake and crude extract solution was studied using a dialysis bag (molecular weight cut off [MWCO] 6-8 kDa, Spectrum Medical Industries Inc) in both 0.1N HCl and phosphate buffer, pH 6.8 maintained at 37±0.5 °C with a paddle rotation speed at 100 rpm. 2 ml of 500 mg/ml concentration of freeze-dried cake and the same concentrated 2 ml crude extract were taken into the separate dialysis bag. The open end of the dialysis bag was tightly bound with threads. The dialysis bag was placed inside the basket of the dissolution machine. Sampling was done by withdrawing 5 ml from the released medium with the help of a pipette and 5 ml of fresh medium was added quickly. Withdrawn samples were analyzed using a UV spectrophotometer at 219.6 nm wavelength. With the help of the standard curve prepared earlier, drug concentration was measured [20].

FTIR analysis

The dried powder of oral lyophilized cake was diluted with potassium bromide in the ratio of 1:100 and the spectrum was recorded at the mid-IR region, 2-20 µm in IRPrestige-21 Fourier-transform Infrared Spectrophotometer using the diffuse reflectance accessory [21].

Stability analysis of formulation

Stability studies of the formulated oral lyophilized cake were carried out by storing the formulations at three different temperatures such as refrigerated condition (2-8 °C), room temperature (25±2 °C), and elevated temperature (40±2 °C) for 6 mo as per ICH guideline. Stored formulations were evaluated visually for its appearance, broken units, and drug content.

CNS depression activity test of formulation

The developed oral lyophilized cake (F_4) was further tested by the hole cross and hole board model with a dose of 200 mg/kg body weight on mice to evaluate CNS depression activity.

RESULTS

Hole board test of crude extract

In the hole board test, the propensity of head dips significantly ($p<0.001$) lessened in the mice treated with crude extract at the dose of 100, 150, 200, and 400 mg/kg compared with the control group (fig. 2). The analogous effect was found in mice treated with diazepam (1 mg/kg).

Hole cross test of crude extract

We noticed that the hole cross activity significantly ($p<0.001$) abridged by second (30 min) and continued to sixth (150 min) observation period in comparison with the control group (fig. 3). The number of hole cross lessened significantly ($p<0.001$) demonstrated for the fourth (90 min), fifth (120 min), and sixth (150 min) observation period at 200 mg/kg body weight. Also, the positive control group (diazepam 1 mg/kg) decrement showed a similar modification to that observed with the extract.

Thiopental sodium-induced sleeping time test of crude extract

In the thiopental sodium-induced sleeping time test, the onset of sleep significantly ($p<0.05$) decreased (fig. 4). Moreover, total sleeping time significantly ($p<0.05$) increased in a dose-dependent manner in mice treated with crude extract at 100, 150, 200, and 400 mg/kg when compared with the control group (fig. 5).

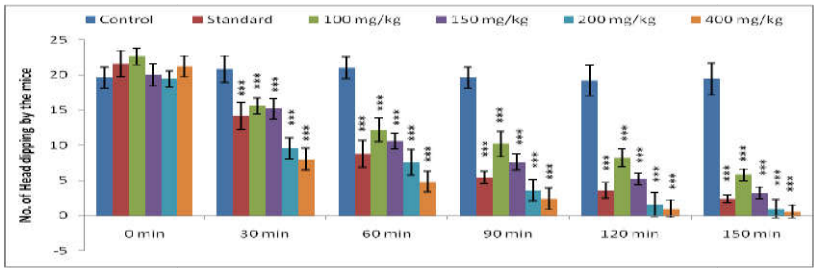

Fig. 2: Effect of AETC fruits on the hole board test model, all values are expressed as the mean±standard deviation of the mean (SDM), n=5. The difference between groups was analyzed by one-way ANOVA. *p<0.05, **p<0.01 and **p<0.001 were considered to be statistically significant

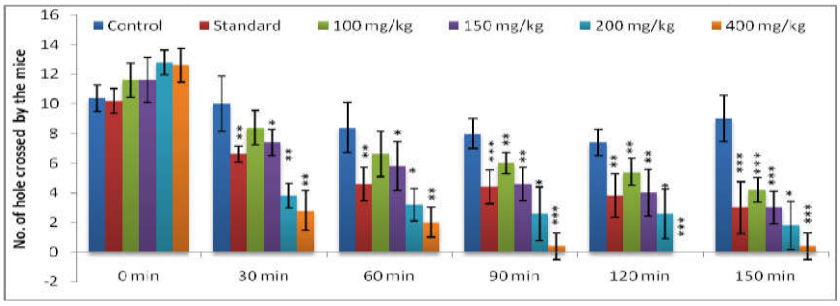

Fig. 3: Effect of AETC fruits on hole cross test model, all values are expressed as the mean±standard deviation of the mean (SDM), n=5. The difference between groups was analyzed by one-way ANOVA. *p<0.05, **p<0.01 and **p<0.001 were considered to be statistically significant

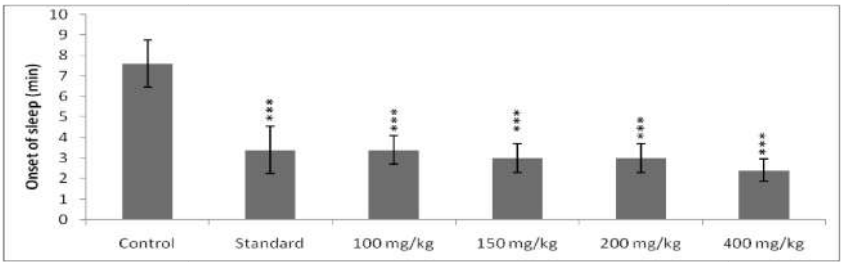

Fig. 4: Time to induce sleep on thiopental sodium induced sleeping time test, all values are expressed as the mean±standard deviation of the mean (SDM), n=5. The difference between groups was analyzed by one-way ANOVA. *p<0.05, **p<0.01 and **p<0.001 were considered to be statistically significant

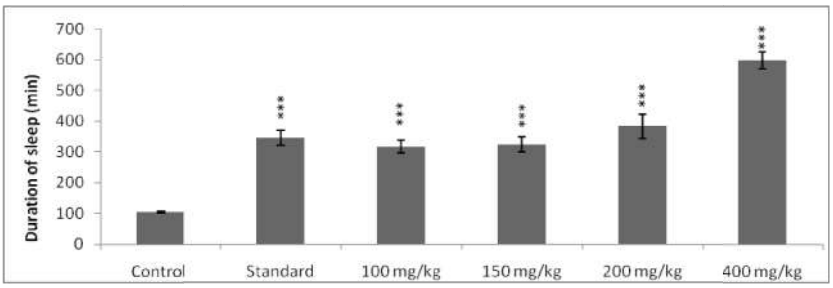

Fig. 5: Duration of sleep produced by AETC fruits on sleeping time test, all values are expressed as the mean±standard deviation of the mean (SDM), n=5. The difference between groups was analyzed by one-way ANOVA. *p<0.05, **p<0.01 and **p<0.001 were considered to be statistically significant

Acute toxicity test

In the oral acute toxicity test, the highest dose (3,000 mg/kg body weight of mice) of the fruit extract did not reveal any mortality and adverse effects.

Human equivalent dose (HED)

According to FDA,

$$\text{HED in mg/kg} = \text{Animal Dose (mg/kg)} \times (\text{Animal Km/Human Km}) = 200 \times (3 \div 37)$$

=16.22 mg/kg

The total dose optimized for a normal, healthy individual is (16.22 × 60) =973.2 mg. So, 1000 mg/60 kg human equivalent dose for CNS depression activity was used for formulation development.

Different formulations of an oral lyophilized cake

Different formulations (F_1, F_2, F_3, and F_4) were prepared using various percentages (% w/v) of mannitol, sorbitol, lactose, and aspartame to formulate oral lyophilized cake of suitable characteristics (table 1). It is well known that the crystalline structure of mannitol gained after freeze-drying provides the required hardness and elegance of the lyophilized cake [17]. For that reason, the concentration of mannitol was increased to prepare different formulations. The resultant lyophilized dry mixture was porous and fluffy and the original starting volume was maintained. This increased the surface area, thus increased dissolution rate.

Appearance and residual humidity analysis

It was found that the resulting formulation F_1, F_2, and F_3 were broken with incorrect appearance. F_4 formulation with a higher percentage of mannitol (6 % w/v) and a lower percentage of sorbitol (2% w/v) contributed no break or split and was regarded as the best formulation with elegant cake. The residual humidity of the formulations was within acceptable specifications (table 3).

Drug content of the oral cake

The drug content of the oral cake at various % w/v of the extract was determined using the calibration curve. Drug content was approximately 99.5% (table 4).

In vitro dissolution studies

The formulated cake showed greater drug dissolution compared to crude extract (fig. 6).

In vitro release studies using dialysis bag

Faster drug release was observed for lyophilized oral cake *in vitro* drug release profile which is depicted in fig. 7.

Table 3: The appearance, broken units, and residual humidity of the formulations

Tests	F_1	F_2	F_3	F_4
*RH%	1.67±0.30	1.48±0.22	1.41±0.25	1.3±0.25
Broken unit	4	3	1	0
Visual aspect	Fluffy	Acceptable	Good	Moderate

*All values are expressed as the mean±standard deviation of the mean (SDM), n=3.

Table 4: Drug content of the oral cake

% w/v of sample	Concentration of AETC (mg/ml)
2.5%	24.81±1.03
5%	50.25±1.49
10%	99.52±1.21

All values are expressed as the mean±standard deviation of the mean (SDM), n=3.

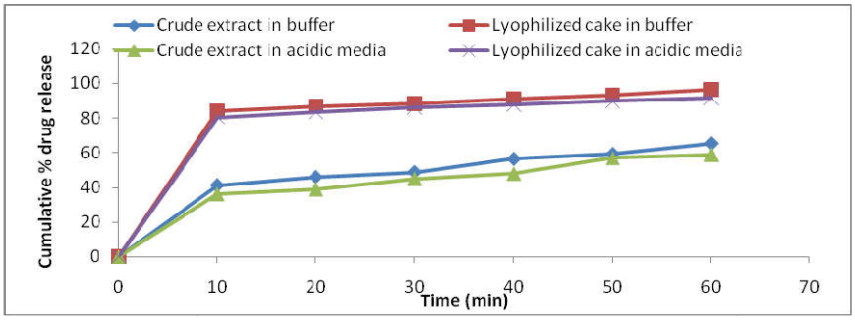

Fig. 6: *In vitro* dissolution studies

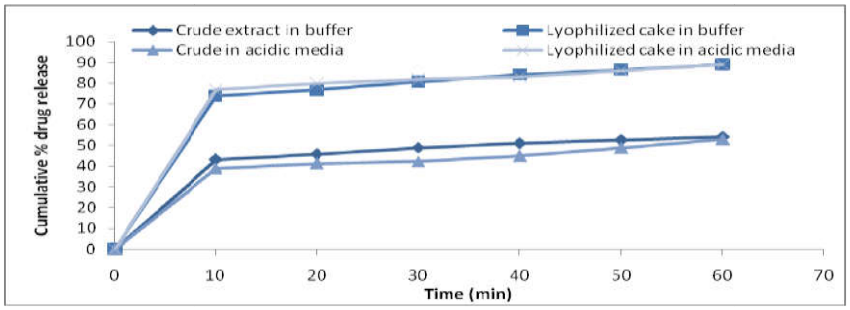

Fig. 7: *In vitro* drug release profile using dialysis bag

FTIR analysis

The formulation reveals all essential peaks of extract, which confirm no drug-excipient interaction, as shown in fig. 8.

Stability analysis of F_4 formulation

Stability analysis complying with the ICH guidelines reported the product stability (table 5).

Table 5: Stability analysis of F_4 formulation

Properties	1st month	3rd month	6th month
*Drug content (%)	99.5±1.65	99.0±1.83	98.7±1.88
Broken unit	0	0	0
Visual aspect	Good	Good	Good
Color change	No	No	No

*All values are expressed as the mean±standard deviation of the mean (SDM), n=3.

Hole board test of F_4 formulation

The lyophilized product was also evaluated for CNS activity through the hole board test at the dose of only 200 mg/kg body weight. In the hole-board test, the propensity of head dips significantly ($p<0.001$) lessened in a time-dependent manner in the mice treated with developed formulation at the dose of 200 mg/kg while compared with the control group (fig. 9).

Hole cross test of F_4 formulation

In the case of developed formulation, the number of hole crosses decreased significantly ($p<0.001$) in the second (30 min), third (60 min), fourth (90 min), fifth (120 min), and sixth (150 min) observation period at 200 mg/kg body weight (fig. 10).

DISCUSSION

Medicinal plants contribute to the major constituents of indigenous medicines. Thus, the quality and effectiveness of indigenous medicinal preparations depend solely on the genuineness and quality of the medicinal plants or products that are used in their preparation. So it is very important to ensure that these medicinal plants or their products possess the claimed properties and exert the desired therapeutic effects.

The freshly prepared extract was subjected to evaluate CNS depression activity. The extract showed significant CNS depressant effect in the hole board and hole cross tests in comparison to control in a dose-dependent manner. In the hole board test, the head-dipping behavior of the animals is directly related to their emotional state [22]. Present finding of the hole board test revealed that the extract caused a dose-dependent reduction in head-dip response in the mice from 2nd observation of the experiment and lasted to the final, suggesting that the extract possesses sedative activity (fig. 2). In hole cross test, agents with sedative activity will cause a reduction in the number of movements and interruption in the curiosity of the new environment [23]. In the present study, the oral administration of test extract at the doses 100, 150, 200, and 400 mg/kg caused a marked decline in the number of hole crosses and lethargy to a new environment (fig. 3). The suppression effect was found at 30 min and continued up to 150 min after administration of the extract. All tested doses twisted significant inhibition of locomotion. The suppression of locomotors by inducing crude extract referred to its potentiality to depress the central nervous system. Present findings of CNS depressant activity were further supported by the results observed in the thiopental sodium-induced sleeping time determination test (fig. 4 and fig. 5). This test is a classical method in behavioral pharmacology to investigate the sedative and hypnotic properties of medicinal plant extract [24]. In the present study, different doses of the extract significantly decreased the latency to induce sleep while increased the duration of hypnosis induced by thiopental sodium. As expected, similar types of effects were observed by the administration with diazepam used as standard.

Spontaneous locomotor activity is considered as an index of attentiveness and can be helpful to corroborate the general depressive activity of any drug. The decrease in motor activity gives a hint of the level of excitability of the CNS and this decrease may be related to sedation resulting from depression of the CNS. It is reported that GABA, an inhibitory neurotransmitter, is involved in the pathophysiology of depression [25]. The extract may act by potentiating GABAergic inhibition in the CNS by membrane hyperpolarization, which diminishes the firing rate of vital neurons in the brain [26]. The sedation may also be due to improved affinity for GABA or a boost in the duration of the GABA-gated channel opening [27].

Literature reviews confirmed the presence of alkaloids, glycosides, flavonoids, carbohydrates, saponins, steroids, and tannins in the crude extract of *Terminalia chebula* fruits [6]. There are several reports which demonstrated that the alkaloids, glycosides, and flavonoids rich plant and plant extracts possess sedative property mediated through their affinity (*in vitro*) with benzodiazepine site of GABAergic complex system or are direct or indirect modulators of this receptor [28, 29]. Datta *et al.*, 2004 [30] described that the flavonol glycoside quercetin-3-O-(6″-feruloyl)-β-D-galacto-pyranoside, isolated from the aerial parts of *Polygonum viscosum* presented CNS depressant activity. Rutin is a glycoside derivative that exerted a significant CNS depression activity at 10 mg/kg [31]. Both the quercetin and rutin were found to be present in *Terminalia chebula* [32]. Besides, nonspecific CNS depression can also be attributed to tannin [33]. Therefore, it appears that the above-mentioned phytochemicals present in the extract may contribute to the sedative effects on the CNS.

The experimental screening method is imperative to establish the safety and efficacy of traditional and herbal products and also to set up the active components of the herbal products. In the acute toxicity test of the extract, there was no mortality or any signs of behavioral changes or toxicity observed after oral administration of extract up to the dose level of 3000 mg/kg body weight in mice. The results of the present study have shown that acute administration of extract may be safe up to 3000 mg/kg body weight.

The oral lyophilized cake can be considered as an interesting fast dissolving oral dosage form [17]. Fast dissolving lyophilized cakes offer the combined advantages of performance, convenience [16], rapid onset of action, patient compliance, and allow administration of an oral solid dosage form in the absence of water or fluid intake [34]. F_4 has been selected as the best formula among four formulations which has a crystalline structure containing an elegant cake. The prepared oral lyophilized cake was evaluated for various pharmaceutical parameters and results were mentioned in table 3. According to the results, it is evident that the formulated lyophilized cake showed good physicochemical properties. When placed on the tongue, lyophilized cake disintegrates instantaneously, releasing the drug, which dissolves or disperses in the saliva [35]. The formulation exhibited fast dissolution of drugs depicted in fig. 6 and 7. All essential peaks of the extract obtained from the FTIR imaging of the formulation ensure no drug-excipient interaction as shown in fig. 8.

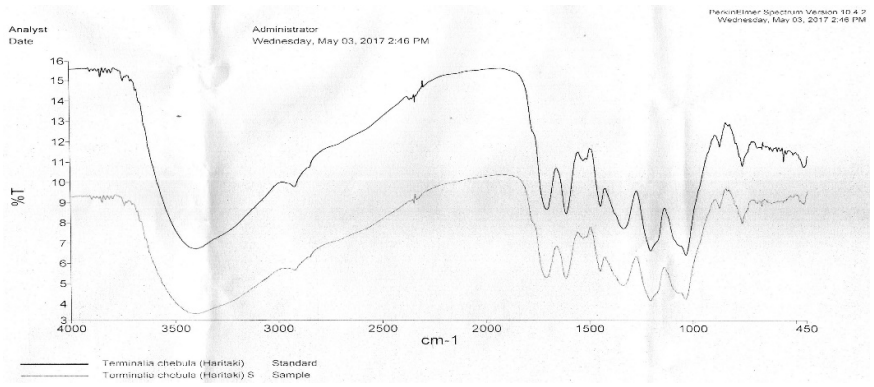

Fig. 8: Combined FT-IR

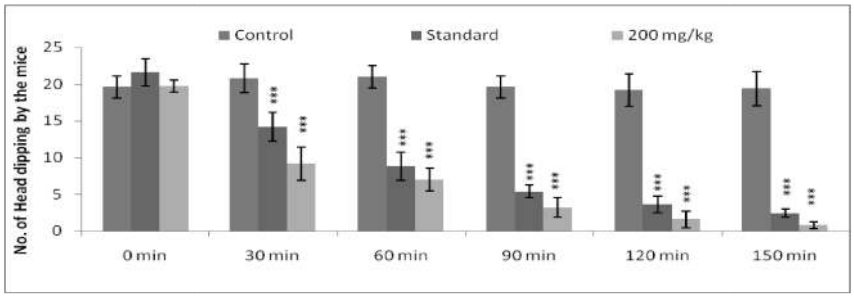

Fig. 9: Effect of AETC fruits from oral lyophilized cake on hole board test, all values are expressed as the mean±standard deviation of the mean (SDM), n=5. The difference between groups was analyzed by one-way ANOVA. *p<0.05, **p<0.01 and **p<0.001 were considered to be statistically significant

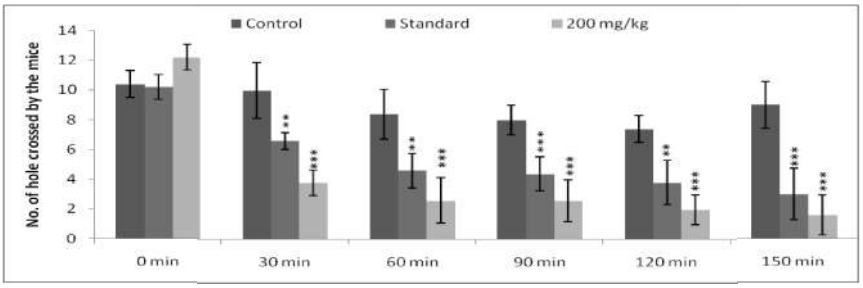

Fig. 10: Effect of AETC fruits from oral lyophilized cake on hole cross test, all values are expressed as the mean±standard deviation of the mean (SDM), n=5. The difference between groups was analyzed by one-way ANOVA. *p<0.05, **p<0.01 and **p<0.001 were considered to be statistically significant

The lyophilized product was also evaluated for CNS activity through the hole board and hole cross tests at the dose of only 200 mg/kg body weight. There were no significant differences in the CNS activities in both methods. In the hole board method, the number of head dipping (fig. 9) by mice using crude extract and the formulated cake was about to equal. On the other hand, in hole cross test (fig. 10) almost similar result was found in the case of crude extract and formulated cake. According to CNS result and FTIR analysis, it can be ensured that active ingredients of crude extract were as usual as in the formulated product.

CONCLUSION

The present study revealed that rapid dissolving and long-lasting new aqueous lyophilized cake of *Terminalia chebula* fruits may contribute to benefit in the health care sector due to its significant CNS depression activity. As per ICH guidelines, the stability analysis ensures the stability of newly formulated herbal preparation. The typical F_4 formulation was found to have all the desirable properties. The effect is rapid, long-lasting, and statistically significant at the experimental doses tested. However, further studies are needed to isolate the bioactive compound(s) and explicate the precise molecular mechanisms in order to establish a safe and effective dosage. Furthermore, it is required to verify the possibility of its use in the prevention and treatment of neurological disorders in humans.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Pharmacy Discipline of Khulna University and Modern Pharmaceuticals Ltd. for their laboratory support.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

The study was designed by Md. Monirul Islam and Md. Anisuzzman. All the authors have contributed equally to the research work, calculations, interpretations, and manuscript preparation.

CONFLICT OF INTERESTS

There are no conflicts of interest to declare.

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