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Antidiarrheal and Antipyretic Activity of Ethyl Acetate and Hydro-Alcoholic Extracts of *Diplazium esculentum* Leaves

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ABSTRACT

The present study was aimed at evaluating antidiarrheal and antipyretic activities of the ethyl acetate and hydro-alcoholic extract of the leaves of *Diplazium esculentum*. Antidiarrheal and Antipyretic activity was evaluated in rodent animals at doses of 250 and 500 mg/kg B.W. The antidiarrheal activity was investigated by the effect of extracts on castor oil-induced diarrhea in rats and the activities were compared to that of loperamide. Antipyretic activity was estimated using Brewer's yeast-induced hyperpyrexia in rats and the activities were compared to that of paracetamol.Hydroalcoholic extracts showed the highest percentageinhibition of defecation (71.91%) was recorded for leaf extract (500 mg/kgb w) of *D. esculentum*. Hydroalcoholic extract at the doses of (500 mg/kg p.o.) significantly decreased the rectal temperature of the rats. The study corroborates the significant antidiarrheal and antipyretic activities of hydroalcoholic leaf extract of *D. esculentum* and raise the demand of further scientific investigation.

KEY WORDS: *DIPLAZIUM ESCULENTUM*, HYDRO-ALCOHOLIC EXTRACT, ANTIDIARRHEAL, ANTIPYRETIC.

INTRODUCTION

Diarrhea is the passage of abnormal liquid or unformed stool at increased frequency. Infectious agents, certain medications, plant and animal toxins, gastro-intestinal disorders and substances that increase gastrointestinal tract secretions may cause it. It can also be caused by the ingestion of poorly absorbable materials, or inflammatory and dysmotility problems of the gastro-intestinal tract (Palombo, 2006; Meite et al., 2009). Diarrheal diseases are one of the leading causes of morbidity and mortality in developing countries and are responsible for the death of millions of people each year. There are a number of epidemiological and experimental evidences worldwide related to acute diarrheal disease, which is one of the principal causes of death in the infants. Around 2.5 million children die each year worldwide and 80% of which are reported in developing countries (Walker et al., 2011). Diarrhea is most common in crowded living conditions coupled with poor hygiene and malnutrition (Gutiérrez et al., 2007).Antibiotics used as antidiarrheal drugs

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sometimes provoke adverse effects and microorganisms tend to develop resistance toward them. Therefore, the search for safe and more effective agents from plant origin has continued to be an important area of active research, (Junejo et al., 2018, Anand et al 2019).

Pyrexia or Fever is defined as an elevation of body temperature. It is a response due to tissue damage, malignancy, and inflammation or graft rejection. Cytokines, interleukin, interferon and Tumor Necrosis Factor α (TNF- α) are formed in large amount under this condition, which increase PGE2 which in turn triggers hypothalamus to elevate body temperature (Rajani et al., 2011).Fever is associated with symptoms of sickness behavior which consist of lethargy, depression, anorexia, sleepiness and inability to concentrate. This increase in set point triggers increased muscle tone & shivering. However antipyretic medication can be effective at lowering the temperature which may include the affected persons' comfort (Duraisankar and Ravichandran, 2012). Antipyretics are drugs which can reduce elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus which regulate the set point of body temperature. Drugs like paracetamol do not influence body temperature when elevated by factors such as exercise or increase in ambient temperature (Gupta et al., 2008).

Diplazium esculentum (Retz.) Sw. (Athyriaceae) is a wild edible rhizomatous fern mainly consumed as vegetables which are probably the most consumed fern along the hill tribe of North Eastern India and Western Ghats (Archana et al., 2012). The young fronds are used in myriad of way to prepare local dishes including stir-fried and salads. The fern is believed to contain various medicinal properties and some of them are evaluated and confirmed by research. It act as mast cell stabilizer and can prevent anaphylactic shock (Das et al., 2012), decoction of the plant can be used to treat hemoptysis and cough (Rahmat et al., 2004). The plant is also reported to use traditionally for the treatment of dysentery, glandular swellings, indigestion, diarrhea and various skin infections (Lense, 2011). In context of our research endeavor, we have planned to study the antidiarrheal and antipyretic activity of ethyl acetate and hydro-alcoholic extracts of *Diplazium esculentum* leaves.

MATERIALS AND METHODS

Collection and extraction of plant material: Fresh leaves of *Diplazium esculentum* (Retz.) Sw. were collected in March 2014 from Dibrugarh forest, Dibrugarh district, Assam, India. The plant species were identified and authenticated by Botanical Survey of India, Eastern Regional Centre, Shillong, India, and a voucher specimen (BSI/ERC/2014/Plant identification/360) was deposited. Air-dried powdered material of previously collected plant, *Diplazium esculentum* was packed in a Soxhlet extractor and extracted successively with the following solvents: petroleum ether (60- 80oC), chloroform, ethyl acetate, methanol and water. Each time before extracting with the next solvent, the powdered material was air dried first and then oven dried below 50°C. Finally, the marc was macerated with chloroform water (ratio) for 24 hours to obtain the aqueous extract. Each extract was concentrated by distilling off the solvent (in a rotary vacuum evaporator) and then evaporated to dryness on the water bath (Annan et al., 2013; Junejo et al., 2018). Reagent and chemicals All chemicals used in the study were of analytical grade, manufactured by Rankem Fine Chemicals Limited (RFCL), Mumbai and Himedia Laboratories, Mumbai.

Acute oral toxicity study: Acute oral toxicity test was performed as per OECD) guidelines 423. The animals were used with the approval of the Institutional Animal Ethics Committee (Approval No.IAEC/DU/50 Dated 24.09.2013, Registration No. 1576/Go/a/11/CPCSEA dated 17.02.2012)and the study was conducted following internationally accepted principles forlaboratory animal use and care. Experiments were performed using healthy young adult wistaralbino rats(both male and female), nulliparous, non-pregnant and weighing 150 to 250 gm(Junejo et al., 2014; Junejo et al., 2017).

Antidiarrheal activity: Healthy adult Wistar albino rats of either sex (200-250gm) were used for the antidiarrheal study. The animals were obtained from the Laboratory Animal Resources, Dibrugarh University (Approval No. IAEC/DU/50 dated 24.09.2013, Registration No. 1576/Go/a/11/CPCSEA dated 17.02.2012) and acclimatized to normal laboratory conditions for one week prior to study and provided with pellet diet and tap water ad libitum. Castor oil-induced diarrhea was done according to the previously described methods(Shoba and Thomas, 2011; Uddin et al., 2005). Rats of either sex were divided into four groups of five rats each. The animals were fasted for 18 h prior to the test. Group I animals were treated with normal saline (10 ml/kg), which served as control, while Group II received loperamide (50 mg/kg). Groups III, IVand V, VI were treated with ethyl acetate and hydro-alcoholic extracts of *Diplazium esculentum* leaves at 250 mg/kg and 500 mg/kg doses respectively. The activity of each group was expressed as percent inhibition (%) of diarrhea. The percent inhibition of defecation was calculated using the formula:

$$\%\text{Inhibition of defecation} = [(A-B)/A] \times 100$$

Where 'A' indicates mean number of defecation caused by castor oil and 'B' indicates mean number of defecation caused by drug or extract.

Antipyretic activity: The antipyretic activity of the tested extracts was screened in adult albino rats (200- 250g bw) by using yeast-induced hyperpyrexia model. The animals were divided in six groups (n = 6). All groups were kept at fasting and allowed free access of drinking water. Group I received saline as control and group II received paracetamol as standard drug while III-VI groups received 250 and 500 mg/kg of ethyl acetate and hydro-Alcoholic extracts of *Diplazium esculentum* leaves. Normal temperature was recorded using digital

thermometer and then pyrexia was induced in all animals by injecting 20% aqueous suspension of Brewer's yeast (10 ml/kg s.c.). After 24 h, rectal temperature was recorded and groups 3-8 were injected with above doses. After drugs administration, rectal temperature was again recorded periodically at 1, 2, 3 and 4 h of drugs administration (Khan et al., 2014; Kang et al., 2008).

Statistical analysis: The results are expressed as the mean ± standard error of the mean (SEM). Statistical analysis has been carried out with comparison between standard and treated groups. Differences were considered to be statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Both ethyl acetate and hydro-alcoholic extracts showed considerable antidiarrheal effect in castor oil-induced diarrhea test in rats. Hydro-alcoholic extract significantly inhibited the frequency of defecation when compared with untreated control rats ($p<0.05$). Results are shown in Table 1. Both extracts decreased the total number of wet feces produced upon administration of castor oil when compared to the castor oil treated rats. Hydro-alcoholic extract showed 53.97 and 71.91% inhibition of defecation at the doses of 250 and 500 mg/kg, respectively. Standard drug loperamide (50 mg/kg) also increased onset of diarrhea and exhibited 74.67inhibition of defecation.

Table 1. Antidiarrhea activity of leaf extracts of *Diplazium esculentum*

Group	Treatment Group	Dose (mg/kg B.W)	Number of diarrheal faces in 4 hours	% Inhibition of defecation
I	Blank control	10 ml/kg	12.32 ± 1.36	---
II	Loperamide (Standard)	50 mg/kg	3.12 ± 0.95	74.67
III	Ethyl acetate Extract	250 mg/kg	10.21 ± 1.75**	17.12
IV	Ethyl acetate Extract	500 mg/kg	8.11 ± 2.15	34.17
V	Hydro-Alcoholic Extract	250 mg/kg	5.67 ± 1.81*	53.97
VI	Hydro-Alcoholic Extract	500 mg/kg	3.46 ± 1.42	71.91

Data are expressed as the means ± SEM. *p < 0.05, **p < 0.01 vs control group

Table 2. Antipyretic activity of leaf extracts of *Diplazium esculentum*

Group	Treatment Group	Dose (mg/kg B.W)	Rectal temperature (°C) After administration of drug					
			Normal	after 24h	1h	2h	3h	4h
I	Saline	10ml	37.12± 1.53	39.34± 1.37	39.35± 0.99	39.59± 1.23	39.65± 0.79	39.67± 1.45
II	Paracetamol (Standard)	150mg	37.03± 2.14	39.11± 0.97	38.65± 1.48	37.89± 1.36*	37.77± 1.68	37.22± 1.64
III	Ethyl acetate Extract	250mg	36.23± 1.46	38.89± 1.75	38.58± 1.13	38.46± 1.29*	38.47± 1.05	37.33± 1.89
IV	Ethyl acetate Extract	500mg	37.05± 1.65	39.75± 2.42**	39.58± 1.75	39.46± 0.88	38.49± 1.46	38.11± 1.18
V	Hydro- Alcoholic Extract	250mg	36.13± 0.87*	38.15± 1.53	38.11± 1.42	37.08± 2.12	37.07± 1.66**	36.77± 1.71
VI	Hydro- Alcoholic Extract	500mg	37.34± 1.02	39.44± 0.95	38.67± 1.11	38.33± 1.53	37.54± 0.83*	37.48± 1.32

Data are expressed as the means ± SEM. *p < 0.05, **p < 0.01 vs control group

The inhibition was dose dependent and remained significant up to 3h of administration as shown in Table 2. The maximum antipyretic effect of hydro-alcoholic extract was observed at 500mg/kg i.e. 37.48 ± 1.32while, the antipyretic effect of paracetamol was 37.22 ± 1.64. Administration of 50 mg/ml dose of loperamide (standard) showed (3.12 ±0.95) diarrheal feces in 4 hours causing 74.67% inhibition of defecation. Ethyl acetate extract of *Diplazium esculentum* administered at 250 mg/kg dose showed (10.21 ± 1.75) number of diarrheal feces

in 4 hr causing 17.12% inhibition of defecation. When administered at a higher dose (500 mg/ml), anti-diarrheal activity increased to 34.17%. Similarly, the hydro-alcoholic extract at 250 mg/kg concentration showed 53.97% inhibition while at 500 mg/kg, 71.91% inhibition was observed. Thus with increasing concentration of the drug dose, antidiarrheal activity also increases. The subcutaneous injection of brewer's yeast evoked pyrexia by ultimately increasing synthesis of prostaglandin and is considered as a valuable in-vivo screening test for the assessment ofantipyretic potential (Muhammad et al., 2012; Wan et al., 2013).

Yeast-induced pyrexia is called pathogenicfever. Its etiology could be the production of prostaglandins. Theinhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclooxygenase enzyme activity (Igbe et al., 2009). The antipyretic effect of test extract is related with several mediators which cause pyrexia especially prostaglandins (Muhammad et al., 2013). The intraperitoneal administration of *Diplazium esculentum* leaf extracts significantly attenuated rectal temperature of yeast induced febrile rats. Thus, it can be postulated that *Diplazium esculentum* leaf extracts interferes with the release of prostaglandins at any stage.

CONCLUSION

Finally we conclude that the test extract may be useful in the protection against antidiarrheal and Antipyretic diseases. In comparison with the standard drug loperamide and paracetamol, hydro-alcoholic extracts of *Diplazium esculentum* leaves showed significant antidiarrheal and antipyretic efficacy. A more detailed and in-depth phytochemical investigation is necessary to identify the novel chemical entity responsible for the bioactivity of *Diplazium esculentum* leaves.

Conflict of Interest: None

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