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In vivo hypoglycemic, antinociceptive and *in vitro* antioxidant activities of methanolic bark extract of *Crataeva nurvala*

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ABSTRACT

Objective: To rationalize the folkloric use of hypoglycemic, antinociceptive and antioxidant potentials with phytochemical screening of methanolic bark extract of *Crataeva nurvala* (*C. nurvala*) *in vivo* and *in vitro*.
Methods: The collected bark was dried and grinded. The coarse powder was soaked in 2 000 mL of 90% methanol for several days then filtrated. At 40 °C the volume of crude methanolic extract (CME) was reduced by a vacuum rotary evaporator, then the aqueous methanol extract was separated into petroleum ether, carbon tetrachloride, and aqueous soluble fractions by Kupchan protocol. Then the extracts were subjected to evaluate *in vivo* analgesic, hypoglycemic activities in Swiss albino mice model and antioxidant *in vitro*.
Results: In quantitative phytochemical analysis, total phenolic content was found maximum (235.94 mg of GAE/g) in aqueous soluble fraction; in case of antioxidant potentials, DPPH free radical scavenging assay showed IC_{50} value of 9.25 µg/mL exhibited by aqueous soluble fraction in comparison to ascorbic acid (8.27 µg/mL) as a reference standard. The CMEs potentially ($P < 0.05$) reduced the acetic acid-induced writhing and increased ($P < 0.05$; $P < 0.01$) latency period in the tail immersion method at a dose dependent manner. The CME significantly reduced blood sugar level of diabetic rat induced by alloxan monohydrate.
Conclusions: This study was conducted to validate the extensive use of *C. nurvala* bark as folk medicine with antinociceptive, hypoglycemic and antioxidant effects. It can be concluded that the bark of *C. nurvala* possesses good antinociceptive, moderate hypoglycemic and antioxidant activities. However, further chemical and pharmacological revise are needed to elucidate the detail mode of action behind this and identify the responsible active principles.

1. Introduction

Medicinal plants are always providing new substances, *e.g.* antibiotics, alkaloids, cardiac glycosides, quinines, phenols, flavonoids, saponins having many biological active functions[1]. These may ensure the pharmacological activity of many medicinal plants. *Crataeva nurvala* (*C. nurvala*), belonging to family of Capparidaceae, is a potentially curative plant and extensively present in Bangladesh, India and other steamy areas of the world[2,3]. It is commonly known as 'Barun tiktoshak'. Different parts of this plant are used as an ingredient in different formulations of Ayurveda to treat prostatitis, prostate enlargement, other inflammatory conditions, thyroid problems, paralysis and urinary tract infection[4,5]. It is used traditionally in the treatment of urolithiasis, carbuncle, nephritic disorders, breast cancer and also used as an oral contraceptive[6-10]. Phytochemical analyses of *C. nurvala* revealed the presence of various types of compound in its different parts. Lupeol is a constituent of *C. nurvala* bark which showed antioxidant[11,12], cardio-protective[13], chemopreventive and chemotherapeutic properties[14]. The bark part also exhibited antidiabetic[15], antidiarrhoeal[16], antifertility[17], and analgesic properties[18]. The leaves of this plant have antidiarrhoeal, analgesic and diuretic activities[19]. Antibacterial, anthelmintic and wound healing properties were observed in the root part of this plant[20,21]. The current experiments aimed to investigate the methodical proof for using it as folk medicinal values as antioxidant, hypoglycemic, antinociceptive activities.

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The studies with the animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee and the methods regarding animal handling were approved (Grant No. WUBPS # 05512).
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2. Materials and methods

2.1. Collection and identification of bark materials

The stem bark of *C. nurvala* was collected from Agailjhara,

Barisal in July 2016 and identified and registered (DACB Accession No. 43485) in Bangladesh National Herbarium.

2.2. Preparation of bark extracts

Collected bark was dehydrated and grinded. The grinded powder (200 g) was soaked in 2 000 mL of 90% methanol for 14 days then filtrated. The volume of crude methanolic extract (CME) was reduced by a vacuum rotary evaporator at 40 °C. Then the aqueous methanol extract was separated into petroleum ether, carbon tetrachloride, and aqueous soluble fractions by Kupchan and Tsou protocol[22] and modification of VanWagenen *et al.* method[23].

2.3. Chemicals and reagents

Alloxan monohydrate and glybenclamide was warmly gifted by ACI Pharmaceuticals Ltd. and Square Pharmaceuticals Ltd. Diclofenac Na was purchased from the local market. Phosphate buffer, sodium hydroxide, hydrochloric acid, sodium carbonate, acetyl salicylic acid, solvents (methanol, petroleum ether, carbon tetrachloride) and also other necessary chemicals and reagents were of analytical grade.

2.4. Phytochemical screenings

All extracts were qualitatively tested for the presence of phytoconstituents such as alkaloids, glycosides, steroids, flavonoids, tannins, proteins, resins, quinines, phenols and saponins. Phytoconstitutes were characterized by using standard method of Sofowora[24].

2.5. Determination of total phenolic content

In this study, methods of Dewanto *et al*. and Karim *et al*. were followed with light modification to analyze the total phenol contents[25,26]. Briefly, 0.3 mL of the CME and their fractions were poured into different small volumetric flask having 2.7 mL of Folin-Ciocalteu (1:10) phenol suspension. After 5 min, 2 mL of 7.5% sodium carbonate solution was added to each test tube and mixed well. Then they were preserved at 25 °C for 30 min in shady place after heat at 45 °C. A calibration curve was prepared by using a series of solutions of gallic acid. Absorbance for test and standard solutions were taken at 725 nm with UV spectrophotometer against the blank solution. The entire phenol contents of extracts were measured from extrapolation of gallic acid standardized curve.

2.6. 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity

The free radical scavenging potential of the extracts was calculated *in vitro* by DPPH test[26,27]. An aliquot of 2.0 mL of CME and extracts at different concentrations of 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562 5, 0.781 25 μg/mL were added in 3.0 mL of a DPPH methanol solution (20 μg/mL). The suspension was allowed to put at 25 °C for 30 min in shady place and the absorbance was taken at 517 nm against methanol as blank by UV spectrophotometer. The IC_{50} value of the fractions was evaluated against ascorbic acid, which was used as the standard.

2.7. Experimental animal

For the study, Swiss albino mice of 6–7 weeks of age, between 30–35 g of weight were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh, Mohakhali, Dhaka, Bangladesh. Mice were kept under standard environmental conditions [temperature: (27.0 ± 1.0) °C, relative humidity: 55%–65% and suitable light/dark cycle] and allowed free access to food and drinking water. The animals were allowed to adapt to laboratory environment for several days before conducting test. The studies with the animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee and the methods regarding animal handling were approved (Grant No. WUBPS # 05512).

2.7.1 Antinociceptive activity evaluation

Antinociceptive potential was observed by acetic acid induced writhing and tail immersion methods.

2.7.2. Tail immersion study

The tail-flick test was done as per the method, which is developed by D'Amour and Smith[28]. In this study, mice were subdivided into four groups as 1, 2, 3, 4 respectively, each group composed of five mice. To avoid thermal burn, immersion of tail in warm water for assessment, allowed only for 5 s instead of 10 s. In this study, the CME at 100, 200, 400 mg/kg and diclofenac Na at 15 mg/kg body weight were respectively given intraperitoneally, with diclofenac Na used as reference drug. Any physical changes were observed after from 0, 30, 60 and 90 min. Then about 3 cm of the tail of the mouse was immersed in a hot water bath at (55.0 ± 0.5) °C. The time of tail withdrawn from warm water was noted. A positive analgesic response was found due to increase in reaction time of tail against control animals[29].

2.7.3. Acetic acid induced writhing test

Koster *et al.* described the acetic acid writhing test in mice[30], which applied with slight modification in acetic acid induced writhing test. Mice were subdivided into four groups as 1, 2, 3, 4 respectively, with each group composed of 5 mice. The amount of 10 mL/kg of 1% Tween 80 was given intraperitoneally to the first group of mice which was considered as control. In Group 2, standard diclofenac Na has given to mice as dose of 15 mg/kg of body weight. Groups 3 and 4 received CME at 200 mg/kg and 400 mg/kg of body weight after each mouse was injected intraperitoneally with 0.7% acetic acid at doses of 10 mL/kg of body weight after 30 min. Writhing was observed which was induced by just after 30 min intra-peritoneal administration of 0.7% acetic acid to all experimental animals. The number of writhing responses was observed and recorded for each mouse during a subsequent 5 min period up to 30 min. The occurrences of abdominal contraction, elongation of the body and twisting of the trunk were considered as absolute writhing. The percentage of protection against writhing was used to estimate the degree of analgesia and was calculated using the formula:

$$\% \text{ Inhibition of writhing} = [(W_c - W_t)/W_c] \times 100$$

where, Wc is the average writhing of control group and Wt is the average writhing of treated group (*i.e.* extract or standard).

2.7.4. Hypoglycemic activity test

Twelve healthy mice were separated into four groups. Mice of all groups were fasted for 12 h. Alloxan monohydrate (120 mg/kg body weight in normal saline) was used intraperitoneally to induce hyperglycemia in fasting conditions. About 10% glucose solution was thereafter given intraperitoneally to produce instant hypoglycemia. Group I was given 1% Tween 80 rich normal saline and considered as diabetic control. Group II was given glibenclamide (10 mg/kg body weight) as standard drug for reference groups. Groups III and Group IV received extracts at 200 mg/kg and 400 mg/kg body weight, respectively. Blood sample was collected at 0, 30 min, 48 h and after 90 h, respectively from the tail vein of mice and glucose level was measured by Freestyle glucometer and followed by evaluated with control and reference groups.

2.8. Statistical analysis

All statistical analyses were designed and the evaluated data were calculated using SPSS software, version 20, and data were expressed as mean ± SEM with their communicating *P* values. Values of investigations were compared with test sample and control or standard was performed by One-way ANOVA followed by Dunnett's *t*-test.

3. Results

3.1. Phytochemical screening test

The qualitative phytochemical screenings revealed the presence of alkaloids, glycosides, steroids, flavonoids, tannins, proteins, resins, quinones, phenols and saponins.

3.2. Total phenolic contents determination

The antioxidant potential was found by total phenolic contents of CME and different extracts of *C. nurvala* ranging from 9.25 mg to 55.62 mg of GAE/g (Table 1).

Table 1

Total phenol compounds and free radical scavenging potential of CME and its fractions of *C. nurvala*.

Sample/Standard	Total phenol compounds (mg of GAE/g)	DPPH free radical scavenging activity (IC_{50} μg/mL)
AQSF	235.94	9.25
CTSF	150.13	13.15
CME	210.06	15.97
PESF	249.06	55.62
Vitamin C	-	8.27

AQSF: Aqueous soluble fraction; CTSF: Carbon tetrachloride soluble fraction; PESF: Petroleum ether soluble fraction.

3.3. Antioxidant activity

The antioxidant activity was determined in terms of DPPH free radical scavenging activity of *C. nurvala* which showed IC_{50} values within the range of 9.25 μg/mL to 55.62 μg/mL. Among the CME and fractionates, the highest free radical scavenging activity was shown by AQSF (9.25 μg/mL) and followed by CTSF (13.15 μg/mL), CME (15.97 μg/mL), and PESF (55.62 μg/mL) in comparison with ascorbic acid (8.27 μg/mL) as a standard (Figure 1 and Table 1).

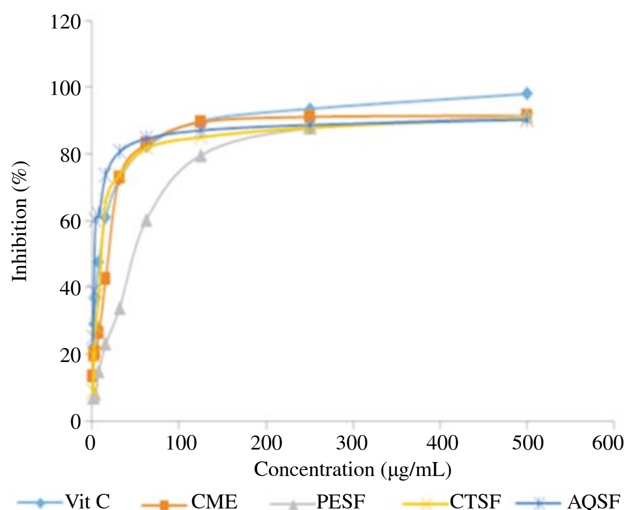

Figure 1. Determination of IC_{50} value against DPPH free radical scavenging activity of CME and its fractions of *C. nurvala*.

3.3.1. Central analgesic activity

The CME of *C. nurvala* showed increase in latency period in a dose reliant increasing mode and results were compared with the standard (Table 2). At 30, 60 and 90 min of experimental period, the elongation percentages of tail flicking by methanolic extract were 44.49%, 68.03% and 93.88% for 200 mg/kg body weight and 113.14%, 152.42% and 184.17% for 400 mg/kg body weight, respectively, which were statistically significant ($P < 0.05$).

Table 2

Antinociceptive effect of CME of *C. nurvala* in the acetic acid induced pain in mice model.

Group	Latency time (s)		
	30 min (% of elongation)	60 min (% of elongation)	90 min (% of elongation)
Control	2.36	2.69	2.78
Diclofenac Na (15 mg/kg)	5.45 (130.93)**	8.37 (211.15)*	9.77 (251.44)
Methanolic extract (200 mg/kg)	3.41 (44.49)*	4.52 (68.03)	5.39 (93.88)
Methanolic extract (400 mg/kg)	5.03 (113.14)*	6.79 (152.42)	7.90 (184.17)

Values are expressed as mean ± SD (n = 5) $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant difference when compared with the corresponding value of standard, done by One-way ANOVA followed by Dunnett's *t*-test.

3.3.2. Acetic acid induced writhing test

Results of peripheral antinociceptive potential of CME of *C. nurvala* in Swiss-albino mice have shown in Table 3. In this study we observed that all doses of (200 and 400 mg/kg body weight) CME manifested the percent inhibition of writhing responses were 45.875% and 66.625%, respectively, which was induced by the acetic acid. Then results were comparative to reference drug (Diclofenac Na 2 mg) (75.000%).

Table 3

Analgesic activity of methanolic extract of *C. nurvala* in tail immersion test in mice model.

Group	No. of writhing (%)	Inhibition of writhing (%)
Control	26.200 ± 2.010	
Diclofenac Na (2 mg)	25.010 ± 1.500**	75.000
Methanolic extract (200 mg)	54.125 ± 1.500*	45.875
Methanolic extract (400 mg)	33.375 ± 1.500**	66.625

Values are expressed as mean ± SD (n = 5), $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant difference when compared with the corresponding value of control, done by One-way ANOVA followed by Dunnett's *t*-test.

Table 4
Hypoglycaemic activity of CME of *C. Nurvala* in mice model.

Group	Dose	Plasma level of glucose (mg/dL)				
		0 min	25 min	24 h	48 h	72 h
Group I	Diabetic control	123.66	203.40	151.74	144.54	132.66
Group II	Diabetic rat + Glibenclamide	97.74	174.60	119.34	111.24	93.60**
Group III	Diabetic rat + Plant extract (200 mg/kg)	118.26	196.74	146.34	126.00	119.34
Group IV	Diabetic rat + Plant extract (400 mg/kg)	113.40	178.74	134.46	117.54	109.26*

Values are expressed as mean ± SD (n = 5); $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant difference when compared with the corresponding value of standard, done by One-way ANOVA followed by Dunnett's *t*-test.

3.4. Hypoglycemic activity

In this study we found that the *C. nurvala* reduced the rise of glucose level in blood. Results are given in Table 4. The crude extract of *C. nurvala* at doses of 200 mg/kg and 400 mg/kg body weight has moderated blood glucose lowering activity as 119.34 mg/dL and 109.26 mg/dL, respectively ($P < 0.05$).

4. Discussion

Crude methanol extracts of bark of *C. nurvala* and fractions were under taken to ensure the presence of different bioactive substances. Different phytochemicals such as alkaloids, glycosides, steroids, flavonoids, tannins, proteins, resins, quinine, phenols and saponins were tracted out in the tested fractionates. Phytoconstitutes characterized in the current experiment are subject to be beneficial in pharmaceutical sciences[31]. The knowledge of this initial investigation can be anticipated as an interpretation in the exploration of a novel and financially valued drug molecules[32,33].

4.1. Total phenolic content

The total phenol compounds are exposed in the Table 3. The decision of quantifying total phenolic compound in the fractions was taken after assurance of presence of phenols or polyphenols in the preliminary phytochemical investigation. The PESF exhibits the highest phenolic content (249.06 mg of GAE/g) where AQSF showed the comparatively less amount of phenolic substance (210.06 mg of GAE/g) among all the Kupchan fractions. But the other factions also showed the considerable quantity of phenolic compounds. Answers show the existence of satisfactory amount of lipid soluble phenolic substance.

4.2. Antioxidant activity

Currently, interest has vigorously enhanced in the naturally occurring antioxidants from natural source due to their safety margin in the reduction of human oxidative stress and low side effects[34,35]. *In-vitro* DPPH free radical scavenging potential was evaluated in order to determine the antioxidant potential of different organic soluble bark extracts of *C. nurvala*. In our current study, aqueous soluble fraction exhibited the highest antioxidant capacity with 9.25 (IC_{50} μg/mL) which was close to the value of standard vitamin C (8.27 μg/mL). The order of IC_{50} value of different Kupchan was as follows: carbon tetrachloride (13.15 μg/mL) > CME (15.97 μg/mL) > petroleum ether (55.62 μg/mL) (Table 1 and Figure 1), illustrating these results accordingly.

4.2.1. Analgesic activity

Many analgesic drugs such as non-steroidal anti-inflammatory drug, narcotics drugs, and steroids are available for many years for the effectively pain management and their harmful effects. Non-steroidal anti-inflammatory drugs cause gastrointestinal irritation, and ultimately cause peptic ulcer, narcotics drugs can develop addiction, and steroids have severe adverse effects affecting hormonal imbalance[36]. Many peripherally or centrally acting pain reducing agents have been separated from natural sources especially from plants and needs analysis to find out more pain reducing agents from natural sources[37].

Tail flicking test is the most extensively used experiment for the neurologic pain sensation, and central analgesic agents can potentially increase the latency period in warm water. Diclofenac Na was used as standard which inhibits pain by the interference of prostaglandin production through the competitive inhibition of cyclooxygenase. At the dose dependent mode, reduction of gastrointestinal contraction by the extract, proofs *C. nurvala* have antispasmodic activity. Diclofenac sodium was exhibited satisfactory percent antispasmodic activity (251.44%) than the plant extracts at both 200 mg/kg body weight (93.88%) and 400 mg/kg body weight (184.17%).

In acetic acid prompted writhing technique, pain was induced by the intraperitoneally given acetic acid which produced pain by the increased synthesis of PGE2 and PGF2a[38], and lipoxygenase derived eicosanoids in the peritoneal region[39]. The releases of these pain modulators are responsible for the pain sensation. The CME significantly reduce the acetic acid induced writhing at 200 mg/kg body weight (45.875%) and 400 mg/kg body weight (66.625%) in comparison to standard (75.000%).

4.2.2. Hypoglycemic test

Diabetes is a chronic metabolic disorder which may be due to initial stage of carbohydrate metabolism, characterized by high blood glucose, resulting from complete or less secretion of insulin. High diabetes causes excessive problems, disturbing the vascular system, eyes, nerves, and kidneys and leading to peripheral vascular disease, nephropathy, neuropathy, retinopathy, *etc*.[40]. The CME showed significant dose dependent anti-diabetic activity in comparison to standard. The outcome may be due to the extract of *C. nurvala* reduced hepatic output of glucose by increasing glycogenesis, uptake of glucose from blood, conversion of glucose into fatty acid and decreased glycogenolysis, gluconeogenesis. The extract of *C. nurvala* also may reduce peripheral glucose level by increasing glucose transport, utilization of glucose and glycogenesis, which was found at fasting state in diabetic animals[41].

Conflict of interest statement

We declare that we have no conflict of interest.

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