SJIF Impact Factor (2023): 8.574| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 8 | Issue: 3 | March 2023 - Peer Reviewed Journal

IN-VIVO ASSESSMENT OF HYPOGLYCEMIC AND ANXIOLYTIC ACTIVITIES ON SWISS ALBINO MICE AND ANTHELMINTIC ACTIVITY ON EARTHWORM OF METHANOLIC EXTRACT OF *MICROCOS PANICULATA* LEAVES

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Article DOI: https://doi.org/10.36713/epra12619
DOI No: 10.36713/epra12619

ABSTRACT

The aim of the present study is to investigate the hypoglycemic, anxiolytic and anthelmintic activities of the methanolic extract of Microcos paniculata Leaves. The hypoglycemic activity was evaluated by measuring the blood glucose level of swiss albino mice, and anxiolytic activity was evaluated by hole board and elevated plus-maze tests on swiss albino mice. The anthelmintic activity was evaluated by measuring the paralysis and death times of adult earthworms. The methanolic extract of M. peniculata showed a highly significant ($p< 0.001$) hypoglycemic effect by reducing blood glucose levels with time, and the reduction is also dose-dependent. In the hole board test, the extract caused a large increase in the number of head dips. In the elevated plus-maze test, the extract caused a large increase in the amount of time spent in the open arm and a decrease in the amount of time spent in the closed arm. The extract showed highly significant ($p< 0.001$) anthelmintic activity by causing paralysis and death of earthworms in a short period, which is inversely proportional to increasing the concentration of extract. The evaluated results indicate the presence of hypoglycemic, anxiolytic and anthelmintic activities of this plant extract. Further investigation needs to be done to pick out the phyto-constituents responsible for these activities.

KEYWORDS: *Hypoglycemic, Anxiolytic, Anthelmintic, Microcos paniculata Leaves, Swiss albino mice, Earthworms*

INTRODUCTION

Many plant species have antidiabetic properties [1] and these plants are the precursors for the synthesis of drugs due to therapeutic values [2]. Diabetes mellitus is indicated by hyperglycemia resulting from defects in insulin secretion, and chronic hyperglycemia affects carbohydrate, lipid and protein metabolism that ultimately causes malfunction and defect of organs, including eyes, kidneys, nerves, heart and blood vessels [3]. Diet, obesity, and sedentary lifestyle, high family aggregation, insulin resistance, nutritional status, age, and lifestyle change for urbanization are all risk factors for diabetes [4]. The recent treatment of diabetics, such as insulin and various oral hypoglycemic agents i. e sulfonylureas, metformin, glucosidase inhibitors, troglitazone, etc., show serious adverse side effects such as diarrhea, liver problems, lactic acidosis [5] those effect is increasing alarmingly in the whole world [6]. Due to the undesirable side effects of currently available antidiabetic drugs, there is an urgent need for novel molecules to treat diabetes [7, 8] along with the sufficiency and few adverse effects of herbal medicine used in taking care of and regulating diabetes in the current world [9-11]. Neurological disorders affect people, and recent life stress associated with suffering and tribulations is liable for the perpetration of a variety of psychiatric disorders. Many plants have effects against C.N.S. disorders, and they decrease human suffering in comparison with drugs that act by initiating pharmacological and psychological effects in the intrinsic system of the body [12]. However, conventional medicines used for the treatment of neurological diseases have side effects. For example, benzodiazepines' deterioration of cognitive function, physical dependence tolerance, respiratory, digestive, and immune systems, etc [13]. Parasitic worms (Helminths) produce harmful effects on humans and other animals around the world, mainly in third-world countries in the tropical region [14]. Men are to be infected with soil-transmitted helminths, while helminth infection is also a serious problem in livestock growth, causing significant economic losses and threatening food safety [15, 16]. The helminthic infection causes

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various problems, including respiratory symptoms, dermatological consequences, and epilepsy, and this infection may also annihilate immune activities against pathogens of other diseases such as malaria and tuberculosis, H.I.V. [14]. Drugs used to treat helminths, such as synthetic anthelmintic drugs, exhibit resistance to parasite [17] along with a lack of efficacy of these drugs [15]. *Microcos paniculata* L., locally known as 'Kathgua' or 'Fattashi' in Bangladesh, contains Tiliaceae family and traditionally, the plant possess a wide range of activities such as analgesic, antidiarrheal, anti-inflammatory, antipyretic, antimicrobial, brine shrimp lethality, cytotoxic, free radical scavenging, insecticidal, larvicidal, neuropharmacological, nicotinic receptor antagonistic and α-glucosidase inhibition activities [18]. Therefore, the current study was designed to investigate the hypoglycemic, anthelmintic and anxiolytic activities of the methanolic extract of *Microcos paniculata* leaves.

MATERIALS AND METHODS

Collection of Plant Materials

For this study, plant sample was collected from Brahmanbaria, Bangladesh. The collected plant sample was identified and authenticated by the expert of the National Herbarium, Mirpur, Dhaka, Bangladesh, and given Accession Number of the plant was **35348.** All other ingredients used in this evaluation were analytical grades collected from the laboratory of the Department of Pharmacy, Noakhali Science and Technology University, Bangladesh.

Preparation of *Microcos paniculata* leaves extract

After collection, plant samples were cleaned and sundried for 7 days. After drying, the samples were grounded into a coarse powder using a high-capacity grinding machine. Then the powder was soaked in 95% methanol for 14 days with occasional shaking and stirring. The whole mixture was filtrated by cotton and then Whatman filter paper (Bibby RE200, Sterilin Ltd., U.K.). The filtrate was then dried at room temperature and found the methanolic fraction of *Microcos paniculata* leaves extract was stored in the refrigerator at 4^0C until use.

Preparation of Animals

For this study, Swiss Albino Mice (25-35g) of either sex, 3-4 weeks of age, were collected from the animal house of Jahangirnagar University, Savar, Dhaka and all experiments were approved by the NSTU research cell committee, Noakhali Science and Technology University. Animals were nursed in animal houses at an ambient temperature 25°C and 45-60% humidity to acclimatize to the environment for seven days prior to the experimentation beginning. They had free access to standard pellets such as basal diet and water ad libitum.

Drugs & Chemicals

Glibenclamide, Diazepam, Albendazole were collected from Square Pharmaceutical Limited, Bangladesh and glucose and 1% Tween-80 were collected from Lab, NSTU.

Hypoglycemic Activity Test

Hypoglycemic activity test is performed according to Osadebe *et al.,* 2014 [19] with slight modification. Mice were grouped randomly, containing four mice in each group. Group-I was given 1% Tween-80 with normal saline orally, whereas the standard drug glibenclamide (5mg/kg body weight) was administered in Group-II mice. The experimental groups, Group-III and Group-IV were treated with methanolic extract of *Microcos paniculata* leaves orally at the dose of 200 mg/kg and 400 mg/kg body weight, respectively. After 60 minutes of administration of test samples, the mice of all groups were orally treated with 20 mg/ml glucose solution (200 mg/kg body wt). The blood glucose level of the experimental animals was then determined using a glucometer by collecting blood samples from the tail vein right before and after 1, 2, 3, and 4 hours of glucose administration.

Anxiolytic Activity Test

Hole board and Elevated plus maze test are used to evaluate the anxiolytic activity of the methanolic extract of *Microcos paniculata* leaves.

Hole board test was performed according to Somani et al., 2010 [20] with slight modification in a wooden box (40 x 40 x 25 cm) with 16 holes (each of diameter 3 cm) and elevated to the height of 35 cm. The mice were grouped randomly, containing four mice in each group. Group-I was given distilled water (10 ml/kg), whereas Group-II was treated with diazepam (1 mg/kg) and experimental group-III and IV were given plant extract 200 and 400 mg/kg body weight, respectively, orally. After one hour of treatment, each mouse was placed in a head dipping box, and the number of head dips for 5 min period was counted for the individual mouse.

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An elevated plus maze test was performed according to Thippeswamy et al., 2011 [21] with slight modification, and mice were grouped randomly, containing four mice in each group. Group-I was treated with distilled water (10 ml/kg, p. o) and diazepam (1 mg/kg, i.p) was administered to group-II whereas group-III and IV were given plant extracts (200 and 400 mg/kg, i.p), respectively. After one hour of treatment, mice were individually placed in the centre square facing either one of the open arms and the time spent in both the open and closed arms was recorded for 5 min period for an individual mouse.

In-Vitro Anthelmintic Activity Test

Anthelmintic activity of methanolic extract of *Microcos paniculata* leaves was evaluated according to Ajaiyeoba *et al.*, 2001 [22] with slight modifications. Adult earthworms (*Pheretima posthuma)* were used to evaluate the anthelmintic activity due to their anatomical and physiological resemblance with the intestinal roundworm parasite of human being [23, 24] and also the availability of them [25]. After the collection of earthworms, they were washed with saline water. Five concentrations of methanol extract of *Microcos paniculata* leaves, i. e 20, 40, 60, 80 and 100 mg/ml were used as test samples; normal saline was used as the control group, and albendazole 10mg/ml was used as the standard. Earthworms were put in a Petri dish with 15 ml of sample solution. The time it took for each worm to become paralyzed and die was timed. Paralysis is considered to exist when no movement is observed in response to forceful shaking of worms. The conclusion of death was reached when the worms did not move in response to forceful shaking or immersion in 500°C water.

RESULTS

Assessment of hypoglycemic activity test

After administration of glucose, at 0 hours, blood glucose level at 200 mg/kg and 400 mg/kg was 3.10 mmol/l and 5.10 mmol/l, but after 1 hour, that increased at 8.35 mmol/l and 10.35 mmol/l, respectively. Then the blood glucose level reduced gradually and reached at 2.98 mmol/l and 3.18 mmol/l, respectively, at a dose of 200 mg/kg and 400 mg/kg body weight after 4 hours when compared to the control group. The blood glucose level of the standard drug glibenclamide was 6.72 mmol/l at 0 hour and 10.95 mmol/l after 1 hour, which was reduced to 3.50 mmol/l after 4 hours (Table 1). It was noticed that there is a highly significant ($p< 0.001$) reduction of blood glucose level with time when compared with control, and also the reduction of blood glucose was more at a dose of 400 mg/kg than 200 mg/kg body weight which confirmed dose dependent reduction of blood glucose.

Table 1: Hypoglycemic activity of methanolic extract of *Microcos paniculata* leaves on mice

Group	Blood Glucose concentration (mmol/l) at different time				
	0 h	1 h	2 h	3 h	4 h
Control 0.2 ml/ 10 g	6.92±0.28	17.42±0.41	15.88±0.30	12.65±0.417	9.20±0.23
Glibenclamide 5 mg/kg	6.72±0.25	10.95±0.5***	7.85±0.30***	5.57±0.17***	3.50±0.27***
***Extract*-200 mg/kg**	3.10±0.16***	8.35±0.13***	6.42±0.27***	4.32±0.06***	2.98±0.14***
***Extract*-400 mg/kg**	5.10±0.34*	10.35±0.12***	8.35±0.12***	4.65±1.08**	3.18±0.05***

Values are presented as mean ± S.E.M. Data was analyzed using one way ANOVA followed by Dunnett's t-test, and groups were compared with the control. (n=4) ***p< 0.001; **p< 0.01; *p< 0.05

Assessment of Anxiolytic Activity

In the hole board test, methanolic extract of *Microcos paniculata* leaves increases the number of head dipping 48 ± 6.5 and 64 ± 7.4 at a dose of 200 mg/kg and 400 mg/kg body weight respectively, when compared to the control group, which are statistically highly significant ($p<0.001$) (Table-2). In the elevated plus-maze test, the extract also increased the time spent in the open arm 93.2 sec and 95.5 sec. at a dose of 200 mg/kg and 400 mg/kg body weight, respectively, when compared to the control group, which indicates the statistically highly significant ($p<0.001$) effect (Table-3).

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Table 2: Effects of the methanolic extract of *Microcos paniculata* on the number of head dipping in hole board test in mice

Treatment Group	Number of head dipping (Mean ± S.E.M)
G-I (Control: 10 ml/kg)	40.5 ± 1.3
G-II (Diazepam: 1 mg/kg)	34.83 ± 1.6***
G-III (Extract: 200 mg/kg)	48 ± 6.5***
G-IV (Extract: 400 mg/kg)	64 ± 7.4***

Values are presented as mean ± S.E.M., Data was analyzed using one way ANOVA followed by Dunnett's t-test and groups were compared with control. (n=4) ***p< 0.001; **p< 0.01; *p< 0.05

Table-3: Effects of methanolic extract of *Microcos paniculata* leaves on time spent in open and enclosed arms in elevated plus-maze test in mice

Treatment Group	Time (s) spent in	
	open arm	Enclosed arm
G-I (Control: 10 ml/kg)	32.8 ± 5.4	233.5 ± 7.8
G-II (Diazepam: 1 mg/kg)	103.0 ± 10.5	169 ± 12.4
G-III (Extract: 200 mg/kg)	93.2 ± 28.52 ***	206.5 ± 28.52
G-IV (Extract: 400 mg/kg)	95.5 ± 28.95 ***	204.7 ± 28.95

Values are presented as mean ± S.E.M., Data was analyzed using one-way ANOVA followed by Dunnett's t-test and groups were compared with the control. (n=4) ***p< 0.001; **p< 0.01; *p< 0.05

Assessment of Anthelmintic Activity Test

The methanolic extract of *Microcos paniculata* leaves exhibits not only paralysis but also death in earthworms. Among the five used concentrations, the highest anthelmintic activity exhibited by the extract at the highest concentration of 100 mg/ml includes 22.34±0.98 minutes for paralysis and 25.17±0.60 minutes for the death of the worms. The shortest times are required at the highest concentration for the paralysis and death of the worms. On the other hand, the standard drug required 35.17±0.31 min. and 67.34±0.99 min. for paralysis and death of worms, respectively (Table 4). So it is said that the anthelmintic activity of different concentrations of the extract is inversely proportional to the paralysis and death time of the earthworms and also in a dose-dependent manner.

Table-4: Paralysis and Death Time of methanolic extract of *Microcos paniculata* leaves on earthworms

Groups	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
Control	-	No Paralysis	No Death
Standard	10	35.17±0.31	67.34±0.99
ME Extract	20	96.67±0.98***	117.33±0.67***
	40	83.83±0.91***	102.83±0.48***
	60	32.34±0.98	38.5±0.57***
	80	27.17±0.75**	33.17±0.65***
	100	22.34±0.98***	25.17±0.60***

Values are presented as mean ± S.E.M.; Data was analyzed using one way ANOVA followed by Dunnett's t-test and groups were compared with control. (n=6) ***p< 0.001; **p< 0.01; *p< 0.05

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DISCUSSION

Polysaccharide is converted into monosaccharide by the alpha-amylase enzyme, and due to this, diabetes can be managed by inhibiting this alpha-amylase enzyme; however, carbohydrate digestion is aggravated, and glucose absorption from starch is also decreased [26, 27]. Phytoconstituents such as saponins, alkaloids, terpenoids, flavonoids, tannins, steroids, phlorotannins, and anthraquinones might be liable for restricting the alpha amylase enzyme [28]. A previous literature study showed that *Microcos peniculata* extract contains phyto-constituents such as flavonoids, tannins, carbohydrates, alkaloids, saponins, triterpenoids, and glycosides [29] and plants that contain safonins and flavonoids have potent antidiabetic activity [30]. As a result, our experimented plant extract showed antidiabetic activity in mice by reducing blood glucose levels, which may be due to the presence of safonins and flavonoids that ultimately hinder the alpha amylase enzyme. Tannins have an anthelmintic effect and cause the death of parasites by energy depletion due to uncoupling of the oxidative phosphorylation reaction or binding with glycoprotein on the cuticle of the parasite [31,31]. The experimental plant shows anthelmintic activity due to the presence of tannins in this plant.

C.N.S. activity of any drug evaluated on the locomotor activities of animals and investigation of excitability of the C.N.S. refer to the locomotor activity of the animal. An increase in alertness is considered to be locomotor activity, and a decrease in locomotor activity is considered to be sedative effect [32]. C.N.S. depression may be due to the reduction of locomotor activity and sedation, and C.N.S. depressant drugs exhibit their activity via GABAA receptor [33]. A higher concentration of GABAA receptor in the brain shows a C.N.S. depressant effect [34]. C.N.S. depressant drugs bind to the GABAA receptor as well as its subtypes and have explicit anxiolytic, sedative, and amnesic effects [35, 36]. Flavonoids and tannins contained in plants have explicit activity against C.N.S. disorders [37] and *M. peniculata* extract show anxiolytic effects owing to the binding of such phyto-chemicals to the $GABA_A$-Benzodiazepine receptors. It is also reported that plants having flavonoids, saponins, sterols, tannins have explicit anxiolytic activity [38]. In the elevated plus-maze test, *M. peniculata* extract revealed that time spent in the open arm increased, but time spent in the enclosed arm decreased, which may be a sign of the anxiolytic action of this plant extract.

CONCLUSION

From the investigation of the above study, it can be summarized that the methanolic extract of *M. peniculata* has significant hypoglycemic, anxiolytic and anthelmintic activities, but the further investigation needs to be done on the higher animal to find out and segregate the compounds responsible for these activities.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgement

The authors are thankful to the department of pharmacy of Noakhai Science and Technology University for their laboratory support and cooperation during this study.

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