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Role of *Hydnora africana* (Sub. Family-Hydnoraceae) root extract for antidiarrhoeal activity

Shibanjan Paul Roy

Abstract
By this study I conducted the evaluation of the antidiarrhoeal activity of hydromethanolic root extract of *Hydnora africana* induced diarrhea model in castor oil, Dinoprostone induced secretion model and by charcoal marker it's antimotility activity seen using charcoal as a marker.
Methods: The animals were randomly allocated in all the three models into five groups of six animals each and mice were received 1 ml/100 gm normal saline in group 1 and group 2 used standard drug for treated them as a positive control whereas group 50, 100 and 200 mg/kg extract doses given to the group 3, 4 and 5, respectively statistical significance of differences in the mean of fluid content of faces, number of defecation, intestinal fluid accumulation ratio, intestinal fluid weight and distance travelled by charcoal between groups was analyzed by software.
Results: The *Hydnora africana* (Sub. Family-Hydnoraceae) at 100 and 200 mg/kg doses showed statistically significant ($p<0.05$) weight difference of the fluid content and inhibition of the frequency of defecation of the fluid content of the feces compared to the negative controls. The percentage inhibition for those doses of diarrheal feces was 43.65 and 53.54% respectively. In the standard drug the antisecretary of the extract in terms of fluid accumulation ratio was not found statistically significant compared to the negative control.
Conclusion: Root of *Hydnora africana* (Sub. Family-Hydnoroideae) has shown promising anti-diarrhoeal activity validates its use. Therewithal studies are needed and possibly the plant may serve as a strong source of new agent in the therapeutic armamentarium of diarrhea.

Keywords: *Hydnora africana* (Sub. Family-Hydnoraceae), antidiarrhoeal, castol oil and ethnopharmacology

Introduction
Background: The passage of abnormally liquid or unformed stools associated with increased frequency of abdominal pain and defecation. Reduction of mortality and morbidity worldwide diarrhea still counts for more than 2million deaths annually. In this world 40% childhood deaths from diarrhea occur in Africa.
For this type of disease always usually preferred medicinal plants to treat Gastrointestinal disorders. E.g. Diarrhoea, constipation.
WHO recommended the use of modern and folk medicine for controlling of health problem. As the ethnopharmacological survey reports of plants therapeutic effects for stomach ache and diarrhea and *in vivo* antidiabetic activity.

Methods
(a) Plant Material: The roots of *Hydnora africana* (Sub. Family-Hydnoraceae) were collected by lab from Malbazar. The taxonomic identification was done by me.

(b) Experimental Animals: The swiss albino mice which is healthy weighing between 24-30 gm and 7-8 weeks old, were obtained in the lab. The animals maintained under standard condition of relative temperature humidity 12 h light/12 h dark cycle and given water and food. Then acclimatized all mice to the working environment 1week before beginning of pharmacologic activities evaluation.

(c) Procedure of Extraction: *Hydnora africana* (Sub. Family-Hydnoraceae) were throughly washed with distilled water to remove soil and dirt and dried under optimal ventilation and shade for 15 days. The chopped the dried roots into small pieces and reduced to powder using electronic miller. In the maceration extraction procedure the coarsely powdered roots were subjected using 80% methanol (for 74 hr at room temperature) as a menstruum and this was

done three times. By using whatman no-1 filter paper the respective extract was filtered and the solvent was evaporated in an oven under reduce pressure at 40 °C. The dried extract finally was stored at 4 °C in refrigerator.

(d) Phytochemical Prelimnary Screening
The absence and presence of secondary plant metabolites in the crude hydromethanolic root extract of *Hydnora africana* (Sub. Family-Hydnoraceae) was screened by precipitation and color forming assays using standard procedures.

(e) Acute Toxicity: Acute oral toxicity was determined by using the limit dose of 1000mg/kg body weight of the mice as per the OECD guideline.

(f) Animal grouping and dosing
The mice were randomly grouped (n=6) in all models and the substances administered were as follows:
Group I: received 1 ml/100 mg normal saline (NS).
Group II: treated with standard drug, 3 mg/kg loperamide (3 mg/kg Lop) in both castor oil induced antidiahreal test and misoprostol induced antisecretary test and atropine sulfate 0.1ug/g IP for gastrointestinal motility test.
Group III: treated with 50 mg/kg extract (50 mg/kg ISP).
Group IV: treated with 100 mg/kg extract (100 mg/kg ISP).
Group V: treated with 200 mg/kg extract (200 mg/kg ISP).
The acute toxicity study result based on the test doses of the extract were selected. It also determined based on OECD guideline as the test doses of the volume to be administered. As we know the African people use the preparations of plant via oral route by using water as vehicle the study was conducted using except the intraperitoneal injection of atropine, standard drug and oral route of administration.

(g) In this model castor oil induced: The animals which were found diarrhea when they taken 0.5 ml castor oil for initial screening test also induced for 24 hr the mice fasted by using stop watch and allocated to five groups of six animals each. 0.5 ml castor oil was given for the induction of diarrhea for each mouse 30min before treatment. The floor of which was lined with transparent paper and every hour the floor lining was changed when each animal was then placed in individual cage. Total number of fecal outputs within in the 4hr period was recorded onsets of diarrhea. By using the weight difference of the fresh and dry stool (dried for 24hr at room temperature in a shaded area) even total fluid content of the faces was determined. On stool consistency evacuation classification based on stool consistency was assigned as follows:
Normal stool=1, semi-solid stools and watery stool=3 and mean of evacuation index was calculated for each group. The percentage inhibition of diarrhoea for all groups was also calculated compared to the negative controls.

(h) By Charcoal meal the Gastrointestinal Motility test performance
For 20 hr the mice were fasted but had free access to water. The respective groups were treated mentioned above after the grouping. Each animal was loaded with 1ml of 3% deactivated Charcoal in normal saline after 1hour and then waited for 1hour and dissected. The small intestine (To Caecum from Pylorus) was removed and it's length was measured. By Charcoal to the total length between the pylorus and Caecum the intestinal Charcoal transit was expressed. Atropine was used as a standard drug for positive control group to known spasmolytic agent.

(i) Antisecretary Assay: The induce of prostaglandins (PGE2) of intestinal secretion and this is helpful to evaluate antisecretory activity of different chemical compounds. The mice were fasted for 24 hr in this model and then received Dinoprostone 0.05 mg/ kg for the induction of intestinal secretion. Just like the castor oil induced diarrhoea model after 1hour each group of mice were treated. After 24hrs the animals were then sacrificed by cervical dislocation laparotimized and then the caecal and pyloric ends of the small intestine were removed and tied. For each animal determination done for the each animal fluid accumulation ratio and antisecretory activity was expressed in percentage of inhibition. The removed intestine then weight for the fluid content of subtracting the weight of the intestine before and after milking of the removed intestine.

(j) Statistical Analysis: Mean±standard error means was expressed by all the experimental data. For data analysis and processing SPSS statistical software was used. This software assessed statistical significance of differences between groups. P-value less than 0.05 considered by the results.

Result
(a) Extract Material: *Hydnora africana* (Sub. Family-Hydnoraceae) which is final dried a hydromethanolic root extract was brown powder in it"s colour and the yield was 12.56%.

(b) Phytochemical Screening Result: The selected secondary plant metabolites are summarized by the preliminary phytochemical screening test result in Table 1.

Table 1: Preliminary phytochemical screening result of crude hydromethanolic root extract of Hydnora Africana

Test	Result
Alkaloids	Present
Tannins	Present
Flavonoids	Present
Saponins	Present
Steroids	Present
Terpenoids	Present

(c) Oral Acute Toxicity: By using the limit dose of 1000mg/kg body weight of the mouse of the oral toxicity test the mouse found safe and the animals did not show any observable behavioural and physical changes, confirming that the LD_{50} of the extract greater than 1000 mg/kg.

(d) Antidiarrhoeal Activity Evaluations
(1) Effect of the extract on the castor oil induced diarrhoea
After castor oil supplementation considering the latency defecation only the 200mg/kg extract dose demonstrated significant ($p<0.01$) For treated groups delay compared to the negative controls. This group is found significantly ($p<0.05$) different compared to the 50 and 100 mg/kg extract dose treated groups by the onset of defecation. The standard drug, the hydromethasnolic crude extract doses (100 and 200 mg/kg) of root of *Hydnora africana* (Sub. Family-

Hydnoraceae) as shown in Table 2 statistically significant (p<0.05) inhibition both total weight of the fluid content and frequency of defecation of the faces compared to the negative control. The percentage inhibition of diarrhoea by 50, 100 and 200mg/kg doses of the extract was determined 22.52 m 43.65 and 53.54%, respectively and this inhibition, especially from the largest dose of extract was comparable with inhibitory effect 51.05% of Loperamide. Nor the extract treated groups exhibited statistically significant difference in the mean evacuation index compared to the exposed groups of normal saline. As the difference among any groups the mean evacuation index didn't show.

(2) Gastrointestinal motility test

All the extract dose treated groups didn't show significant difference compared to the negative control and amongst each other regarding spasmolytic activity. The treated group is atropine treated group showed statistically significant (p<0.001) inhibition of intestinal motility compared to both all the extract treated groups and the negative control as it is illustrated in Table 3. The percentage inhibition of intestinal transit from atropine and the largest dose of the extract (200mg/kg) were found 68.48 and 11.27%, respectively as compared to the normal saline exposed group.

(3) Antisecretary Activity

GI secretion induced by antisecretary activity on the extract on Dinoprostone according to the fluid accumulation ratio unlike that of Loperamide was found insignificant statistically compared to the negative control. The percentage inhibition of intestinal fluid accumulation ratio of the extract is inversely related to its dose shown in Table 4. The weight of the intestinal fluid in all the respective found (p<0.01) significantly lower than the negative control and with the Loperamide treated group this was comparable.

Table 2: Effect of crude hydromethanolic extract of root of *Hydnora africana* on castor oil induced diarrhea in mice

Group	Onset of diarrhea (min)	Total number of faeces in 4 h (frequency of defecation in 4 h)	Mean evacuation index	Fluid content of the faces (g)	% Inhibition of diarrhea
NS	63.36±5.61	11.86±1.33	2.42±0.12	0.81±0.16	0.00
3 mg/kg Lop	99.20±18.17	5.86±0.78*	2.32±0.18	0.39±0.08*	51.05
50 mg/kg IS	71.86±5.70	9.20±0.73	2.37±0.09	0.64±0.14	22.52
100 mg/kg IS	75.36±4.89	6.70±1.55*	2.20±0.11	0.35±0.12*	43.65
200 mg/kg IS	121.20±12.21*[ab]	5.53±0.70*	1.99±0.19	0.38±0.11*	53.54

Values are mean±S.E. (n = 6), * for p<0.05 compared to the negative control, [a] for p<0.05 compared to 50 mg/kg IS and [b] for p<0.05 compared to 100 mg/kg IS

Table 3: Effect of crude hydromethanolic root extract of root of *Hydnora africana* on intestinal motility using charcoal as a marker in mice

Group	Total intestinal length (cm)	Distance travelled by charcoal (cm)	% Intestinal transit of charcoal	% Inhibition
NS	55.70±2.19	49.70±2.09††	89.26±1.29††	0.00
Atropine	57.86±1.17	16.36±1.81	28.18±2.93	68.48
50 mg/kg IS	54.20±2.18	47.70±1.48††	88.24±1.65††	1.17
100 mg/kg IS	53.20±1.57	45.20±1.92††	85.16±3.50††	4.62
200 mg/kg IS	58.36±1.39	46.20±1.97††	79.23±3.14††	11.27

Values are mean±S.E. (n = 6), †† refers p<0.001 compared to the positive control

Table 4: Effect of crude hydromethanolic extract of root of *Hydnora africana* on dinoprostone induced GI secretion in mice

Group	Weight of small intestine (g)	Intestinal fluid accumulation ratio	Weight of intestinal fluid (g)	% Inhibition of intestinal fluid accumulation ratio
NS	1.97±0.55	0.112±0.011	0.4570±0.14	0.00
3 mg/kg Lop	1.41±0.84	0.087±0.007*	0.1570±0.04*	22.84
50 mg/kg IS	1.49±0.38	0.091±0.007	0.1370±0.06*	19.24
100 mg/kg IS	1.48±0.70	0.093±0.009	0.1386±0.04*	17.30
200 mg/kg IS	1.62±0.44	0.099±0.008	0.1653±0.06*	11.84

Values are mean±S.E. (n = 6), * for p<0.05 compared to the negative control

Discussion

In this research the medicine root of *Hydnora africana* is used for the treatment of diarrhoea by using water as vehicle and in this study 80% methanol because hydromethanolic solvents (especially 80% methanol) are more efficient in extracting and usually better the most important bioconstituents plant material which is owing to their expanded polarity range.

In Table C the dose increased by two fold the response also increased by more than two fold hence the extract still do have dose dependent activity in this model. Only the intestinal fluid accumulation ratio of loperamide treated group was found statistically significant compared to the negative controls in the antisecretary activity. The inhibition of intestinal fluid percentage accumulation by 50, 100 and 200 mg/kg doses extract was determined to be 19.24, 17.30 and 11.84% respectively. This research always tells as the noticeable presence between response and the dose of the extract.

In the research of preliminary phytochemical screening I found here alkaloids, tannins, flavonoids, saponins and steroids are present in hydromethanolic root extract of *Hydnora africana* (Sub. Family-Hydnoraceae) and shown a good antidiarrhoeal activity.

Conclusion

In this research 1000 mg/kg hydromethanolic extract of root Hydronora Africana (Sub. Family-Hydnoraceae) on castor oil induced diarrhoea in mice. This study accordingly validates traditional use root of the plant in diarrhoea but this new research guide us to use it as a strong source of new agent in

the therapeutic use of diarrhoea.

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