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A Study on *Hevea Brasiliensis* for evaluation of phytochemical and pharmacological properties in Swiss Albino Mice

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ABSTRACT

Hevea brasiliensis, a plant belonging family Euphorbiaceae. In Brazil this plant is not only use for medicinal purpose but also for cosmetics purpose. The present study was aimed to study analgesic and antidiarrheal activity of methanol extract of *Hevea brasiliensis.* Analgesic activity was evaluated by acetic acid- induced writhing method and antidiarrheal by gastrointestinal motility method (charcoal meal test) in mice. Phytochemical evaluation was carried out by qualitative analysis. For analgesic evaluation, the extract (250 mg and 500 mg) showed significant activity compared to control diclofenac Na. On the other hand, for antidiarrheal activity the extract (250 mg and 500 mg) significantly reduce charcoal propulsion. The phytochemical evaluation showed significant presence of Alkaloids, Carbohydrates, Glycosides, Saponins, Phytosterols, Proteins and amino acids, Fats & fixed oils. It's concluded that the extract possesses both analgesic and antidiarrheal activity and containing wide range of phytochemicals.

Keywords: Analgesic, Phytochemicals, Antidiarrheal, *Hevea brasiliensis*

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INTRODUCTION

Hevea brasiliensis (H. brasiliensis), better known as the rubber tree, is the primary source of natural rubber. *Hevea brasiliensis*, a plant belonging to the family Euphorbiaceae, is widely used for the production of rubber. Several rubber producing countries are expanding cultivation of rubber to areas outside the scope of optimum weather conditions in order to meet increasing demand for natural rubber, capture otherwise 'waste' land, enhance economic activities of rural dwellers and boost of export trade to earn foreign exchange. In addition, *H. brasiliensis* is cultivated in non-traditional areas as escape zone for some devastating diseases such as the South American Leaf Blight in Brazil.[1] Rubber tree is a quick-growing tree which is the most economically important member of the genus *Hevea*. It is of major economic importance because the milky latex extracted from the tree is the primary source of natural rubber. As a latex producing crop, the bark is regularly tapped.[2,3] Latex, the source of hevea or para rubber, is obtained by tapping the trunks of the trees. It is cultivated for rubber, food, apiculture, fibre, timber, lipid, fuel etc. It also cultivated for soap, insect repellent in Brazil. The extract from *H. brasiliensis* possesses antimicrobial activities against *E. coli, Klebsila pneumoniae and Pseudomonas aeruginosa*.[3,4] Proteins present in natural rubber latex may cause allergic reactions.[5] In this study, we aimed to evaluate the analgesic and antidiarrheal activity of methanol extract of *Hevea brasiliensis.*

MATERIALS AND METHODS

Plant collection and identification

The roots of *Hevea brasiliensis* was collected from Fatikchari, Chittagong, July, 2013. It was taxonomically identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh (Accession number-38637) and also deposited there for further study.

Preparation of Hevea brasiliensis extract

After collection, the roots were cut into pieces, dried under sun for three weeks and finally ground to coarse powder by a suitable grinder. According to cold extraction method, dried and powdered roots (500 gm) were soaked in distilled methanol at room temperature for two weeks. The filtrate was concentrated by evaporation. Dried extract was stored at 4°C in air tight container and was diluted with methanol prior to any pharmacological screening.

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Chemicals and Drugs

Diclofenac Na and Loperamide were obtained from Square Pharmaceuticals Ltd, Bangladesh.

Experimental Animals

Young Swiss-albino mice aged 4-5 weeks, average weight 20-25gm were used for the experiment. The mice were purchased from Jahangirnagar University, Dhaka. They were kept in standard environmental condition for one week for adaptation before tests. They were provided with the standard rodent pellet diet, water *ad libitum* andfasted 18 h prior to their use. Experiments were carried out according to animal ethics guidelines.[6] Animals were marked for their proper identification.

Acetic Acid Induced Writhing Test

Analgesic activity of the methanol roots extract of *Hevea brasiliensis* was tested using the method of Koster et al., 1959.[7] Experimental animals were randomly selected and splitted into four groups, i.e. control, positive control and the two doses of the extract, consisting of 6 mice in each group. Each group received a particular treatment. Test groups received the methanol roots extract at the doses of 250 and 500 mg/kg in oral route. Standard analgesic drug diclofenac sodium (25 mg/kg, p.o.) was administered orally to the positive control group. Control group orally received 1% tween-80 in saline at the dose of 10 ml/kg. After the interval of 40 min, each animal was given an intraperitoneal (i.p.) injection of 0.1%acetic acid at the dose of 10 ml/kg to induce the characteristic writhing. 5 minutes after the administration of acetic acid, number of writhing were counted for each mouse for ten minutes. The percent writhing inhibition was calculated and compared with control to assess analgesic activity.

% writhing Inhibition = Mean No. of Writhes in control-Mean No. of Writhes in test / Mean No. of Writhes in control × 100.[8]

Intestinal motility test

Albino Swiss mice were kept in standard conditions for 10 days before performing the experiment. They had free access to water and a normal commercial laboratory diet. On the test day, the animals were divided into four groups of 5 mice each. They were weighed and deprived of food, with free access to water. Three hours after food deprivation, the animals in group A received 10ml/kg of normal saline, while those in group B received orally by gavage 5mg/kg of loperamide as positive control. The *Hevea brasiliensis* methanolic roots extracts (250 and 500 mg/kg, p.o.) were provided to the test groups. After 90min, 0.3ml of an aqueous suspension of 5% charcoal in 10% water was administered to each animal orally by gavage. The animals are killed 45 min later to open the abdomen and remove the small intestine (from the pylorus to the caecum) to determine the length of the intestine and distance travelled by charcoal meal as a fraction of the length of intestine.[9]

Phytochemical screening

Methanol roots extract of *Hevea brasiliensis*was subjected to different preliminary phytochemical tests to detect major phytochemicals like alkaloids, carbohydrates, glycosides, phytosterols, proteins, flavonoids, tannins, saponins, phenols, terpenes, fats & fixed oils. Phytochemical examinations were carried out for all the extracts as per the standard methods.[10]

Statistical analysis

The results of all experiments were expressed as mean values±Standard Error of Mean (SEM) and group data comparisons were evaluated by Student's t-test. The results were considered statistically significant when $P < 0.001$.

RESULT

Analgesic activity

The methanol roots extract of *Hevea brasiliensis* exhibited dose dependent inhibition of writhing. The doses of 250 and 500 mg/kg extract showed 21.62% and 36.53% inhibition of writhing significantly ($P < 0.001$). Positive control diclofenac sodium showed strong analgesic activity with 45.02% inhibition of writhing, compared to control. Activity of the extract was strongly comparable with Diclofenac sodium. Result showed in Table 1.

Intestinal motility test

The methanol roots extract of *Hevea brasiliensis* significantly ($P < 0.001$) and dose dependently decreased the intestinal transit charcoal meal as compared with control. Percentage of distance travelled by charcoal for control, loperamide, extract (250 mg/kg) and extract (500 mg/kg) were 89.32%, 40.19%, 62.61% and 56.98% respectively. The results indicated a reduction in peristaltic activity and ultimately reduction in gastrointestinal motility. Result showed in Table 2.

Phytochemical screening

The preliminary phytochemical analysis of methanolic extract confirmed the presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, proteins, fats and fixed oils. Result showed in Table 3.

Table 1 **Analgesic effect of methanolic roots extract of *Hevea brasiliensis***

Animal group (n=6)	Treatment	Writhing count (Mean±SEM) (%writhing)	% Inhibition of writhing
I (Control)	1%tween-80 solution	12.95± 0.68 (100)	---
II (Positive control)	Diclofenac sodium (25 mg/kg)	7.12 ± 0.57 [a] (54.98)	45.02
III (Test group)	Methanol extract (250 mg/kg)	10.15 ± 0.93 [a] (78.38)	21.62
IV (Test group)	Methanol extract (500 mg/kg)	8.22 ± 0.58 [a] (63.47)	36.53

N = number of mice, S.E.M = standard error of mean, [a]P<0.001, Values are expressed as Mean±S.E.M

Table 2 **Effects of methanol roots extract of *Hevea brasiliensis* on charcoal meal-stimulated gastrointestinal transit in mice**

Group (n=5)	Mean intestinal length (cm) Mean ± SEM	Mean distance travelled by charcoal (cm) Mean ± SEM	Gastrointestinal Transit (%)
Control	51.13 ± 1.34	45.67 ± 1.98	89.32
Atropine	56.67 ± 1.27	22.78 ± 1.86	40.19
Extract(250mg/kg)	57.50 ± 1.50	36.00 ± 0.50	62.61
Extract(500mg/kg)	59.75 ± 0.55	34.05 ± 0.45	56.98

n=number of mice, S.E.M = standard error of mean P <0.001, Values are expressed as Mean ± S.E.M

Table 3 **Results of phytochemical screening of methanol roots extract of *Hevea brasiliensis***

Phytochemicals	Methanolic roots extract of *Hevea brasiliensis*	Phytochemicals	Methanolic roots extract of *Hevea brasiliensis*
Alkaloids	+ ve	Fats & fixed oils	+ ve
Carbohydrates	+ ve	Flavonoids	- ve
Glycosides	+ ve	Phenols	- ve
Saponins	+ ve	Terpenes	- ve
Phytosterols	+ ve	Tannins	- ve
Proteins and amino acids	+ ve		

"+ ve" indicate the presence and "- ve" indicate absence

DISCUSSION

Medicinal plants have a long history of serving people in many regions worldwide. 80% of the world population still uses herbal and medicinal plants in treating various ailments because such plants possess different types of phytoconstituents which exert a variety of pharmacological effects in human body.[6] According to the previous studies, phytochemical screening on most of the medicinal plants revealed the presence of several phytochemicals like alkaloids, carbohydrates, tannins, phenol, terpenes, fats and fixed oils. These phytochemicals are responsible for various biological actions including antioxidant activity.[6,10]

Analgesic activity of the extract was evaluated by acetic acid-induced writhing method. Acetic acid-induced writhing method was used to assess peripherally acting analgesic activity of the plant extract in which writhing results from the sensitization of pain receptors by prostaglandins release. The released prostaglandins, mainly prostacyclin (PGI_2) and prostaglandin-E have been reported to be responsible for pain sensation by exciting the Ad-fibres. Activity in the Ad-fibres cause a sensation of sharp well localized pain.[11,12] Since the methanol roots extract of *Hevea brasiliensis* significantly inhibited the acetic acid-induced writhing in mice it suggests that the analgesic effect of the extract may be peripherally mediated.

Previous study shows that activated charcoal avidly absorbs drugs and chemicals on the surface of the charcoal particles thereby preventing absorption.[13] Thus, gastro-intestinal motility test

with activated charcoal was carried out to find out the effect of methanol roots extract of *Hevea brasiliensis* on peristaltic movement. The results showed that, intestinal motility was decreased with increasing the dose of the plant extract. The methanol roots extract of *Hevea brasiliensis* significantly delayed gastrointestinal transit of charcoal meal, compared to control. So, the extract might have ability in greater extent to decrease gastrointestinal motility. The inhibition of peristaltic movement with methanol roots extract of *Hevea brasiliensis* may be due to the anti-histaminic and anticholinergic actions.[13,14] From these models we can suggest that methanol roots extract of *Hevea brasiliensis* nonspecifically inhibit diarrhea by decreasing intestinal motility. Loperamide was used as positive control. The effects of this positive control on gastrointestinal motility were investigated.

The phytochemical evaluation of the extract confirmed the presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, proteins, fats and fixed oils. Either one or combination of these may be responsible for the observed analgesic and anti-diarrhoeal effect.

CONCLUSION

It can be concluded that, methanol roots extract of *Hevea brasiliensis* contains some bioactive agents, which comprise of analgesic and anti-diarrheal effects. It requires further investigations to use in folk medicine.

CONFLICT OF INTEREST

Authors has no conflict of interest.

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