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Analgesic, Anti-inflammatory and CNS Depressant Activities of the Methanolic Extract of *Abelmoschus esculentus* Linn. Seed in Mice

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Author's contributions

This work was carried out in collaboration between all authors. Author MLN designed the study, performed the statistical analysis. Author MMI wrote the protocol and wrote the first draft of the manuscript. Authors MSM and SM managed the analyses of the study. Authors MNN and MRI managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The study was carried out to assess the analgesic, anti-inflammatory and CNS depressant activity of the methanolic extract of *Abelmoschus esculentus* Linn. Seeds.
Study Design: The Present study was designed to observe pharmacological activities of the crude extract of the plant *Abelmoschus esculentus seeds.* The study consisted of hot extraction of the seeds of the *A. esculantus* with methanol. Afterwards, Methanolic crude extract was filtered and the filtrate was evaporated. Finally, screening of analgesic, anti-inflammatory and CNS depressant activity of crude extracts of *A. esculantus* on Swiss Albino mice.
Place and Duration of Study: Department of Pharmacy, Atish Dipankar University of Science and Technology, Dhaka, Bangladesh. January, 2013- July 2013.
Methodology: The animals are divided into Four groups and each group consists of five

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mice. Analgesic activity was performed by acetic acid-induced writhing model and formalin induced licking and biting in mice. Anti-inflammatory effects of *Abelmoschus esculentus* seed extract were done by carrageenan induced anti-inflammatory method at the dose of 100 and 200 mg/kg b.wt., (p.o). The CNS depressant activity was evaluated by observing the reduction of locomotor and exploratory activities in the hole cross and opens field tests at the dose of 100 and 200 mg/kg body weight.

Results: In statistical analysis, the dose (200 mg/kg) was found to exhibit (significant p=0.05) better analgesic activity (65.16% and 54.38%) against both acetic acid and formalin induced pain in mice which is about similar to standard drug Indomethacin. The extract of *A. esculentus* (100 and 200mg/kg) also showed sustained inhibition (54.97% and 65.56%) of paw edema at the 4th hour compared to Indomethacin (74.17%). Besides this *A. esculentus* (significant p=0.05) seed extract (100 and 200mg/kg p.o.) also possesses depressant activity at 90min in both methods. Conclusion: this study recommends that the methanolic extract of the *Abelmoschus esculentus* seeds has significant CNS depressant, analgesic and anti-inflammatory properties.

Keywords: Abelmoschus esculentus; analgesic; Formalin induced pain; anti-inflammatory; CNS Depressant.

1. INTRODUCTION

Abelmoschus esculentus, (*A. esculentus*L , family-Malvaceae) is an annual or perennial herbaceous plant and its height up to 2 m. *A. esculentus* (common okra) is most widely cultivated in south and east Asia, Africa and the southern USA [1]. Okra plays a significant role in the human diet due to presence of carbohydrate, minerals and vitamins. K, Na, Mg, Ca, Fe, Zn, Mn and Ni [2,3]. Okra seeds could serve as substitute rich sources of protein, fat, fiber and sugar [4,5,6]. The natural phenolic content of okra seeds has been stated [3,6,7]. The pods, flowers, leaves and fruits of *A. esculentus* are used as therapeutic diets due to the presence of nutrient content. In many countries such as Turkey and Cyprus, the plants are used in making medicinal remedies and diminish swelling; inflammation [8]. The consumption has been described to reduce serum cholesterol, triacylglyceride and blood pressure [9]. In menstrual pains and hypertension, the parts are used as part of therapeutic diet in any part of Africa such as Ethiopia [10]. The fruit of *A. esculentus* has emollient, demulcent and diuretic activity and a vulnerary activity [11,12]. The two flavonol glycosides were isolated from the fruits of *A. esculentus,* have the strong ability for scavenging DPPH and FRAP free radical by the experiment of antioxidant activities [13]. In the above consideration, the pharmacological rationale for some of the reporters and traditional uses of the plant, the methanolic extract of *Abelmoschus esculentus* Linn. seeds (MEAES) were evaluated for analgesic, anti-inflammatory and CNS depressant activity in mice.

2. MATERIALS AND METHODS

2.1 Plant Material

For this present investigation, the fresh seeds of *A. esculentus* collected from the area of Tangail, Bangladesh and were identified by the experts of Bangladesh National Herbarium, Dhaka, where a voucher specimen has been retained. The collected plant parts were dried for one week and pulverized into a coarse powder with the help of a suitable grinder. The

powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

2.2 Preparation of Extracts

About 150 gm of powdered material was taken in a clean, flat bottomed glass container and soaked in 200 ml of 85% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate (methanol extract) obtained was evaporated using a rotary evaporator. It rendered a gummy concentrate of reddish black color. The gummy concentrate was designated as a crude extract of methanol. The extract was transferred to a closed container for further use and protection.

2.3 Animals

Swiss albino mice of either sex weighing about 25-35 gm were used for the experiment. The mice were purchased from the animal Research Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B) were used for the evaluation of analgesic activity, anti-inflammatory and CNS depressant. The animals were housed under standard laboratory conditions (relative humidity55–65%, room temperature 23.0±2ºC and 12-h light, 12-h dark cycle). The animals were fed with a standard diet and water ad libitum in all animal experiments; the guidelines of the Animal Experimentation Ethics Committee, ICDDR, B were followed. Each group consists of five mice and the animals are divided into four groups.

2.4 Chemicals

Indomethacin and Ibuprofen were obtained from Square Pharmaceuticals Ltd., Bangladesh, Acetic acid was collected from Merck, Germany. Normal saline water (0.9%) Sodium chloride was brought from Beximco Infusion Ltd. Bangladesh. BDH Chemicals Ltd provided Tween 80. Formalin, carageenan and all other chemicals were of analytical grade.

2.5 Photochemical Screening of the Extract

The extract of *Abelmoschus esculentus* Linn seed was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, tannins, proteins and flavonoids (Table 1).

Table 1. Phytochemical screening of methanolic extracts of *Abelmoschus esculentus* Linn seed

Test	Glycoside	Phytosterol	Protein	Tannins	Flavonoid	Alkaloid	Carbohydrate
Result	-	+	+	+	+	-	+

Here, (+) ve= presence, (-) ve =absence

2.6 Analgesic Activity

2.6.1 Acetic acid-induced writhing method

The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice [14]. Test samples (100 and 200 mg/kg body weight), vehicle (1% tween 80 in water) and Indomethacin (10mg/kg) were administered orally 30 min before intraperitoneal administration of 0.1% acetic acid. Then the mice were observed for specific contraction of the body referred to as 'writhing' for the next 20 min [15]. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group while Indomethacin (10mg/kg) was used as a reference substance (positive control).

The percent inhibition (% analgesic activity) was calculated by % inhibition = {(A-B) /A} X 100.

Where, A= Average number of writhing of the control group; B= Average number of writhing of the test group.

2.6.2 Formalin test

The antinociceptive activity of the drugs was determined using the formalin test described by [15]. The control group received 5% formalin. 20 µl of 5% formalin was injected into the dorsal surface of the right hind paw 60 min after administration of MEAES (100 and 200 mg/kg, p.o.) and Indomethacin (10mg/kg, p.o.). The mice were observed for 30 min after the injection of formalin, and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is referred to as the early phase and the period between 15 and 30 min as the late phase. The total time spent licking or biting the injured paw (pain behavior) was measured with a stop watch.

2.7 Anti-inflammatory Activity

2.7.1 Carrageenan-induced paw edema method

The mice were divided into five groups, each containing 5 mice. Acute inflammation was induced by injecting 0.1 ml of (1%) carrageenan into the plantar surface of the mouse's hind paw [16]. The MEAES (100 and 200 mg/kg), normal saline (1ml/kg) and Indomethacin at a dose of (10 mg/kg/i.p) as referral agent were administered 30 min before carrageenan injection. The paw volume was measured at 1h, 2h, 3h, and 4h using a vernier caliper to determine the diameter of edema. The difference between the readings at time 1 h and different time interval was taken as the thickness of edema.

2.8 CNS Depressant Activities

2.8.1 Hole cross test

The method was carried out as described by [17]. A steel partition was fixed in the middle of a cage having a size of 30×20×14 cm. A hole of 3 cm diameter was made at a height of 7.5

cm in the center of the cage. Twenty animals were divided into four groups with five mice in each group. The group I animals received vehicle (1% Tween 80 in water, 10 ml kg-1 p.o.), animals of Group II received diazepam at 1 mg/kg body weight (p.o.) while Group III and Group IV were treated with 100 and 200 mg/ kg body weight (p.o.) of the MEAES. The number of passages of a mouse through the hole from one chamber to another was counted for a period of 3 min on 0, 30, 60, 90 and 120 min after oral administration of test drugs.

2.8.2 Open field test

The animals were treated as discussed above. The experiment was carried out according to the methods described by [18]. The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had 40 cm height a wall. The number of squares visited by the animals was counted for 3 min for 0, 30, 60, 90 and 120 min after oral administration of test drugs.

2.9 Statistical Analysis

Results are expressed as the mean±SEM. Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the vehicle control group; p=0.05 was considered as statistical significant.

3. RESULTS

3.1 Phytochemical Screening of *Abelmoschus esculentus* Linn Seed

The methanolic extract of *A. esculentus* Linn seed has shown the presence of phytosterol, protein, tannins and Flavonoid and absence of Glycoside and carbohydrate.

3.2 Analgesic Activity

3.2.1 Acetic acid induced writhing in mice

The result of the effect of *A. esculentus* against acetic acid induced writhing in mice is shown in (Fig. 1). The *A. esculentus* (100 and 200 mg/kg) dose dependently reduced acetic acid induced abdominal constrictions and stretching. The reduction was significant (p=0.05) when compared to control. The effect of the extract (200 mg/kg) was parallel to that of the standard drug, Indomethacin (10 mg/kg).

3.2.2 Formalin induced hind paw licking in mice

The result of the effect of the *A. esculentus* against formalin induced hind paw licking in mice is shown in (Fig. 2). The *A. esculentus* (100 and 200 mg/kg) pretreated animals showed a significant (p=0.05) dose-related reduction of the hind paw licking caused by formalin at the second phase which is compared to control. *A. esculentus* (200 mg/ kg) showed better activity in comparing to standard (Indomethacin 10 mg /kg) at the late phase.

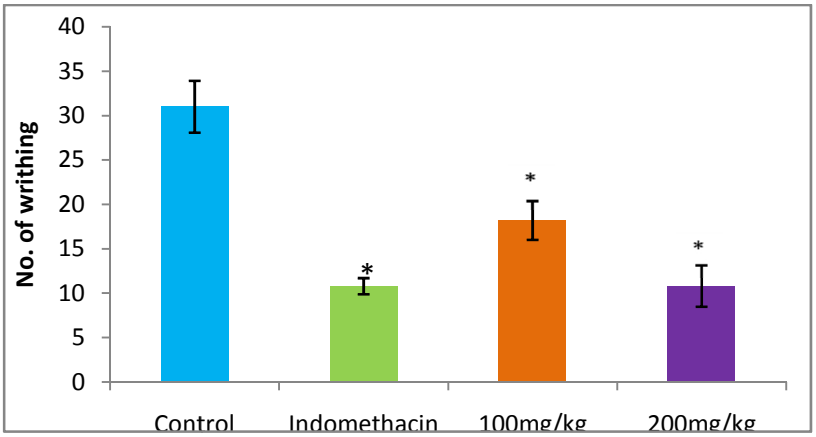

Fig. 1. Graphical presentation of the methanolic extract of *A. esculentus* seeds on acetic acid-induced writhing in mice.

*Values are mean ± SEM, (n = 5); *p=0.05 as compared to vehicle control (One way ANOVA followed by Dunnet test). Group I animals received vehicle (1% Tween 80 in water), Group II received Indomethacin 10 mg/kg body weight, Group III and IV were treated with 100 and 200 mg/kg body weight (p.o.) of the MEAES respectively.*

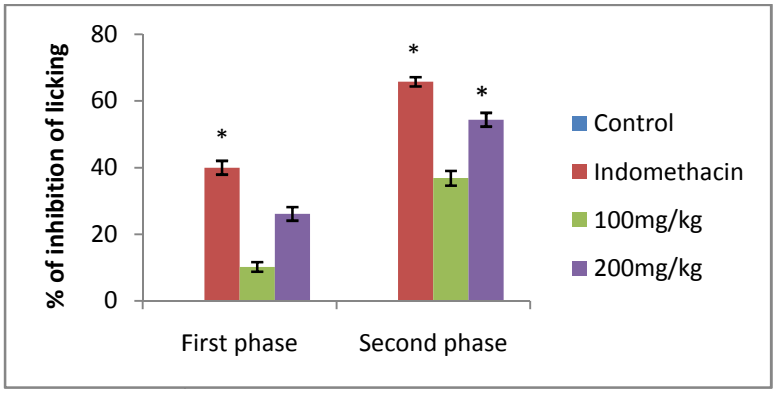

Fig. 2. Graphical presentation of the Percent of inhibition of methanolic extract of *A. esculentus* seeds on formalin induced hind paw licking in mice.

*Values are mean± SEM, (n = 5); at first (0-5min) & second (15-30min) phase *p=0.05 as compared to vehicle control (One way ANOVA followed by Dunnet test). Group I animals received vehicle (1% Tween 80 in water), Group II received Indomethacin (10 mg/kg) body weight, Group III and IV were treated with 100 and 200 mg/kg body weight (p.o.) of the MEAES.*

3.3 Carrageenan Induced Paw Edema in Mice

The result of the effect of *A. esculentus* on carrageenan-induced edema is shown in (Table 2). The *A. esculentus* exerted a significant (p=0.05) anti-inflammatory effect at the dose of 100, 200 mg kg^{-1} at 4h which was comparable to that of the control group. The percentage inhibition activity of *A. esculentus* (100, 200 mg kg^{-1}) and standard (Indomethacin) 10 mg/kg were found to be 45.33%, 27.55% and 34.22%, respectively.

Table 2. Effect of methanolic extract of *A. esculentus* seeds on carrageenan induced paw edema in mice.

Group	Dose	Oedema diameter(mm)				Inhibition (%)			
		1h	2h	3h	4h	1h	2h	3h	4h
Control	Vehicle	3.5 ±0.48	3.56 ±0.48	3.44 ±0.52	4.7 ±0.7				
Indomethacin	10mg/kg	2.96 ±0.54*	2.86 ±0.51*	2.76 ±0.72*	2.46 ±0.63*	16.85	19.66	19.77	45.33
MEAES	100mg/kg	3.48 ±0.47	3.44 ±0.41	3.36 ±0.43	3.26 ±0.37*	2.25	3.37	2.33	27.55
MEAES	200mg/kg	3.28 ±0.44	3.24 ±0.43	3.18 ±0.39	2.96 ±0.41*	7.87	8.99	7.56	34.22

*Probability values (calculated as compared to control using one way –ANOVA followed by Dunnet's test): *P=0.05.All values are Mean of individual data obtained from four mice(n=5);± indicates Standard error mean;, Group III and IV were treated with 100 and 200 mg/kg body weight (p.o.) of the MEAES.*

3.4 CNS Depressant Activities

3.4.1 Hole-cross test

Results of the hole-cross test of *A. esculentus* are given in (Table 3). The Depressant activity of standard and extract were statistically significant (P=0.05) of all dose levels at 90 and 120min and followed a dose-dependent response. The depressing effect of 100 and 200mg/kg was a better effect than standard.

Table 3. Effect of methanolic extract of the *A. esculantus* seeds in hole cross test in mice

		Number of Movements				
Group	Dose	0 min	30 min	60 min	90 min	120 min
Group-I	10ml/kg,	8.40±1.72	7.2±1.61	6.4±1.44	9.4±1.51	6.6±1.72
Group-II	1mg/kg,	7.00±1.25	4.20±1.22*	3.8±0 .91	4±1.45*	3.2±1.39*
Group-III	100 mg/kg	7.80±1.28*	5.0±1.19	4.6±1.35	4.6±1.23*	2.4±1.07*
Group-IV	200 mg/kg	7.20±.91*	4.80±0.91	2.8±1.33*	3.60±1.16*	1.8±0.91*

*Values are mean ± SEM, (n = 5); * p<0.05, Dunnet test as compared to vehicle control. Group I animals received vehicle (1% Tween 80 in water), Group II received diazepam 1 mg/kg body weight, Group III and Group IV were treated with 100 and 200 mg/kg body weight (p.o.) of the MEAES.*

3.4.2 Open-field test

Results of the open-field test of *A. esculentus*are given (Table 4). The *A. esculentus* extract exhibited a decrease in the movements of the test animals at all dose levels. The results of Stansard and extract (100 and 200 mg/kg) were statistically significant (P=0.05) at 90min followed a dose-dependent response. The extract 200mg/kg exhibited similar depressant effect to standard (Diazepam) at 4th phase.

Table 4. Effect of methanolic fruit extract of *A. esculantus* on Open Field test in mice

Group	Dose	Number of Movements			
		0 min	30 min	60 min	90 min
Group-I	10ml/kg	217±9.27	195±3.43	185±5.44	179±6.77
Group-II	1mg/kg	204±6.67	88±1.23*	70±1.92*	64±1.96*
Group-III	100 mg/kg,	252±10.19	230±7.20	136±8.95	77±7.84*
Group-IV	200 mg/kg,	206±5.37	149±5.20	94±5.19*	64±4.55*

*Values are mean ± SEM, (n = 5); * p<0.05, Dunnet test as compared to vehicle control. Group I animals received vehicle (1% Tween 80 in water), Group II received diazepam 1 mg/kg body weight, Group III and Group IV were treated with 100 and 200 mg/kg body weight (p.o.) of the MEAES.*

4. DISCUSSION

Members of Abelmoschus have been reported to reveal diverse medicinal properties ranging from antidiabetic, antimicrobial, anticancer, analgesic, antioxidant and antiplasmodial activities. Though there have been reports that some members of this genus exhibit toxic effect, extracts from the leaves, fruits and roots have shown no harmful effects on living cells [19]. Powder of fruit of the plant showed the presence of carbohydrate, gums and mucilages, proteins, phytosterols, flavonoids, tannins and phenolic compounds and volatile oil [20]. Acetic acid induces pain by enhancing levels of PGE_2 and $PGF^{2\alpha}$ [21] at the receptors of peritoneal cavity [22,23], which mean the acetic acid acts indirectly by increasing the release of endogenous mediators, leading to stimulation of the nociceptive neurons which are sensitive to most of the non-steroidal anti-inflammatory drugs. *A. esculentus* containing scopoletin is capable of ameliorating clinical symptoms of rat adjuvant-induced arthritis, by reducing numbers of new blood vessels in the synovium and the production of important endogenous angiogenic inducers [24]. The two different doses (100 & 200 mg/kg b. wt.) of crude extract showed 41.29% and 65.16% of inhibition while 200 mg/kg was found to exhibit more analgesic activity (significant *p=0.05, Fig. 1) against acetic acid induced pain and this percent of inhibition is similar to the reference drug Indomethacin (65.16%). This result suggests the taking part of peripheral mechanisms of analgesia.

The formalin test is another important model of analgesic which is better related to clinical pain [25,26]. This method elucidates central and peripheral activities. Formalin-induced nociception is biphasic in which first phase involves direct stimulation of sensory nerve fibers representing neuropathic pain and second phase involves inflammatory pain mediated by prostaglandin, serotonin, histamine, bradikinin and cytokines such as IL-1β, IL-6, TNF-α, eicosanoids and NO [27,28,29,30,31,32]. In our previous study the methanol extract of *A. esculentus* roots have shown analgesic and CNS depressant activity [33]. But in our study seed extract of *A. esculentus* showed inhibition against pain at the second phase of formalin induced nociception in mice. The extract (100 and 200 mg/kg) caused percent of protection (36.84% and 54.38% respectively) against licking and biting and Inhibition of percentage (*p=0.05) at the dose 200mg/kg is closest to standard, Indomethacin (65.79%, Fig. 2). So, the inhibition against licking response at the late phase of formalin test demonstrate specifying analgesic effect of the extract (Fig. 2). The suppression of neurogenic and inflammatory pains by the extract might imply that it contains active analgesic principles that may be acting both centrally and peripherally. This is an indication that the extract can be used to manage acute as well as chronic pain.

Carageenan –induced paw edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic in which the early phase (1-2) of

the carageenan model is mainly mediated by histamine, serotonin, and increased synthesis of prostaglandins in the damaged tissue surroundings and the late phase is sustained by prostaglandin release and mediated by bradikinin, leukotrienenes, polymorpho nuclear cells, and prostaglandins produced by tissue macrophase [34,35]. Further, in our study the crude methanol extract (100 and 200 mg/kg) of *A. esculentus* seeds exhibited (*P=0.05) inhibition (27.55% and 34.22%) of paw edema at the 4th hour while the standard (Indomethacin) reported 45.33% inhibition at the same hour (Table 2). The possible mechanism of the observed anti-inflammatory activity might be its ability to reduce the release of histamine, serotonin or kinin like substances or biosynthesis of prostaglandins which is consistent with the test of analgesic activity.

Locomotor activity considered as an increase in alertness and decrease in locomotor activity indicated sedative effect [36]. Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs are elucidated their action through GABA, therefore it is possible that extracts of *A. esculentus* may act by potentiating GAB Aergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extracts [37]. Many research presented that plant containing flavonoids, saponins and tannins is useful in many CNS disorders [38]. In the hole cross test, all doses showed significant (*P=0.05) effect at 60 and 90 min (Table 3) and depressant activity of extracts were nearer to reference drug. But in case of open field test the plant extract (200 mg/kg) exhibited specific activity at 60 min but at 90 min (100 mg/kg and 200 mg/kg) revealed significant (*P=0.05) depressant activity (Table 4).Earlier investigation on phytoconstituents and plants suggests that many flavonoids and neuroactive steroids were found to be ligands for the GABA receptors in the central nervous system; which led to assume that they can act as benzodiazepine like molecules [39].

5. CONCLUSION

The methanolic extract of *A. esculentus* seeds showed low activity at the dose 100mg/kg. But in case of 200mg/kg dose exhibited significant antinociceptive, anti-inflammatory and CNS depressant activity. The present work was a preliminary effort which will require further detailed investigation, including characterization of active compounds and requires preformulation studies for development of a potential dosage form.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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