

Molecular & Clinical Pharmacology



CNS depressant and analgesic activities of Okra (*Abelmoschus esculentus* Linn.)

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Received: 29 August 2012, **Revised:** 8 October 2012, **Accepted:** 13 October 2012

Abstract

Okra (*Abelmoschus esculentus*) is used in folk medicine in the management of diabetes, diarrhea and inflammation. The present study was designed to evaluate the central nervous system (CNS) depressant and analgesic activity of the methanolic extract of *Abelmoschus esculentus* root (MAER). CNS depressant study of the extract (100 and 200 mg/kg, p.o.) was done by open field and hole cross test whereas acetic acid writhing test and formalin induced pain was done for analgesic activity. A statistically significant ($p < 0.05$) decrease in locomotor activity was observed at all doses in the open-field and hole-cross tests. The extract significantly ($p < 0.05$) and dose dependently reduced the writhing reflex in the acetic acid-induced writhing test as well as linking response in the formalin induced inflammatory pain. The finding of this study suggested that *Abelmoschus esculentus* possesses good CNS depressant activity along with high analgesic activity.

Keywords: CNS depressant; analgesic; *Abelmoschus esculentus*

Introduction

The use of the medicinal plants is increasing in many countries where 35% of drugs contain natural products. At present, thousands of plant metabolites are being successfully used for the treatment of variety of diseases (Rakh & Chaudhari, 2010). The investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant based drugs (Kumara, 2001). In Bangladesh thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. The beneficial medicinal effects of plant materials typically results from combinations of

secondary product present in plant such as alkaloids, steroids, tannins, phenol compounds, resins, gums, flavonoids and fatty acids which are capable of producing definite physiological action on body (Joshi *et al.*, 2009).

Okra (*Abelmoschus esculentus* L.) is an important vegetable which is widely distributed from Africa to Asia. Okra plays an important role in the human diet by supplying carbohydrate, minerals and vitamins. K, Na, Mg and Ca were found to be the principle elements, with Fe, Zn, Mn and Ni also present (Moyin-Jesu, 2007; Arapitsas, 2008). Okra seeds could serve as alternate rich sources of protein, fat, fiber and sugar (Oyelade *et al.*, 2003; Deters *et al.*, 2005; Adedokun *et al.*, 2009a). The natural phenolic content of okra seeds has been reported (Huang *et al.*, 2007; Arapitsas, 2008; Adedokun *et al.*, 2009b). With a view to find the pharmacological rationale for some of the reported and traditional uses of the plant, the methanolic extract of *Abelmoschus esculentus* Linn. root (MAER) was evaluated for central nervous system (CNS) depressant and analgesic activity in mice.

Materials and Methods

Plant materials and extraction

The roots of *Abelmoschus esculentus* plant were collected from Rajshahi, Bangladesh in the month of September 2011 and identified by DR. M.A. Razzaque Shah PhD, Tissue Culture Specialist, BRAC Plant Biotechnology Laboratory, Bangladesh. The dried and coarsely powdered leaves (400 g) were extracted with methanol at room temperature for 72 h. The filtrate was evaporated to dryness under reduced pressure (45 °C) to afford the crude extract (yield ca. 6%) used in pharmacological screening.

Animals

Swiss albino mice of either sex weighing about 25-35gm were used for the experiment. The mice were purchased from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B) were used for the evaluation of CNS depressant and analgesic activity. The animals were housed under standard laboratory conditions (relative humidity 55–65%, room temperature 23.0±2.0°C 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.

Drugs and chemicals

Folin-chiocaltu phenol reagent, were purchased from E. Merck (Germany). Tween-80 was obtained from BDH Chemicals, UK. Formalin was purchased from CDH, India. Normal saline solution was purchased from Beximco Infusion Ltd., Bangladesh. Diclofenac Na and diazepam was obtained from Square Pharmaceuticals Ltd., Bangladesh. All chemicals used were of analytical reagent grade

Preliminary phytochemical analysis

The crude extract of *Abelmoschus esculentus* root (MAER) was subjected to a preliminary phytochemical screening for the presence of alkaloid, polyphenol, tannins and glycoside (Harborne, 1984).

The amount of phenolic compounds and flavonoids

The total phenolic and flavonoid content of MAER was determined using Folin-ciocalteu reagent (Yu *et al.*, 2002) and aluminium chloride colorimetric method (Chang *et al.*, 2002), respectively. The content of total phenolics in MAER was calculated from regression equation of the calibration curve ($y=0.013x+0.127$, $r^2=0.988$) and is expressed as galic acid equivalents (GAE) and the flavonoid contents of the extract was expressed in terms of quercetin equivalent (the standard curve equation: $y=0.009x-0.036$).

Acute toxicity test

Test animals were divided into groups ($n = 6$ per group) which were administered different doses of the crude extract (62.5, 125, 250, 500, 1000, 2000 and 4000 mg/kg p.o.), while the control group received only the vehicle (1% Tween 80 in water, p.o.). The general signs and symptoms of toxicity were observed for 24 h and mortality was recorded for each group at the end of this period (Lorke, 1983).

CNS depressant activity

Hole Cross Test

The method used was done as described by Takagi *et al.*, (1971). The animals were divided into control, standard and test groups ($n = 6$ per group). The control group received vehicle (1% Tween 80 in water at the dose of 10 ml/kg p.o.) whereas the test group received MAER extract (at the doses of 100 and 200 mg/kg p.o.) and standard group received diazepam at the dose of 1mg/kg body weight orally. Each animal was then placed on one side of the chamber and the number of passages of each animal through the hole from one chamber to the other was recorded for 3 min on 0, 30, 60, 90, 120, 180 and 240 min during the study period.

Open Field Test

This experiment was carried out as described by Gupta *et al.*, (1971). The animals were divided into control standard and test groups ($n = 6$ per group). The control group received vehicle (1% Tween 80 in water at the dose of 10 ml/kg p.o.). The test group received the crude extract (at the doses of 100 and 200 mg/kg p.o.) and standard group received diazepam at the dose of 1mg/kg body weight orally. The animals were placed on the floor of an open field (100 cm×100 cm×40 cm h) divided into a series of squares. The number of squares visited by each animal was counted for 3 min on 0, 30, 60, 90, 120, 180 and 240 min during the study period.

Analgesic activity

Acetic acid induced writhing method

The analgesic activity of the samples was studied using acetic acid-induced writhing model in rats. Test samples (at the doses of 100 and 200 mg/kg) and vehicle (1% tween 80 in water) were administered orally to rats (n=6) 30 minutes prior to intraperitoneal administration of 0.7% v/v acetic acid solution (0.1ml/10g). The positive control group received Diclofenac-Na at the dose of 10 mg/kg p.o. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min (Ahmed *et al.*, 2006).

Formalin test

The analgesic activity of the drugs was determined using the formalin test described by Dubuission and Dennis (1977). Control group received 5% formalin. 20 μ l of 5% formalin was injected into the dorsal surface of the right hind paw 60 min after administration of MAER (100 and 200 mg/kg, p.o.) and Diclofenac Na (10 mg/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.

Statistical Analysis

All data were expressed as mean \pm S.E.M. One-way ANOVA followed by Dunnett's multiple comparison tests was used to analyze the data obtained from *in vivo* experiments. All statistical analyses were performed with Prism 4.0 (GraphPad software Inc., San Diego, CA). $P < 0.05$ was considered to be significant.

Results

Preliminary phytochemical analysis and acute toxicity

Results of the preliminary phytochemical analysis carried out on the crude methanol extract indicated the presence of alkaloid, polyphenol, tannins and glycoside. No lethal effects were observed within 24 h after the administration of the extract at any of the doses used, even at the highest dose tested (4000 mg/kg). Therefore, the lethal dose (LD₅₀) of the extract in mice could not be determined.

Total phenolic and flavonoid contents

Table 1 represents the content of both groups in MAER extract. The content of total phenolics in the extract of *A. esculentus* was determined using the Folin-ciocalteu assay, calculated from regression equation of the calibration curve ($y=0.013x+0.127$, $r^2=0.988$) and is expressed as galic acid equivalents (GAE) and the flavonoid contents of the extract was expressed in terms of quercetin equivalent (the standard curve equation: $y=0.009x-0.036$).

Table 1. Yield, total amount of plant phenolic compounds and flavonoids of methanolic extract of *Abelmoschus esculentus* root.

Sample	Yield (%)	^a Total phenols mg/g plant extract (in GAE)	^b Total flavonoids mg/g plant extract (in QA)
MPBL	39.92	82.33 ± 1.02*	40.16 ± 0.61*

The GAE, and QA values are expressed as Means±SEM of triplicate experiments. ^aGalic acid equivalents (GAE, mg/g of each extract) for the total phenolic content, ^bQuercetin equivalent (QA, mg/g of each extract) for the total flavonoid content.

Table 2. Effect of methanolic extract of *Abelmoschus esculentus* root on hole cross test in mice.

Group	Dose	Number of Movements				
		0 min	30 min	60 min	90 min	120 min
Group-I	10ml/kg,	110.4 ± 1.20	105 ± 1.30	109.4 ± 0.50	107.4 ± 1.16	108 ± 0.70
Group-II	1mg/kg,	111.2 ± 1.15	61.6± 0.43*	33.8± 0.58*	14.8± .86*	8.6 ± 0.50*
Group-III	100 mg/kg	108.4 ± 0.81	70.8±1.02*	49.8±1.35*	38.8± .02*	26 ± 0.71*
Group-IV	200 mg/kg	112.8 ± 1.43	65.8± .06*	38.6± .92*	18.6±0.92*	11.6± 0.60*

Values are mean ± SEM, (n = 6); * 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 MAER.

Table 3. Effect of methanolic extract of *Abelmoschus esculentus* root on Open Field test in mice

Group	Dose	Number of Movements				
		0 min	30 min	60 min	90 min	120 min
Group-I	10ml/kg	11.8 ± 1.15	10.3 ± 1.41	10.6 ± 0.92	11.2 ± 0.86	12 ± 0.54
Group-II	1mg/kg	10.2 ± 0.58	5 ± 0.70*	4.0 ± 0.83*	2.4±0.81*	1.2 ± 0.37*
Group-III	250 mg/kg,	11 ± 0.70	8.8 ± 0.58*	6.4±0.50*	5.2±0.37*	4.2 ± 0.37*
Group-IV	500 mg/kg,	11.2 ± 0.66	7.2 ± 0.37*	5.0 ± 0.70*	4.8±0.37*	2.4 ± 0.40*

Values are mean ± SEM, (n = 6); * 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 MAER.

CNS depressant activity

Open-field test

In the open-field test, MAER extract exhibited a decrease in the movements of the test animals at all dose levels tested. The results were statistically significant for all doses and followed a dose-dependent response (Table 2).

Hole-cross test

Results of the hole-cross test followed a similar trend to the ones observed in the open-field test. They were statistically significant for all dose levels and followed a dose-dependent response. The depressing effect was most intense during the second (60 min) and third (90 min) observation periods (Table 3).

Analgesic activity

Acetic acid-induced writhing test

Table 4 shows the effects of the extract of on acetic acid-induced writhing in mice. The oral administration of both doses of MAER significantly (p<0.001) inhibited writhing response induced by acetic acid in a dose dependent manner.

Table 4. Effect of methanolic extract of *Abelmoschus esculentus* root on acetic acid induced writhing in mice.

Groups	Dose	No. of writhing	Percent inhibition
Group-I	0.1 ml/10gm	33.33 ± 0.55	
Group-II	10mg/kg	10.03 ± 1.22*	69.90
Group-III	100mg/kg	21.0 ± 1.69*	36.99
Group-IV	200mg/kg	12.12 ± 0.76*	63.63

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 Diclofenac Na 10 mg/kg body weight, Group III and Group IV were treated with 100 and 200 mg/kg body weight (p.o.) of the MAER.

Table 5. Effect of methanolic extract of *Abelmoschus esculentus* root in hind paw licking in the formalin test in mice.

Groups	Dose	Early phase (Sec)	% protection	Late phase (Sec)	% protection
Group-I	10 ml/kg,	36.67 ± 1.38	-	42.0 ± 1.03	-
Group-II	10 mg/kg,	12.83 ± 0.90*	65.01	12.83 ± 0.70*	69.45
Group-III	100 mg/kg,	29.5 ± 0.76*	19.55	23.05 ± 0.95*	45.11
Group-IV	200mg/kg	17.17 ± 0.65*	53.17	14.0 ± 1.46*	66.66

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 Diclofenac Na 10 mg/kg body weight, Group III and Group IV were treated with 100 and 200 mg/kg body weight (p.o.) of the MAER.

Formalin test

MAER (100 and 200 mg/kg, p.o.) significantly ($P < 0.001$) suppressed the licking activity in either phase of the formalin-induced pain in mice in a dose dependant manner (Table 5).

Discussion

Locomotor activity considered as an increase in alertness and decrease in locomotor activity indicated sedative effect (Verma *et al.*, 2010). Extracts of *A. esculentus* decreased locomotor activity indicates its CNS depressant activity. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs are elucidation their action through GABA, therefore it is possible that extracts of MAER may acts by potentiating GABAergic 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 (Kolawole, Makinde & Olajide, 2007). Many research showed that plant containing flavonoids, saponins and tannins are useful in many CNS disorders (Bhattacharya & Satyan, 1997). Earlier investigation on phytoconstituents and plants suggests that many flavonoids and neuroactive steroids were found to be ligands for the GABA_A receptors in the central nervous system; which led to assume that they can act as benzodiazepinelike molecules (Verma *et al.*, 2010). Phytochemical investigations also showed the presence of alkaloids, flavonoids, and tannins in the extract, so might be this phytoconstituents are responsible for its CNS depressant activity.

Acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics and represents pain sensation by triggering localized inflammatory

response. Such pain stimulus leads to the release of free arachidonic acid from the tissue phospholipids (Ahmed *et al.*, 2006). The response is thought to be mediated by peritoneal mast cells (Ribeiro *et al.*, 2000), acid sensing ion channels (Voilley, 2004) and the prostaglandin pathways (Hossain *et al.*, 2006). The organic acid has also been postulated to act indirectly by inducing the release of endogenous mediators, which stimulates the nociceptive neurons that are sensitive to NSAIDs and narcotics (Adzu *et al.*, 2003). It is well known that non-steroidal anti-inflammatory and analgesic drugs mitigate the inflammatory pain by inhibiting the formation of pain mediators at the peripheral target sites where prostaglandins and bradykinin are proposed to play a significant role in the pain process (Hirose *et al.*, 1984). In addition, it was suggested that non narcotic analgesics produce their action by interfering with the local reaction to peritoneal irritation thereby reducing the intensity of afferent nervous stimulation in the acetic acid induced writhing test, a model of visceral pain (Vogel & Vogel, 1997).

The formalin model normally postulates the site and the mechanism of action of the analgesic (Chau, 1989). This biphasic model is represented by neurogenic (0-5 min) and inflammatory pain (15-30 min), respectively (Hunnskaar & Hole, 1987). Drugs that act primarily on the central nervous system such as narcotics inhibit both as steroids and NSAIDs suppress mainly the late phase (Adzu *et al.*, 2003). The suppression of neurogenic and inflammatory pains by the extract might imply that it contains active analgesic principle 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. The mechanism by which formalin triggers C-fibers activation remained unknown for a relatively long time. Recently, however, McNamara *et al.* (2007) demonstrated that formalin activates primary afferent neurons through a specific and direct on TRPA1, a member of the transient receptor potential family of cation channels, expressed by a subset of C-fiber nociceptors, and this effect is accompanied by increased influx of Ca^{2+} ions. TRPA1 cation channels at primary sensory terminals were also reported to mediate noxious mechanical stimuli (Kerstein *et al.*, 2009). These experiments suggest that Ca^{2+} mobilization through TRPA1 cation channels is concomitant with noxious chemicals and mechanical stimuli as they produce their analgesic action. It is likely that the inhibitory effect of MAER to pain response is due to inhibit the increase of the intracellular Ca^{2+} through TRPA1, presumably evoked by formalin. So, the extract of *A. esculentus* may contain substances that affect the metabolism of Ca^{2+} . Literature survey revealed that tannins, and flavonoid are the major phytoconstituents of *A. esculentus*. (Huang *et al.*, 2007; Arapitsas, 2008; Adalakun *et al.*, 2009b). Flavonoids, for example, have been found to suppress the intracellular Ca^{2+} ion elevation in a dose dependent manner, as well as the release of proinflammatory mediators such as TNF α . (Kempuraj *et al.*, 2005).

Our preliminary pharmacological studies on the methanol extract of *A. esculentus* roots provide in part scientific support for the use of this species in traditional medicine, particularly in various ailments related to CNS disorders, and pain. However, further pharmacological investigations are required to understand its underlying mode of action on the CNS, and mechanism of pain inhibition. In addition, future bioactivity-guided phytochemical work should be carried out to identify any active constituent(s).

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