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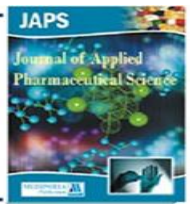

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Antidiuretic and antidiarrhoeal activities of polar and non-polar extract *of Brassica oleracea*

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ABSTRACT

Diuretic activities of both polar and non-polar extract of leaves of *Brassica oleracea* were investigated on male white rabbits and male Sprague-Dawley rats. Anti diarrheal activity of the same extract was investigated on male and female swiss albino mice. Both polar and non-polar extract exhibited anti diuretic activities on both rats and rabbits. Polar and Non-polar extract also showed anti diarrheal activity on male and female mice. Anti diarrheal activity affects both latent period and number of stools.

Keywords: *Brassica oleracea*, Anti diuretic, Anti diarrheal activity, Polar extract, Non-polar extract.

INTRODUCTION

Brassica oleraceae var. capitata (Brassicaceae) is round shape in size and is composed of leaf layers that are imposed one after another. It is a member of the food family traditionally known as cruciferous vegetables and is related to kale, broccoli, collards. It has a long history of using as a food and traditional treatment option in diarrhea (Gani, 2003). Important properties of this plant prove the versatility in recent time. Antioxidant property (Eberhardt MV *et al.,* 2005 & Bidchol AM *et al.,* 2011) of *B. oleraceae* studied over which is also responsible for its anticholinesterase activity (Boga M *et al.,* 2011). Both analgesic and anti inflammatory (Choudhary A *et al.,* 2010) properties of the plant were also found. Aqueous extract of the capitata variety of *B. oleracea* shows antiulcerogenic property (Carvalho CA *et al.,* 2011) whereas both aqueous and non-aqueous extract of acephala DC variety provide protection from the oxidation of LDL and VLDL resulting in improvement of cardiovascular diseases (Kural BV *et al.,* 2011). Thrombolytic (Anwar MS *et al.,* 2011) activity is also alternative to common anti platelet drugs. Non-aqueous fraction of *B. oleracea* plays hypolipidemic and hepatoprotective effect on rats (Asadujjaman M *et al.,* 2011). Antitumor (Cakar J *et al.,* 2012) activities of *B. oleracea* are also

screened for possible trypanocidal properties (Igweh AC *et al.,* 2002) of this plant. In ancient time Roman utilized *Brassica sp.* in the treatment of injuries (Balbach A, 1992). *B. oleacea* is taken as vegetables and have been traditionally used in retension of urine (Yusuf M, 1994). In Bangladesh, aside from B. oleacea's seasonal role as vegetables it is also used in diarrhoeal treatment (Khan MS, 1975 & Uddin SN *et al.,* 2004). Therefore, the present study was designed to investigate anti diuretic activity of *B. oleracea* on rats and rabbits and anti diarrheal property of both polar (ethanolic) and non-polar (carbon tetrachloride) extract on swiss albino mice.

MATERIALS AND METHODS

Plant material collection and specification

This vegetable plant was collected from local (Mirpur) market. Then the leaves of plant *B. oleracea* was identified by Bangladesh National Harberium, Mirpur, Dhaka, Bangladesh. The accession number of this plant is DACB 36403.

Chemicals and Drugs

The chemical reagents used in the experiment were purchased from Sigma Chemical Co. Ltd, (St. Louis, MO, USA) and E. Merck (Germany). The drug product hydrochlorothiazide was collected from Incepta Pharmaceuticals Ltd. and loperamide was collected from Square Pharmaceuticals Ltd. Both pharmaceutical company is local in Bangladeshi market.

Preparation of plant extract

The leaves of *Brassica oleraceae* were separated from the bunch of cabbage. Then it was sliced into small pieces. The sliced leaves were sundried for 2 weeks until a dry mass was found. Dry leaves were crushed in a grinding machine to get powder material. 1 kg powder material was macerated with 3 L of ethanol in a 4 L round bottom flask. The container was sealed with cotton plug and aluminum foil at room temperature for 10 days. It was shaking occasionally for proper mixing. The mixture was finally filtered with cotton and then evaporated to dryness under reduced pressure by rotary evaporator. The crude extract found was 250 gm. 5 gram ethanolic extract was mixed with 90 ml ethanol having 10 ml distilled water. The crude extract was dissolved completely. It was mother solution which was taken in a separating funnel. 100 ml carbon tetrachloride was added and the funnel was shaken for proper extraction and finally kept undisturbed. Then the organic portion was collected and repeated thrice. Thus CCl_4 fraction of ethanolic extract was obtained.

Animals

Male white rabbits (2.0–2.2 kg), male Sprague-Dawley (SD) rats (200–220 g) and twenty swiss albino mice of either sex (20-25 g), were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (icddr, b) for conducting our experiment. Animals were kept in supervision under standard environmental conditions [temperature: (24.0±1.0°C), relative humidity: 55-65% and 12 h light/ dark cycle] and had provided free access to feed and water. The animals were acclimatized one week in laboratory condition prior to our experimental demand. Each animal was used only once in the experiment. Principles of laboratory animal care guidelines (NIH publication number 85–23, revised 1985) were followed.

Diuretic test

Rat

We collected Sprague-Dawley (SD) male rats for diuretic test. Male rats were administered with physiological saline (0.9% NaCl) at an oral dose of 25 ml/kg body weight at first. Only those rats that were excreted 2 ml urine within 2 hours were selected for further diuretic test. The rats were divided into eight groups having 5 rats in each group. Each group were kept fasting for the following 12 hours. Then they were supplied with water and physiological saline (0.9% NaCl) for uniform loading of water and salt. Group I (Control) & Group II (positive control) were supplied with distilled water & hydrochlorothiazide (30 mg/kg body weight) respectively. Group III, V and VII of experimental rats were given 100 mg/kg, 200 mg/kg, 400 mg/kg body weight of polar (Ethanol) extract respectively via intragastric route. Group IV, VI and VIII were given 100 mg/kg, 200 mg/kg, 400 mg/kg body weight of non-polar (Carbon tetrachloride) extract respectively. Then urine collected was measured after 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} hour (Yu C *et al.,* 2012).

Rabbit

We also took Male white rabbits that were again divided into eight groups for the same test. Distilled water, hydrochlorothiazide and both polar and non-polar extract (100 mg/kg, 200 mg/kg, 400 mg/kg BOE) are administered in the same way as in male rat. After 20 minutes the rabbits were intravenously anaesthetized with 3% (w/v) sodium pentobarbital solution at 30 mg/kg body weight. Sterile liquid paraffin was used to lubricate the catheter that was inserted in the bladder to discharge urine from all groups after a brief period of fasting. Again physiological saline (0.9% NaCl) was supplied to the animals at a speed of 2 ml/min by ear vein injection at 10 ml/kg body weight. Catheter was used to collect the urine that was measured after 1^{st}, 2^{nd}, 3^{rd} and 4^{th} hour of treatment (Xu S *et al.,* 2002).

Diarrheal test

Anti diarrheal test of the non-polar carbon tetrachloride fraction of ethanolic extract and polar (Ethanol) extract was conducted by using castor oil induced diarrhea in both sexes of swiss albino mice by the method described by Nwodo and Alumanah, 1991. For this study swiss albino male mice were divided into six groups. Of the experimental groups, Group-I or the Control received only distilled water, Group-II or the positive control received standard anti-motility drug, loperamide (LP) at a dose of 3mg/kg-body weight as oral suspension. The test groups (Group-III, Group-V) were gavaged with suspension of ethanol extract (Polar) of leaves of *B. oleracea* at the oral dose of 250 mg/kg body weight and 500 mg/kg-body weight. But the other test groups (Group-IV, Group-VI) were supplied with carbon tetrachloride extract (Non-polar) of *B. oleracea* at the oral dose of

250 mg/kg body weight and 500 mg/kg-body weight respectively. Prior to the oral administration of castor oil the mice were fed with the samples before 1 hour at a dose of 0.5 ml per mouse respectively for individual animal of each group that was placed in separate cages on adsorbent paper. Then the animals were examined for the presence of diarrhea every hour for 8 hours study after the castor oil administration. Same procedures were applied for female mice for grouping and administering control and sample.

Statistical Analysis

ANOVA test was performed using SPSS 16.0 software. $P < 0.05$ was considered statistically significant.

RESULT AND DISCUSSION

Anti diuretic activities

Both polar and non-polar fraction showed anti diuretic properties on both male white rabbits and male Sprague-Dawley rats. The findings of anti diuretic activities on male rats and rabbits are shown in table-1 and table-2 respectively.

Anti diarrheal activities

Both polar and non-polar extract of leaves of *B. oleracea* exhibited anti diarrheal activity on swiss albino mice. The extract affected both latent period up to eight hours and number of stools in first five hours. The result of anti diarrheal activities is provided in table-3 and table-4 on male and female mice respectively.

Table. 1: The effect of ethanol and carbon tetrachloride extract of leaves of anti diuretic activities of both polar and non-polar extract of leaves of *Brassica oleracea* on urine output in male Rat.

Experiment Group[a]	Dose	TI[b] (h)	Urine Output (ml)	
I-Control	Distilled water	1	2.5±0.4	
		2	3.3±0.5	
		3	4.4±0.2	
		4	5.6±0.3	
		5	6.1±0.1	
II-HCT[c]	30 mg/kg	1	3.5±0.2	
		2	6.3±0.3*	
		3	7.3±0.2*	
		4	8.4±0.3*	
		5	9.0±0.4*	
			Polar	**Non-Polar**
III-BOE[d] & IV-BOE	100 mg/kg	1	2.1±0.5	2.2±0.4
		2	2.9±0.3	3.1±0.2
		3	3.6±0.4	3.7±0.5
		4	4.7±0.2	4.6±0.1
		5	5.1±0.3	5.3±0.2
V-BOE & VI-BOE	200 mg/kg	1	1.9±0.4	2.0±0.2
		2	2.3±0.3*	2.4±0.3*
		3	3.1±0.1*	3.2±0.2*
		4	4.1±0.3	4.0±0.1
		5	4.8±0.4	4.9±0.3
VII-BOE & VIII-BOE	400 mg/kg	1	1.6±0.3	1.7±0.3
		2	2.1±0.2*	2.0±0.2*
		3	2.6±0.1*	2.7±0.3*
		4	3.9±0.4*	3.8±0.4*
		5	4.3±0.2	4.4±0.2

Data are presented as mean ±SE. *$P < 0.05$ was considered as statistically significant. [a]Each Group had n=5; [b]TI=Time Interval, [c]HCT= Hydrochlorothiazide, [d]BOE= *Brassica oleraceae* Extract.

Table 2: Effects of polar and non-polar extract of anti diuretic activities of both polar and non-polar extract of leaves of *Brassica oleracea* on urine output of male Rabbit

Experiment Group[a]	Dose	TI[b] (h)	Urine Output (ml)	
I-Control	Distilled water	1	3.6±0.4	
		2	4.8±0.3	
		3	7.0±0.2	
		4	7.3±0.1	
		0-4	21.8±0.3	
II-HCT[c]	30 mg/kg	1	10.7±0.3*	
		2	12.1±0.2*	
		3	20.5±0.1*	
		4	11.3±0.3*	
		0-4	53.6±0.4*	
			Polar	**Non-Polar**
III-BOE[d] & IV-BOE	100 mg/kg	1	3.1±0.4*	3.2±0.5*
		2	4.2±0.3	4.1±0.2
		3	6.1±0.2	6.2±0.4
		4	6.8±0.4	6.6±0.3
		0-4	19.8±0.4	20.4±0.3

V-BOE &	VI-BOE	200 mg/kg	1	2.8±0.4*	3.0±0.5*
			2	3.8±0.3*	3.9±0.4*
			3	5.3±0.1*	5.2±0.2*
			4	6.2±0.2	6.1±0.3
			0-4	17.8±0.3*	17.9±0.1*
VII-BOE &	VIII-BOE	400 mg/kg	1	2.1±0.3*	2.0±0.3*
			2	3.1±0.2*	3.3±0.1*
			3	4.2±0.3*	4.3±0.1*
			4	5.3±0.1*	5.2±0.3*
			0-4	14.4±0.3*	14.5±0.2*

Data are presented as mean ±SE. *P < 0.05 was considered as statistically significant. [a]Each Group had n=5; [b]TI=Time Interval, [c]HCT= Hydrochlorothiazide, [d]BOE= *Brassica oleraceae* Extract.

Table. 3: Experimental profile to observe the effect of polar and non-polar extract of *Brassica oleracea* on castor oil induced diarrhea in male swiss albino mice.

Group of experiment[a]	Dose	PS[b] (h)	Number of Stools (Mean± SE)		Total Number of Stools (Mean± SE)		Latent Period (h)	
I-Control	0.9% NaCl	1	2.7± 0.2		25.9± 0.1		1.32± 0.1	
		2	3.0± 0.3					
		3	3.5± 0.5					
		4	4.0± 0.2					
		5	4.2± 0.2					
		6	3.3± 0.4					
		7	2.5± 0.3					
		8	2.1± 0.1					
II-LP[c]	3 mg/kg	1	0.5± 0.2*		7.8± 0.3*		4.15± 0.2*	
		2	0.6± 0.1*					
		3	0.7± 0.2*					
		4	1.1± 0.4*					
		5	1.2± 0.2*					
		6	1.0± 0.1*					
		7	0.9± 0.1*					
		8	0.7± 0.2*					
			Polar	**NP[d]**	**Polar**	**NP**	**Polar**	**NP**
III-BOE[e] & IV-BOE	250 mg/kg	1	1.4± 0.3	1.6± 0.1	12.2± 0.1*	14.4± 0.2	2.80± 0.2*	2.75± 0.2
		2	1.6± 0.2*	1.8± 0.3				
		3	1.9± 0.2*	2.0± 0.2				
		4	2.2± 0.3*	2.3± 0.1				
		5	2.5± 0.2*	2.7± 0.2				
		6	1.7± 0.1*	1.9± 0.1				
		7	1.5± 0.2	1.7± 0.2				
		8	1.2± 0.1	1.4± 0.1				
V-BOE &	VI-BOE	500 mg/kg	1	1.2± 0.1*	1.4± 0.1	9.8± 0.2*	10.2± 0.3	3.15± 0.3*	3.50± 0.3
			2	1.3± 0.4*	1.3± 0.3				
			3	1.5± 0.3*	1.6± 0.2				
			4	1.8± 0.1*	1.7± 0.4				
			5	1.9± 0.1*	2.0± 0.1				
			6	1.3± 0.2*	1.4± 0.4				
			7	1.1±0.2*	1.2± 0.2				
			8	1.0± 0.2	1.1± 0.1				

Data are presented as mean ±SE. *P < 0.05, statistically significant different. [a]Each Group had n=5; [b]PS=Period of Study, LP[c]= Loperamide, NP[d]= Non-polar, BOE[e]= *Brassica oleraceae* Extract.

Table. 4: Experimental profile to observe the effect of polar and non-polar extract of *Brassica oleracea* on castor oil induced diarrhea in female swiss albino mice.

Group of experiment[a]	Dose	PS[b] (h)	Number of Stools (Mean± SE)	Total Number of Stools (Mean± SE)	Latent Period (h)
I-Control	0.9% NaCl	1	2.1± 0.3	25.1± 0.1	1.35± 0.1
		2	2.5± 0.2		
		3	3.3± 0.1		
		4	3.9± 0.1		
		5	4.5± 0.2		
		6	3.1± 0.4		
		7	2.7± 0.3		
		8	2.0± 0.1		
II-LP[c]	3 mg/kg	1	0.6± 0.4*	7.1± 0.3*	3.75± 0.2*
		2	0.7± 0.2*		
		3	0.8± 0.3*		
		4	1.2± 0.1*		
		5	1.4± 0.2*		
		6	1.0± 0.1*		
		7	0.9± 0.2*		
		8	0.6± 0.1*		

			Polar	NP[d]	Polar	NP	Polar	NP
III-BOE[e] & IV-BOE	250 mg/kg	1	1.5± 0.3	1.7± 0.2	13.3± 0.2*	14.2± 0.4	2.50± 0.2*	2.65± 0.2
		2	1.7± 0.2*	1.8± 0.1				
		3	1.9± 0.2*	2.0± 0.2				
		4	2.0± 0.1*	2.3± 0.1				
		5	2.5± 0.4*	2.7± 0.3				
		6	1.9± 0.1*	2.1± 0.2				
		7	1.6±0.2*	1.7± 0.2				
		8	1.0± 0.1	1.1± 0.1				
V-BOE & VI-BOE	500 mg/kg	1	1.2± 0.1*	1.1± 0.3	9.2± 0.2*	10.0± 0.3	3.25± 0.2*	3.37± 0.3
		2	1.3± 0.1*	1.4± 0.1				
		3	1.5± 0.3*	1.6± 0.3				
		4	1.6± 0.4*	2.0± 0.2				
		5	1.7± 0.1*	1.5± 0.1				
		6	1.4± 0.3*	1.3± 0.1				
		7	1.1±0.2*	1.2± 0.2				
		8	1.0±0.2*	0.9± 0.1				

Data are presented as mean ±SE. *P < 0.05 was considered as statistically significant. [a]Each Group had n=5; [b]PS=Period of Study, LP[c]= Loperamide, NP[d]= Non-polar, BOE[e]= *Brassica oleracea* Extract.

DISCUSSION

Both polar and non-polar extract of *Brassica oleracea* shows urine retention in both rat and rabbit in our study. Mechanism of anti diuretic function might follow prostaglandin pathway in which both polar and non-polar BOE inhibit prostaglandin on smooth muscle cells resulting in contraction of smooth muscle (Nelson RJ, 2005). The extract also acts in mesangial cells in glomerulus of kidney which affect in decreasing glomerular filtration rate (Vane JR, 1971 & Kim GH, 2008). Both polar and non-polar leaves extract of *B. oleracea* in it's all doses acted in dose dependent manner and reduced the volume of urine significantly ($p<0.5$) in physiological saline (0.9% NaCl) induced male rat and male rabbit.

Castor oil which is used to induce diarrhea in our study, is hydrolyzed in the upper small intestine and convert into ricinoleic acid which is believed to act by irritating the gastrointestinal mucosa and causes the reduction of sodium ion and chloride ion permeability. The volume of the intestinal content increased which forces the peristaltic movement increasing intestinal motility followed by diarrhea (Zavala MA *et al.*, 1998). In our study both polar and non-polar extract show anti diarrheal activity which support the previous uses (Yusuf M, 1994; Khan MS, 1975 & Uddin SN *et al.*, 2004). Both extracts affect on latent period and number of stools. So it is said that the compounds responsible for anti diarrheal effect might be present in both polar and non-polar extracts which are responsible for decreasing peristaltic movement and increasing segmentation of intestinal content.

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