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RESEARCH ARTICLE

ANTI-INFLAMMATORY AND ANTIPYRETIC ACTIVITY OF METHANOL EXTRACTS OF *BRASSICA OLERACEA VAR. CAPITATA RUBRA* IN WISTER RATS

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ABSTRACT

The methanol extract of *Brassica oleracea var. capitata rubra* was investigated for anti-inflammatory and antipyretic activity in animal models. The extract has an anti-inflammatory effect against paw oedema in rats. Brewer's yeast (15%) suspension was used to induce fever in rats. Our results were $P< 0.05$ significant even at lower dosage of 0.50 mg/kg which showed the dominant nature of methanolic extract of *Brassica oleracea var capitata rubra.*

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INTRODUCTION

Red cabbage *(Brassica oleracea var. capitata rubra)*, which belongs to family Brassicaceae, is one of the most important vegetables grown worldwide. It is also known as purple cabbage, red kraut, or blue kraut after preparation. Its leaves are coloured dark red/purple. Numerous studies have highlighted the potential importance of *brassica* vegetables as a source of antibacterial (Kyung and Fleming, 1994; Hu *et al*., 2004; Ayaz *et al*., 2008 and Ayswarya and Sudha Rameshwari, 2015a) and antioxidant substance (Zhou and Yu, 2006; Andarwulan *et al*., 2010; Ayswarya and Sudha Rameshwari, 2015b and Isabelle *et al*., 2010). Cabbage has widespread use in traditional medicine, in alleviation of symptoms associated with gastrointestinal diseases (gastritis, peptic and duodenal ulcers, irritable bowel syndrome) as well as in treatment of minor cuts and wounds and mastitis (Samec, 2011). The Romans utilized brassica species in the treatment of injuries (Balbach *et al*., 1992). *Brassica oleracea* have a lot of health benefits. In order to analyse the dominant nature of red cabbage we chose it as our source of interest. Red cabbage is a rich source of phenolic compounds, with anthocyanins being predominant over other flavanoids (Hasimotto *et al*., 2005; Arapitsas and Turner, 2008). Inflammation is basically a defense phenomenon yet often leading to serious pathological conditions (Arrigoni Martelli 1977). Non steroidal anti inflammatory drugs (NSAID) are the most commonly prescribed drugs. The side effects of these drugs evoked us to search for new anti inflammatory agents from natural botanical sources that may have minimal side effects (Vogel and Gerhard 2002). Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive (Chattopadhyay *et al*., 2005). Search for safe herbal remedies with potent antipyretic activity received momentum recently as the available antipyretics, such as Paracetamol, Aspirin, Nimusulide etc, which have toxic effect to the various organs of the body (Guyton and Hall, 1998). The aim of our study is to evaluate the pharmacologic activities such as anti-inflammatory and antipyretic activity of methanol extracts of *Brassica oleracea var. capitata rubra* in Wistar rats.

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MATERIALS AND METHODS

The present project was carried out in the department of PG Biochemistry at V.V. Vanniaperumal College for Women,

Table 1. Anti inflammatory activity by paw oedema method

Drug and dose	Paw circumference in mm					
	Normal	0 hrs	1 hr	2 hrs	3 hrs	4 hrs
Control Normal saline 5ml/kg	0.39±0.00	0.50±0.02	0.55±0.02	0.57±0.03	0.59±0.03	0.60±0.02
Standard Diclofenac 10mg/kg	0.41±0.12	0.56±0.02	0.53±0.01*	0.51±0.01*	0.49±0.02*	0.48±0.01*
BOME 1 0.25mg/kg	0.44±0.03	0.54±0.07	0.58±0.04	0.56±0.05	0.56±0.05	0.62±0.08
BOME 2 0.50mg/kg	0.49±0.03	0.55±0.03	0.57±0.03	0.58±0.05	0.52±0.02*	0.51±0.03*

Values are expressed as mean±S.D. (n=4); At 95% confidence interval *P<0.05 were considered significant. BOME- Brassica oleracea methanolic extract

Table 2. Anti pyretic activity

Drug & dose	Normal body temp	Rectal temperature in ^{o}C					
		18 hrs	0 hrs	1 hr	2 hrs	3 hrs	4 hrs
Control 5ml/kg	37.2±0.8	37.8±0.7	37.8±0.7	38.2±0.8	38.5±0.7	38.5±0.9	38.3±1.0
Standard paracetamol 10mg/kg	37.4±0.2	37.9±0.2	37.9±0.2	38.3±0.4	37.9±0.4*	37.7±0.3*	37.4±0.3*
BOME 1 0.25mg/kg	37.6±0.1	38.4±0.3	38.4±0.3	38.7±0.2	38.9±0.3	39.2±0.1	39.4±0.2
BOME 2 0.50mg/kg	37.6±0.1	38.4±0.2	38.4±0.2	38.3±0.3*	38.1±0.3*	37.9±0.2*	37.8±0.2*

Values are expressed as mean±S.D. (n=4); At 95% confidence interval *P<0.05 were considered significant. BOME- Brassica oleracea methanolic extract

Virudhunagar, Tamilnadu, India. The preliminary work (extraction) was done in V.V. Vanniaperumal College for Women, Virudhunagar, Tamilnadu, India. The pharmacologic activity of drug on rat study was carried out in the Sankaralingam Bhuvaneswari college of Pharmacy, Annaikuttum, Tamilnadu, India.

Vegetable collection

Red cabbage *(Brassica oleracea var. capitata rubra)* was collected from local markets of Virudhunagar, Tamilnadu, India. The vegetable was washed thoroughly under running tap water to remove dirt and then shade dried at room temperature for a week. They were ground into fine particles after drying and kept in closed container.

Extraction and sample preparation

Ten grams of ground sample of *Brassica oleracea var. capitata rubra*, was weighed and homogenized with 100 ml of methanol. The crude preparation was left for 72 hours in shaker at room temperature. The extract obtained by cold extraction was then concentrated by evaporating the solvent at room temperature

Animal model

Wistar rats of either sex weighing between 130-170 g were procured from animal house of Sangaralinkam Bhuvaneswari College of Pharmacy (Regd. No. 622/ 02/ C/ CPCSEA) used for the present study. They were maintained under standard conditions (28±2◦C; 55–60% RH) and fed a standard diet for rats and given water adlibitum.

Pharmacological Activities

Anti inflammatory activity

Weighed the animals and numbered them. Noted the initial paw circumference (both right and left) of each rat using vernier caliper in mm. Divided the animals into four groups each comprised of at least four rats. First group was given saline orally- 5ml/kg (control), second was injected diclofenac-10mg/kg subcutaneously and the next two groups were given different concentrations of extract (T_1- 0.25 mg/kg, T_2- 0.50 mg/kg) orally. After 30 minutes 0.1 ml of 1% formaldehyde was injected in the plantar region of the left paw of all the rats. The right paw (non-inflamed) served as reference for comparison. The circumference of both legs of all rats was noted at 0, 1, 2, 3, 4 hours after challenge. Statistical analysis was performed by one way ANOVA followed by student's test using SPSS software (Kulkarni, 2008).

Antipyretic activity (Parmer and Shiv Prakash)

Wistar rats of either sex weighing between 130-170 g are divided in groups of four animals each. Their initial rectal temperature was recorded by insertion of a thermocouple to a depth of 2 cm into the rectum. A 15% suspension of brewer's yeast in 0.9% saline was injected subcutaneously in back below the nape of the neck in a dose of 10 ml/kg. The site of injection was massaged in order to spread the suspension beneath the skin. Immediately after yeast injection the food was withdrawn and at 18 hour post challenge the rise in rectal temperature was recorded. The observation was repeated after 30 min. These animals received the test compounds (T_1- 0.25 mg/kg, T_2- 0.50 mg/kg) and standard drug (10 mg/kg) by oral administration and their rectal temperatures were recorded at 0, 1, 2, 3, 4 hours thereafter. Statistical analysis was performed by one way ANOVA followed by student's test using SPSS software.

RESULTS AND DISCUSSION

The plant *Brassica oleracea va.r capitata rubra* was collected from local markets of Virudhunagar, Tamilnadu, India. The material was dried under shade and then powdered. The dried powders of *Brassica oleracea* were extracted with methanol

using cold extraction. The extracts were allowed to evaporate to dryness. Methanolic extract of *Brassica oleracea var capitata rubra* produced a potent anti inflammatory activity against paw oedema in rats (P< 0.05). The potency was found to be inversely proportional to the time taken for reduction in paw oedema. The results obtained by anti inflammatory activity by paw oedema method are tabulated in Table 1.

Inflammation is a dynamic pathological process wherein several changes proceed simultaneously but at different speeds. More mechanistic definition of inflammation are invasion of circulating lymphocytes and macrophages (immune cells) and induction of various locally produced inflammatory mediators such as kinins, cyclooxygenase products and cytokines. Inflammatory mechanism is believed to be biphasic. The first phase (1-2 hr) is due to the release of histamine or serotonin and the second phase of oedema is due to the release of prostaglandin (Britto *et al*., 1998; Saha *et al*., 2007). Histamine and other mediators of inflammation increase vascular permeability at various times after injury. The inflammatory response is a physiological characteristic of vascular tissue. Increased permeability seen in the inflammatory reaction leads to exudation of fluid rich in plasma proteins, coagulation factors and injured tissues with subsequent oedema at the site (Rang, 1996).

Datta debranjan *et al*., 2010 evaluated the anti inflammatory activity of alcoholic extract of seeds of *trigonella–foenum-graceum* (fenugreeks). The acute anti inflammatory activities of trigonella-foenum-graceum (100mg & 200mg/kg body weight) were measured using carrageenan as inflammatory agent, keeping indomethacin (10mg/kg body weight) as reference standard. *Trigonella* had shown significant difference with control ($p<0.05$) at 3rd, 4th, 6th hour. The study indicated that alcoholic extract of *Trigonella* exhibited anti-inflammatory effect on carrageenan induced paw oedema in rats at dosage of both at 100mg and 200mg/kg body weight. The study concluded that fenugreek was more significant than Indomethacin. Exudation which is a consequence of vascular permeability is considered as major features of acute inflammation (Thirupathy *et al*., 2001). The results of this study indicate that methanolic extract of *Brassica oleracea* significantly reduced formaldehyde induced paw oedema in rats. Therefore, the mechanism of action may be by inhibition of histamine, serotonin or prostaglandin synthesis. Effect of methanolic extract of *Brassica oleracea var. capitata rubra* on rectal temperature in rats is presented in Table 2 and is significant ($P<0.05$). Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive (Chattopadhyay *et al*., 2005). Normally the infected or damaged tissue initiates the enhanced formation of proinflammatory mediator's (Cytokines like interleukin 1β, α, β and TNF- α), which increase the synthesis of prostaglandin E2 (PG E2) near peptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature (Spacer *et al*., 1994). As the temperature regulatory system is governed by a nervous feedback mechanism, so when body temperature becomes very high, it dilate the blood vessels and increasing sweating to reduce the temperature; but when the body temperature become very low hypothalamus protect the internal temperature by vasoconstriction (Veugelers *et al.,* 1997). Our results were also P< 0.05 significant even at lower dosage of 0.50 mg/kg which showed the dominant nature of methanolic extract of *Brassica oleracea var capitata rubra*.

Summary and Conclusion

The methanolic extract of *Brassica oleracea var capitata rubra* in the dose 0.50 mg/kg produced significant (P< 0.05) antipyretic and anti inflammatory activity. On the basis of the present results and available reports, it can be finally concluded that *Brassica oleracea var capitata rubra* can be used as an effective herbal medicine for inflammatory, pyretic conditions. This study also indicates that methanol is the most efficient solvent for extraction of polyphenolic compounds from vegetables. Further research possible involves the isolation of individual components and formulation of a potent anti-inflammatory and antipyretic drug from *Brassica oleracea var. capitata rubra.*

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