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Comparative Pharmacological Studies of *Abelmoschuse sculentus* Linn. Fruits and Seeds

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Abstract: *Abelmoschus esculentus* Linn. is very popular and usually used in the traditional system of medicine. The present studies were carried out to investigate the analgesic, anti-inflammatory, CNS depression as well as the anti-diarrheal activity of the methanolic extract of *Abelmoschus esculentus* Linn. fruits(MAEF) and seeds (MAES).MAEF and MAES were used to investigate the analgesic effect by acetic acid induced writhing and formalin induced licking method whereas carrageenan induced inflammation was used for anti-inflammatory activity.CNS depression activities were evaluated in hole-cross and open field test methods. Furthermore castor oil-induced diarrhea and charcoal-induced gastrointestinal motility were used to investigate the anti-diarrheal activity of MAEF and MAES. But the doses of 100 and 200mg/kg body weight p.o., significantly (p<0.05) reduced the writhing caused by acetic acid and the number of licks induced by formalin in a dose dependent manner. At 200 mg/kg doses of MAES showed highest anti-inflammatory activity (% of inhibition, 70.06%) after 4 hrs. A statistically significant CNS depression activity was also observed in both hole cross and open field tests in a dose dependent manner. MAEF and MAES significantly reduced the frequency and severity of diarrhea in test animals throughout the study period in a dose dependent manner and also showed an effective (p<0.05) reduction in the gastrointestinal motility in charcoal meal test. Altogether, these results suggest that the *Abelmoschus esculentus* Linn. has good pharmacological effects conforming the traditional use of this plant.

Key words: Anti-Inflammatory · Analgesic · Anti-Diarrheal · CNS · *Abelmoschus esculentus* linn

INTRODUCTION

Plants have many important potent bioactive compounds such as alkaloids, polyphenol, glycosides, essential oils, fatty oils, resins, mucilage, tannins, gums and others that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs [1]. The active principles differ from plants to plants due to their biodiversity and produce a definite physiological action on the human body that develops interest on their medicinal properties and importance in the pharmaceutical industries. Today about 300 species of medicinal and aromatic plants as well as their secondary metabolites are used worldwide in the pharmaceuticals, foods, cosmetics and perfume industries [2]. 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 [3].

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.

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Abelmoschus esculentus Linn. commonly known as Ladies finger or Orka, is an important vegetable which is widely distributed from Africa to Asia. Studies have shown that the daily consumption of 100 grams of okra provides 20% of the calcium, 15% of the iron, and 50% of the vitamin C of human dietary requirements [4]. It is well known for its nutritional value and healing properties such as anticancer, reduced heart attack, lower blood cholesterol, relieve intestinal disorder, relieve inflammation of the colon, relive diverticulitis, relieve stomach ulcer, neutralize acid, lubricate large intestine, treatment of lung inflammation, treatment of irritable bowel, keep joints limber, as well as the treatment of sore throats, burns, reducing poisonings and psoriasis [5-7]. In some part of Africa such as Ethiopia, the parts are used as part of therapeutic diet against menstrual pains and for hypertension [8]. Okra seeds could serve as alternate rich sources of protein, fat, fiver and sugar. 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. Fruits (MAEF) and seeds (MAES) was evaluated for analgesic, anti-inflammatory, anti-diarrheal and CNS depression activity in *in vivo* methods.

MATERIAL AND METHODS

Plant Material and Extraction: The fresh fruits and seeds of *Abelmoschus esculentus* Linn. were collected from Savar, Dhaka, Bangladesh in July, 2012 and identified by DR. M.A. Razzaque Shah PhD, Tissue Culture Specialist, BRAC Plant Biotechnology Laboratory, Bangladesh and the voucher specimen no. maintained in our laboratory for future reference. The both plant materials were shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve #40 and stored in an air-tight container. The dried powder material of fruits (1.0 kg) and seeds (1.0 kg) was refluxed with MeOH for three hours. The total filtrate was concentrated to dryness; *in vacuo* at 40°C to render the MeOH extract 160 g and 180 g for fruits and seeds respectively.

Chemicals: Acetic acid, formalin and castor oil as well as carrageenan were purchased from E. Merck (Germany). Atropine, Loperamide, Diazepam, Indomethacin, Diclofenac-Na were collected from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals and reagents were of analytical grade.

Experimental Animals: Young Long-Evans rats of either sex weighing about 140-160 gm and Swiss Albino mice (25-30g) were used for assessing biological activity. The animals were maintained under standard laboratory conditions and had free access to food and water *ad libitum*. The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. The animals were divided into different groups, each consisting of five animals which were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee, Atish Dipankar University of Science & Technology, Dhaka, Bangladesh. Animal treatment and maintenance for acute toxicity and analgesic effects were conducted in accordance with the Principle of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and the Animal Care and Use Guidelines of Atish Dipankar University of Science & Technology, Dhaka, Bangladesh.

Acute Toxicity Study: Acute oral toxicity assay was performed in healthy adult male and non-pregnant adult female albino Swiss mice (25-30g) divided into different groups. The test was performed using increasing oral dose of the MSSL and MSSB (50, 100, 200, 500, 1000 mg/kg body weight p.o.), in 20 ml/kg volume to different test groups. Normal group received water. The mice were allowed to feed *ad libitum,* kept under regular observation for 48 hr, for any mortality or behavioral changes [9].

Analgesic Activity

Acetic Acid-Induced Writhing Test: The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice. The animals were divided into eight groups with five mice in each group. Group I animals received vehicle (1% Tween 80 in water, p.o.), animals of Group II received Diclofenac-Na at 10 mg/kg body weight while animals of groups III, IV and V, VI were treated with 100 and 200 mg/kg body weight (p.o.) of the MAEF and MAES respectively. Test samples and vehicle were administered orally 30 min before intra-peritoneal administration of 0.7% v/v acetic acid but Diclofenac-Na was administered intra-peritoneally 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min [10].

Formalin Test: The anti-nociceptive activity of the drugs was determined using the formalin test described by Dubuission and Dennis [11]. Control group received 2.5% formalin, 20 µl of 2.5% formalin was injected into the dorsal surface of the right hind paw 30 min after administration of MAEF and MAES (100 and 200 mg/kg, body weight p.o. respectively) and 15 min after administration of Diclofenac Na (10 mg/kg, body weight 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.

Anti-Inflammatory Activity

Carrageenan Induced Rat Paw Edema: Long-Evan rats (140-160 g) of both sexes were divided into six groups of five animals each. The test groups received 100 and 200 mg/kg body weight p.o. of the extract MAEF and MAES. The reference group received Indomethacin (10 mg/kg body weight, p.o.) while the control group received 3 ml/kg body weight normal saline. After 30 min, 0.1 ml 1% carrageenan suspension in normal saline was injected into the sub-planatar tissue of the right hind paw. The paw volume was measured at 1, 2, 3 and 4 hrs after carrageenan injection using a micrometer screw gauge. The percentageof inhibition of the inflammation was calculated from the formula:

$$\% \text{ inhibition} = (1-D_t/D_o) \times 100$$

where, D_o was the average inflammation (hind paw edema) of the control group of mice at a given time, D_t was the average inflammation of the drug treated (i.e., extract or reference Indomethacin) mice at the same time [12].

CNS Depression Activity

Hole Cross Test: The method used was done as described by Takagi *et al*. [13]. The animals were divided into different group and each group contains 6 animals. The control group received vehicle (1% Tween 80 in water at the dose of 10 ml/kg p.o.) whereas the test group received MAEF and MAES 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 (p.o.) 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 and 120 min during the study period.

Open Field Test: This experiment was carried out as described by Gupta *et al.*[14]. 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 hr) 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.

Anti-Diarrheal Activity

Castor Oil-Induced Diarrhea: The experiment was performed according to the method described by Shoba & Thomas [15]. Briefly, mice fasted for 24 hrs were randomly allocated to four groups of five animals each. The animals were all screened initially by giving 0.5 ml of castor oil. Only those showing diarrhea were selected for the final experiment. Group I received 1% CMC (10 ml/kg, p.o.), Groups III, IV, V, and VI were treated with 100 and 200 mg/kg body weight (p.o.) of the MAEF and MAES respectively. Group II was given Loperamide (3 mg/ kg, *p.o.*) in suspension. After 60 min, each animal was given 0.5 ml of castor oil, each animal was placed in an individual cage, the floor of which was lined with blotting paper which was changed every hour, observed for 4 hrs and the characteristic diarrheal droppings were recorded.

Effect on Gastrointestinal Motility: Animals were divided into four groups of five mice each and each animal was given orally 1 ml of charcoal meal (5% activated charcoal suspended in 1% CMC) 60 min after an oral dose of drugs or vehicle. Group I was administered 1% CMC (10 ml/kg body weight *p. o.*) and animals in groups III, IV and V, VI were treated with 100 and 200 mg/kg body weight (p. o.) of the MAEF and MAES respectively. Group II received atropine sulfate (0.1 mg/kg body weight *p. o.*) as the standard drug. After 30 min, animals were killed by light ether an aesthesia and the intestine was removed without stretching and placed lengthwise on moist filter paper. The intestinal transit was calculated as a percentage of the distance travelled by the charcoal meal compared to the length of the small intestine [16].

Statistical Analysis: All values were expressed as the mean±SEM (Standard Error Mean) of three replicate experiments. The analysis was performed by using SPSS statistical package for WINDOWS (version 16.0; SPSS Inc, Chicago). Results related to the reducing power

activities were statistically analyzed by applying the Student *t*-test and $p<0.05$ were considered to be statistically significant. All *in vivo* data are subjected to ANOVA followed by Dunnett's test and $p<0.05$ were considered to be statistically significant.

RESULTS

Acute Toxicity Studies: The acute toxicity studies mainly aim at establishing the therapeutic index, i.e., the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species. Both extract of MAEF and MAES were safe up to a dose of 1000 mg/kg *(p. o.)* body weight. Behavior of the animals was closely observed for the first 3 hr then at an interval of every 4 hrs during the next 48 hrs. The extract did not cause mortality in mice during 48 hrs observation but little behavioral changes, locomotor ataxia, diarrhea and weight loss were observed. Food and water intake had no significant difference among the group studied.

Analgesic Activity

Acetic Acid Induced Writhing Method: Table 1expressed the effect of MAEF and MAES on acetic acid induced writhing in mice. The analgesic activity of MAEF and MAES was significantly ($p<0.05$) inhibited writhing response induced by acetic acid in a dose dependent manner. MAES showed very good response than MAEF which was almost similar to that of standard.

Formalin Induced Writhing Method: Both MAEF and MAES significantly ($P<0.05$) suppressed the licking activity in either phase of the formalin-induced pain in mice in a dose dependent manner (Fig.1). In second phase, MAES at the dose of 200 mg/kg body weight (p.o.) showed the batter analgesic activity than the standard.

Anti-inflammatory Activity: Fig. 2 represents the anti-inflammatory activity of MAEF and MAES. Both extract showed dose dependent anti-inflammatory activity and statistically significant ($P<0.05$). At 200 mg/kg dose, MAES showed remarkable anti-inflammatory effects (% of inhibition 50.87%) compared with the Indomethacin (% of inhibition 56.14%).

CNS Depression Activity

Hole-Cross Test: In the Hole- cross test, MAEF and MAES extracts exhibited statistically significant ($P<0.05$) of decrease in the movements of the test animals at all dose levels tested and followed a dose-dependent response. The depressing effect was most intense during the second (60 min) and third (90 min) observation periods in both extracts (Table 2).

Open Field 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).

Anti-Diarrheal Activity

Castor Oil-Induced Diarrhea: The extracts significantly reduced the number of diarrheal episodes in a dose dependent manner when compared with the untreated

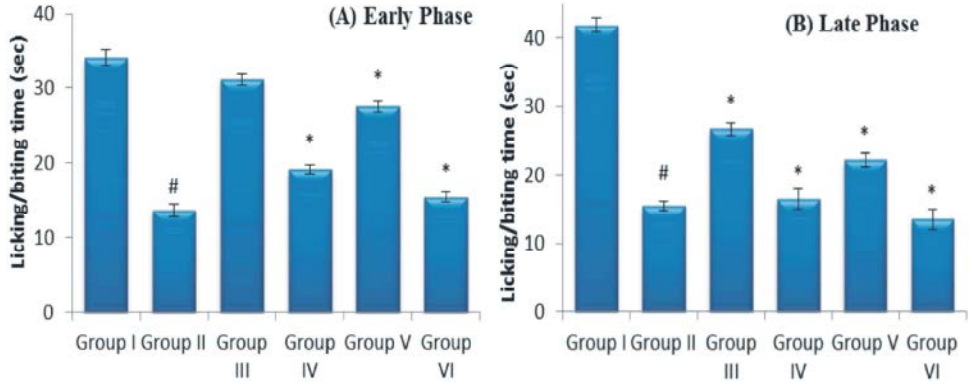

Fig. 1: Effects of methanolic extract of MAEF and MAES in the hind paw licking in the formalin test in mice. Values are mean±SEM, (n = 5); *$p<0.05$ as compared to vehicle control (One way ANOVA followed by Dunnet test). Group I animals received vehicle (1% Tween 80 in water), Group II received Diclofenac Na as standard 10 mg/kg body weight p.o., Groups III, IV and V, VI were treated with 100 and 200 mg/kg body weight (p.o.) of MAEF and MAES respectively.

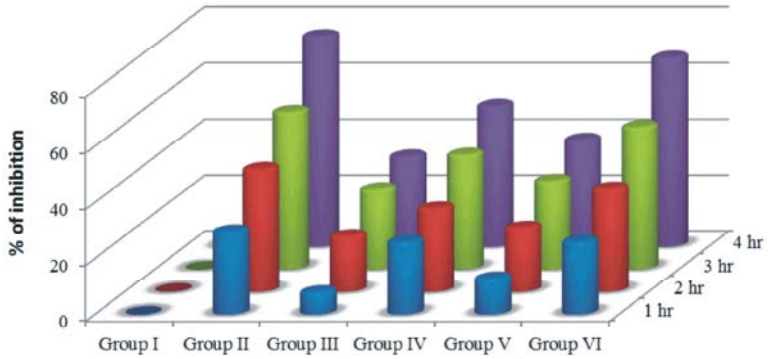

Fig. 2: Effects of the extracts of MAEF and MAES on carrageenan induced paw edema test. Values are mean ±SEM, (n = 5); *P<0.05 **P<0.005 as compared to vehicle control (One way ANOVA followed by Dunnet test). Group I animals received vehicle (1% CMC in water), Group II received Indomethacin 10 mg/kg body weight p.o., Groups III, IV and V, VI were treated with 100 and 200 mg/kg body weight (p.o.) of MAEF and MAES respectively.

Table 1: Effects of methanolic extract of MAEF and MAESon acetic acid-induced writhing in mice

Groups	Dose (mg/kg)	No. of writhing	% inhibition
Group I	Vehicle	28.16	
Group II	10	6.16	78.10*
Group III	100	19.5	30.76*
Group IV	200	9.0	68.04*
Group III	100	11.8	61.68*
Group IV	200	6.9	77.59*

Values are mean±SEM, (n = 5), [a]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 Diclofenac Na as standard 10 mg/kg body weight (p.o.), Groups III, IV, V, and VI were treated with 100 and 200 mg/kg body weight (p.o.) of MAEF and MAES respectively.

Table 2: Effects of methanolic extract of MAEF and MAES on hole cross test in mice

		Number of Movements				
Groups	Dose	0 min	30 min	60 min	90 min	120 min
Group-I	10ml/kg,	12.8±1.15	13±1.41	13.6±0.92	14.2±0.86	14±0.54
Group-II	1mg/kg,	11.2±0.58	6±0.70*	4.0±0.83*	2.4±0.81*	1.8±0.37*
Group-III	100 mg/kg	12±0.70	8.8±0.58*	6.4±0.50*	5.2±0.37*	4.2±0.37*
Group-IV	200 mg/kg	12.2±0.66	6.2±0.37*	4.3±0.70*	2.9±0.37*	2.4±0.40*
Group-V	100 mg/kg	12±0.70	7.8±0.58*	5.4±0.50*	4.2±0.37*	3.2±0.37*
Group-VI	200 mg/kg	12.2±0.66	5.6±0.37*	4.0±0.70*	2.3±0.37*	1.2±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, Groups III, IV and V, VI were treated with 100 and 200 mg/kg body weight (p.o.) of MAEF and MAES respectively.

Table 3: Effects of methanolic extract of MAEF and MAES on Open Field test in mice

		Number of Movements				
Group	Dose	0 min	30 min	60 min	90 min	120 min
Group-I	10ml/kg	118.4±1.20	115±1.30	117.4±0.50	111.4±1.16	110±0.70
Group-II	1mg/kg	110.2±1.15	61.6±0.43*	33.8± .58*	12.8± .86*	8.6±0.50*
Group-III	100 mg/kg	114.4±0.81	70.8±1.02*	55.8±1.35*	35.8± .02*	26±0.71*
Group-IV	200 mg/kg,	112.8±1.43	60.8± .06*	42.6± .92*	24.6±0.92*	16.6±0.60*
Group-III	100 mg/kg	115.4±0.81	68.8±1.02*	51.8±1.35*	33.8± .02*	22±0.71*
Group-IV	200 mg/kg,	114.8±1.43	51.8± .06*	33.6± .92*	18.6±0.92*	10.6±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, Groups III, IV and V, VI were treated with 100 and 200 mg/kg body weight (p.o.) of the MAEF and MAES accordingly.

Table 4: Effects of methanolic extract of MAEF and MAES on castor oil-induced diarrhea in mice

Treatment	Dose	Onset of diarrhea (min)	Animals with diarrhea	No. of faeces in 4 hrs	% inhibition of defecation
Group I	10ml/kg,	24.45±1.19	5/5	22.6±0.68	
Group II	1mg/kg,	160±0.13**	1/5	4.8±0.58**	83.18
Group III	100 mg/kg	52.67±2.73**	3/5	17.2±1.05**	32.74
Group IV	200 mg/kg	69.23±3.03**	2/5	12.1±0.29**	55.30
Group VI	100 mg/kg	45.67±2.03**	3/5	12.4±1.15**	48.51
Group VII	200 mg/kg	64.23±2.03**	2/5	7.4±0.39**	73.27

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 loperamide 1 mg/kg body weight, Groups III, IV and V, VI were treated with 100 and 200 mg/kg body weight (p.o.) of the MAEF and MAES accordingly.

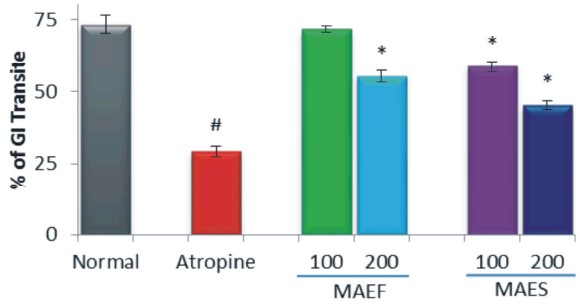

Fig. 3: Effect on gastrointestinal motility of MAEF and MAES values are presented as mean±SEM, (n=5); * p<0.05, respectively, compared to control by student's t-test. Group I animals received vehicle (1% CMC in water), Group II received Atropine sulfate 0.1 mg/kg body weight (p.o.) Groups III, IV and V, VI were treated with 100 and 200 mg/kg body weight (p.o.) of MAEF and MAES respectively.

controls. At 200 mg/kg doses, MAEF showed 55.30% and MAES 73.27% reduction in the number of fecal episodes, whereas Loperamide offered 83.18% protection (Table 4).

Effect on Gastrointestinal Motility: With the gastrointestinal transit experiment, the treated groups showed significant difference compared with control (p< 0.05). The intestinal transit of charcoal meal was 27. 30% in Atropine induced group, but the dose of MAES at 200 mg/kg body weight was 46.00% (Fig. 3).

DISCUSSION

Acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics and represents pain sensation by triggering localized inflammatory response, leads to the release of free arachidonic acid from the tissue phospholipid mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathways [17]. 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 [18] and Flavonoids inhibiting the writhing will have analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [19]. Therefore, it is likely that MAEF and MAES might have exerted its peripheral anti-nociceptive action by interfering with the local reaction caused by the irritant or by inhibiting the synthesis, release and/or antagonizing the action of pain mediators at the target sites. Interestingly, compounds like flavonoids and steroids, triterpenes in part, have been shown to possess anti-inflammatory, analgesic activity and the claim made by Pritam *et al*.[20].

The biphasic formalin model normally postulates the site and the mechanism of action of the analgesic by neurogenic (0-5 min) and inflammatory pain (15-30 min), respectively [21]. Drugs that act primarily on the central nervous system such as narcotics inhibit both as steroids and NSAIDs suppress mainly the late phase [22]. 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. McNamara *et al*. [23] 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. TRPA1cation channels at primary sensory terminals were also reported to mediate noxious mechanical stimuli [24]. It is likely that the inhibitory effect of MAEF and MAES to pain response is due to inhibit the increase of the intracellular Ca^{2+} through TRPA1, presumably evoked by formalin. Flovonoids, 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 TNFα. [25]

and literature survey revealed that tannins and flavonoid are the major phytoconstituents of *A. esculentus* [7] may play the provital role for analgesic activity.

Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic, early phase (1-2hrs) is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings and late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages [26]. Since the extract significantly inhibited paw edema induced by carrageenan in the second phase and this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract and this effect is similar to that produced by non-steroidal anti-inflammatory drugs such as Indomethacin, whose mechanism of action is inhibition of the cyclooxygenase enzyme. Our studies were also supported by Biren *et al*. [27] where the air-dried fruits of *Abelmoschus esculentus* Linn. exhibited potent analgesic activities and also well known for their ability to inhibit pain perception due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation.

Locomotor activity considered as an increase in alertness and decrease in locomotor activity indicated sedative effect [28]. Extracts of MAEF and MAES 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 MAEF and MAES may act by potentiating GABAergic inhibition in the CNS via membrane hyper-polarization which lead 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. Many research showed that plant containing flavonoids, saponins and terpenoidsare useful in many CNS disorders [29]. 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 Benzodiazepine like molecules [28]. Phytochemical investigations also showed the presence of alkaloids, flavonoids and tannins in the extract, so might be these phyto-constituents are responsible for its CNS depressant activity.

In the present investigation, MAEF and MAES at large dose (200 mg/kg, b. wt.) exhibited significant anti-diarrheal effects in one or the other experimental models. With respect to the castor oil induced diarrhea model, the results revealed that MAES showed lightly better protection from diarrhea in the animals as compared with MAEF. It is likely that the extracts bring out the aforementioned action either through their proabsorbtive property that promotes faster fluid absorption in the intestine or through an anti-secretory mechanism. The flavonoids isolated from the extracts of MAEF and MAES were tested for a possible spasmolytic activity. All flavonoids, showed dose dependent (100 and 200 mg/kg body weight) spasmolytic activity. These indicate that the presence of compounds with spasmolytic and calcium antagonist activity may be responsible for the anti-diarrheal effect [30].In the small intestinal transit test, both extracts of MAEF and MAES suppressed the propulsion of charcoal marker in a dose dependent manner. This finding suggests that the extracts act on all parts of the intestine. A decrease in the motility of gut muscles increases the stay of substances in the intestine [31]. This allows moderate water absorption. It is therefore presumed that the reduction in the intestinal propulsive movement in the charcoal meal model may be due to antispasmodic properties of the extracts. It has reported that flavonoids inhibit the intestinal motility in experimental induced diarrhea in rats.

CONCLUSION

In summary, the methanolic extract of *Abelmoschus esculentus* Linn. showed significant analgesic, anti-inflammatory, CNS depression and anti-diarrheal properties. Further investigations are required to find the active component of the extract in order to confirm the mechanism of action in the development of a potent analgesic, anti-inflammatory CNS depression and anti-diarrheal reagent.

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