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Antibacterial, Neuropharmacological and Analgesic Activities of *Garcinia cowa* (Family: Clusiaceae)

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Abstract

The current study was aimed to investigate the antibacterial as well as neuropharmacological and analgesic activities of methanol extract from the leaf and bark of *Garcinia cowa* (Family: Clusiaceae). The antibacterial activity test was performed by determining the zone of inhibition of living microorganisms compared with the standard drug, Ciprofloxacin. The result showed that, the petrolium ether, dichloromethane and methanol extracts from the leaf and bark have mild to moderate antibacterial activity. The neuropharmacological screening was evaluated by hole cross and open field tests where a significant and dose dependent suppression of motor activity and exploratory behavior was observed in the methanol extract of *G. cowa* when treated in Swiss Albino mice with the reference sedative drug, Diazepam. The analgesic activity was evaluated by using acetic acid-induced writhing test and tail immersion method at a dose of 200 and 400 mg/kg body weight. The results displayed 40.68% and 52.31% of inhibition for leaf and 56.18% and 56.91% for bark extract in the acetic acid-induced writhing test which is mild to the reference standard drug, Diclofenac-Na whose writhing inhibition was 78.21%. The analgesic activity in the tail immersion method was dose dependent.

Keywords

Garcinia cowa, Antibacterial Activity, Neuropharmacological Activity, Analgesic Activity, Ciprofloxacin, Diazepam, Diclofenac-Na

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1. Introduction

The medicinal plant, *Garcinia cowa* (Family: Clusiaceae) is mainly tropical regions trees, shrubs, or sometimes herbs containing resin or oil in schizogenousspaces or canals and sometimes black or red glands containing hypericin or pseudohypericin. The Bengali name is Kau, Cowa, Kaglichu, Kao-gola and the English name is Cow Tree. The plant is widely distributed in South Asia. Fruit pericarp is composed of a fat and the seeds yield a wax-like fat consisting of glycerides of stearic, oleic, palmitic, linoleic and myristic acids. Bark contains a gum resin (Ghani, 2003). A new compound 1,3,6-Trihydroxy-7-methoxy-8-(3,7-dimethyl-2,6-octadienyl)-xanthone has been isolated from stems (Rastogi and Mehrotra,1993). Fruits contain vitamin A and vitamin C.Bark is astringent; used in spasm. Fruits are given in headache. Sun-dried slices of the fruits are used in dysentery. Gum resin is drastic cathartic, may produce nausea and vomiting (Yusuf et al., 2009).

2. Materials and Methods

2.1. Chemicals

Drugs and chemicals used in the current study include: Diclofenac-Na, Acetic Acid (Merck, Germany), Methanol (Merck, Germany), Ciprofloxacin, Diazepam.

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2.2. Plant Materials and Extraction

The plant *Garcinia cowa* (Leaves and Bark) was collected from Munshigoanj, Dhaka, Bangladesh in March, 2011. The plant was identified by scientist of Bangladesh National Herbarium Institute, Mirpur, Dhaka. An accession number was given from there and a voucher specimen (DACB: 35585) has been deposited in the herbarium for future reference. Following the method of cold extraction, the powdered leaf (100g) and bark (100g) of *G. cowa* were separately soaked in 500 ml of methanol for 20 days and then all the extracts were filtered through a cotton plug followed by Whatman filter paper number 1 and then concentrated by using a rotary evaporator at low temperature (40-50)°C and reduced pressure to have greenish extracts.

2.3. Animals

For the experiment Swiss albino mice of either 3-4 weeks of age, weighing between 20 to 25g were collected from the Animal Resources Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). Soft wood shavings were used as bedding of cages. Animals were maintained under standard environmental conditions: temperature (24.0±1.0°C), relative humidity (55-65% and 12 hrs. light/12 hrs. dark cycle). Husk and excreta were removed from the cages every day (Asma et al., 2015).

2.4. Antibacterial Activity

The disc diffusion method (Bauer et al., 1966) was used to test antibacterial activity against two Gram positive and two Gram negative bacteria (Table 1). Solutions of known concentration (mg/ml) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs (impregnated with solvents) were used as positive and negative control. These plates were then kept at low temperature (4^{o}C) for 24h to allow maximum diffusion. There is a gradual change of test materials concentration in the media surrounding the discs. The plates were then incubated at 37^{o}C for 24h to allow maximum growth of the organisms. The test material having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment is carried out three times and the mean of the readings is required. Standard disc of Ciprofloxacin (30µg/disc) was used for comparison purpose (Sadia, 2015).

2.5. Neuropharmacological Activity

Drug acting in the central nervous system (CNS) were first discovered by primitive humans and are still the most widely used group of pharmacologic agents for CNS action (Katjung, 1998). The effects of drugs on the central nervous system (CNS) with reference to the neurotransmitters for specific circuits, attenuation should be developed to general organizational principles of neurons. The view that synapses represent drug–modifiable control points within neuronal networks. It requires explicit delineation of the sites at which given neurotransmitters may operate and the degree of specificity with which such site that may be affected (Bloom, 1996).

2.5.1. Open Field Test

The method described by Gupta et al., 1971 was adopted for open field test. Open-field behavioral assays are commonly used to test both locomotors activity and emotionality in rodents. The test group received methanol leaf and bark extracts of *G. cowa* at the dose of 200 and 400mg/kg body weight orally whereas the control group received vehicle (1% tween 80 in water). 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 a wall of 40cm height. The number of squares visited by the animals was counted for 3 minutes at on 0, 30, 60 and 90 minutes after oral administration of the test drugs (Gupta et al., 1971).

2.5.2. Hole Cross Test

The experiment was carried out as described by Takagi et al., 1971. The most consistent behavioral change is a hyperemotional response to novel environmental stimuli. The aim of this study was to characterize the emotional behavior of mice using the hole-board test. The number of head-dips in the hole-board test in single-housed mice was significantly greater. A steel partition was fixed in the middle of a cage having a size of 30 x 20 x 14cm. A hole of 3cm diameter was made at a height of 7.5cm in the center of the cage. Movement of the animals through the hole from one chamber to the other was countered for 3 minutes in this test. The observations were made on 0, 30, 60 and 90 minutes after oral administration of the test drugs (Takagi et al., 1971).

2.6. Analgesic Activity

Analgesic means a drug that selectively relieves pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness. So, analgesic activity means capacity of a substance to neutralize the pain sensation (Rang, 1993). Pain has been officially defined as an unpleasant sensory and emotional experience associated with

actual or potential tissue damage. Pain acts as a warning signal against disturbances of the body and has a proactive function (Tripathi, 1999).

2.6.1. Acetic Acid-Induced Writing Test

The acetic acid-induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice. Test sample and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but diclofenac-Na was administered 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 (Meera et al., 2008).

2.6.2. Tail-Flick Test

The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice. The animals of the control, positive control and test groups were treated with diclofenac-Na (5mg/kg body weight), water (10ml/kg body weight) and test samples at the doses of 200 and 400mg/kg body weight respectively. 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55^0C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20sec was defined as complete analgesia and the measurement was stopped when the latency period exceeded to avoid injury to mice. The latent period of the tail-flick response was taken as the index of antinociception and was determined at 0, 30, 60 and 90 min after the administration of the test drugs and standard (Hasan et al., 2009).

3. Results and Discussion

3.1. Antibacterial Activity of *Garcinia cowa*

The results of antibacterial activity of *G. Cowa* are given in Table 1.

3.2. Neuropharmacological Activity

3.2.1. Open Field Test

The results of neuropharmacological activity of *G. cowa* are given in Table 2 and Table 3.

Table 1. Antibacterial activity of *G. cowa* leaf and bark extracts.

Microorganisms	Diameter of zone of inhibition (mm)						
	Leaf			Bark			
	Petrolium ether	Dichloro methane	Methanol	Petrolium ether	Dichloro methane	Methanol	Ciprofloxacin
Gram positive bacteria							
Bacillus cereus	...	...	17	15	10	18	25
Staphylococcus aureus	14	16	...	7	12	20	30
Gram negative bacteria							
Escherichia coli	16	10	17	20	10	19	28
Shigella boydii	17	...	12	10	...	...	32

"..." indicate- No activity.

All the extracts showed mild to moderate activity against the tested microorganisms.

Table 2. Effect of methanol leaf extract of *G. cowa* on open field test.

Test Group	Route of Administration	Observation			
		0 min	30 min	60 min	90 min
Control	Oral	49.8±6.3	42.8±4.31	32.2±7.17	19.8±5.43
Positive Control	Intraperitoneal	85.4±4.03	50.4±8.54	37±8.01	26.4±3.35
Group I	Oral	76.6±15.32	49±9.8	39.8±7.96	34.4±6.88
Group II	Oral	14±2.8	7.4±1.48	4±0.8	4.6±0.92

Values are expressed as mean±SEM (n=5). Control: Tween-80+Water, Positive control: Diazepam (10mg/kg), Group I: Extract (200mg/kg) and Group II: Extract (400mg/kg).

Table 3. Effect of methanol bark extract of *G. cowa* on open field test.

Test Group	Route of Administration	Observation			
		0 min	30 min	60 min	90 min
Control	Oral	22.4±1.82	11.8±0.74	11.4±0.84	7.8±0.96
Positive Control	Intraperitoneal	15±1.5	7±0.94	4.6±1.04	3.6±0.98
Group I	Oral	71.4±14.28	23.8±4.76	40.4±8.08	21±4.2
Group II	Oral	50.4±10.8	38.6±7.2	23.6±4.72	15.2±3.04

Values are expressed as mean±SEM (n=5). Control: Tween-80+Water, Positive Control: Diazepam (10mg/kg), Group I: Extract (200mg/kg) and Group II: Extract (400mg/kg).

3.2.2. Hole Cross Test

The Hole cross test results of *G. cowa* are given below in the following Table 4 and Table 5.

The most important step in evaluating drug action on CNS is to observe its effect on locomotors activity of the animal. The activity is a measure of the level of excitability of the CNS and this decrease may be closely related to sedation resulting from depression of the central nervous system. Both holecross and open field tests showed that the depressing activity of the methanol leaf and bark extracts were evident from the 2nd observation period in the test animals at the doses of 200 and 400 mg/kg body weight of mice. Maximum depressant effect was observed from 2nd (60 min) to 4th (90 min) observation period at the dose of 400 mg/kg. The results were also dose dependent. The significant CNS depressant activity of the extracts is probably due to increase in concentration of GABA in brain (Afia et al., 2012).

Table 4. Effect of methanol leaf extract of *G. cowa* on hole cross test.

Test Group	Route of Administration	Observation			
		0 min	30 min	60 min	90 min
Control	Oral	22.4±1.82	11.8±0.74	11.4±0.84	7.8±0.96
Positive Control	Intraperitoneal	15±1.5	7±0.94	4.6±1.04	3.6±0.98
Group I	Oral	5.8±1.16	5.8±1.16	1.6±0.32	2.4±0.48
Group II	Oral	14±2.8	7.4±1.48	4.2±0.8	4.6±0.92

Values are expressed as mean±SEM(n=5). Control: Tween-80+Water, Positive Control: Diazepam (10mg/kg), Group I: Extract (200mg/kg) and Group II: Extract (400mg/kg).

Table 5. Effect of methanol bark extract of *G. cowa* on hole cross test.

Test Group	Route of Administration	Observation			
		0 min	30 min	60 min	90 min
Control	Oral	22.4±1.82	11.8±0.74	11.4±0.84	7.8±0.96
Positive Control	Intraperitoneal	15±1.5	7±0.94	4.6±1.04	3.6±0.98
Group I	Oral	71.4±14.28	23.8±4.76	40.4±8.08	21±4.2
Group II	Oral	50.4±10.8	38.6±7.2	23.6±4.72	15.2±3.04

Values are expressed as mean±SEM (n=5). Control: Tween-80+Water, Positive Control: Diazepam (10mg/kg), Group I: Extract (200mg/kg) and Group II: Extract (400mg/kg).

3.3. Analgesic Activity

3.3.1. Acetic Acid-Induced Writhing Test

The extracts of *G. cowa* significantly inhibited writhing response induced by acetic acid in a dose dependent manner. The result was statistically significant and was comparable to the reference drug, diclofenac-Na (Table 6 and Table 7).

Table 6. Acetic acid-induced writhing test of methanol extract from the leaf of *G. cowa.*

Test Group	SEM	Mean ± SEM	% of Inhibition
Control	1.47	41.3±1.47	0
Positive control	1.99	9±1.99	73.36
Group I	0.8	24.5±0.8	40.60
Group II	1.8	20.1±1.8	51.33

Values are expressed as mean ±SEM (n=5)

Table 7. Acetic acid-induced writhing test of methanol extract from the bark of *G. cowa.*

Test Group	SEM	Mean ± SEM	% of Inhibition
Control	1.47	41.3±1.47	0
Positive control	1.99	9±1.99	73.36
Group I	0.9	25.15±0.9	39.10
Group II	1.08	17.8±1.08	56.91

Values are expressed as mean ±SEM (n=5)

Dose dependent reduction in the number of abdominal constriction was observed in animals treated with different concentration of the methanol extract of *G. cowa* at the dose of 200 and 400mg/kgbody weight. For leaf extract, inhibition of writhing response was observed 40.68% and 52.31% respectively and for bark extract, inhibition of writhing response was observed 56.18% and 56.91% respectively whereas the inhibition of writhing response of diclofenac-Na was 73.36%.

3.3.2. Tail Immersion Test

The analgesic activity tests was carried out in the laboratory on five groups of mice by tail-flick method. Time interval for the test was 30 minutes. The tail withdrawal reflex time after administration of the *G. cowa* was found to increase with increasing dose of the extract (Table 8 and Table 9).

Table 8. Effect of the leaf extract of *G. cowa* on tail withdrawal reflex in mice.

Test Group	Route of Administration	Observation			
		0 min	30 min	60 min	90 min
Control	Oral	2.16±0.09	2.07± 0.33	2.66±0.19	2.35±0.35
Positive Control	Intraperitoneal	4.17± 0.32	5.09± 0.39	4.81±0.39	5.24±0.69
Group I	Oral	4.46±0.89	4.496±0.89	4.994±0.99	5.482±1.09
Group II	Oral	5.474±1.09	5.37±1.07	6.188±1.24	7.18±1.44

Values are expressed as mean±SEM (n=5).Control: Tween-80+Water, Positive Control: Diazepam (10mg/kg), Group I: Extract (200mg/kg) and Group II: Extract (400mg/kg).

Table 9. Effect of the bark extract of *G. cowa* on tail withdrawal reflex in mice.

Test Group	Route of Administration	Observation			
		0 min	30 min	60 min	90 min
Control	Oral	2.16±0.09	2.07± 0.33	2.66±0.19	2.35±0.35
Positive Control	Intraperitoneal	4.17± 0.32	5.09± 0.39	4.81±0.39	5.24±0.69
Group I	Oral	5.418±1.08	4.104±0.83	3.968±0.79	5.2032±1.04
Group II	Oral	6.11±1.22	5.228±1.05	4.332±0.87	4.91 ±0.98

Values are expressed as mean±SEM (n=5).Control: Tween-80+Water, Positive Control: Diazepam (10mg/kg), Group I: Extract (200mg/kg) and Group II: Extract (400mg/kg).

The tail withdrawal reflex time following the administration of the leaf and bark extracts of *G. cowa* were found to increase with increasing dose of the sample. The mean reflex time of positive control was 4.17,5.09, 4.81 and 5.24 sec at 0, 30, 60 and 90 minutes respectively. Withdrawal reflex time of leaf extract of *G. cowa* was found 4.46, 4.49, 4.99and 5.48sec at 0, 30, 60, 90 minutes for Group I (200mg/kg) and for Group II (400mg/kg), it was found 5.47, 5.37, 6.18 and 7.18sec at 0, 30, 60 and 90 minutes respectively. The bark extract of *G. cowa* was found 5.41, 4.10, 3.97 and 5.20sec at 0, 30, 60, 90 minutes for Group I (200mg/kg) and for Group II (400mg/kg), it was found 6.11, 5.22, 4.33 and 4.91sec at 0, 30, 60 and 90 minutes respectively.

3.4. Statistical Analysis

All experiments were performed thrice and the data were expressed as mean± SD.

4. Conclusion

It can be concluded that the crude extracts from the leaf and bark of *G. cowa* possess mild to moderate antibacterial activity as well as significant neuropharmacological effect and mild analgesic potential to respective standard drugs. Dose dependant activity was also identified by all the performed pharmacological investigations. These observations also support the use of this plant for medicinal purposes and encourage further investigations for more fruitful results.

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