ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS LEAF EXTRACT OF BRASSICA OLERACEAE LINN VAR. DC (brassicaceae)

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ABSTRACT
The present study explored the anti-inflammatory potential of aqueous leaf extract of Brassica oleraceae in rodents, using standard laboratory models. Brassica oleraceae at doses of 75, 150 and 300 mg/kg were administered orally in carrageenan-induced rat hind paw edema, xylene induced ear edema in mice, and formalin-induced rat hind paw edema (sub-acute 7 days), using indomethacin (10 mg/kg), dexamethasone 1 mg/kg and acetylsalicylic acid (Aspirin, 100 mg/kg) respectively as standard drugs. The study further explored the effect of the extract on some inflammatory mediators, histamine, serotonin and prostaglandin, using only the highest dose of 300 mg/kg. The result obtained showed that aqueous extract of Brassica oleraceae produced a significant ($p<0.01$) dose-dependent anti-inflammatory property. Significant inhibitions of inflammatory mediators were also recorded. Phytochemical analysis revealed the presence of flavonoids, tannins, anthraquinones, alkaloids, cardiac glycosides, saponins, and phlobatanins, some of which have been reported to possess anti-inflammatory property. The pH of the extract was 8.5. The findings justify the traditional use of Brassica oleraceae in the treatment of inflammatory conditions while it also suggests inhibition of histamine, serotonin and prostaglandin synthesis as possible pathways for its observed anti-inflammatory activity.

Keywords: Brassica Oleraceae, Oedema, Rodent, Inflammation.

INTRODUCTION

Inflammation is a response triggered by damage to living tissues. The inflammatory response is a defense mechanism that evolved in higher organisms to protect them from infection and injury. Its purpose is to localize and eliminate the injurious agent and to remove damaged tissue components so that the body can begin to heal. The response consists of changes in blood flow, an increase in permeability of blood vessels, and the migration of fluid, proteins, and white blood cells (leukocytes) from the circulation to the site of tissue damage. Inflammatory diseases are still one of the main health problems of the population. Several modern drugs are used to treat these disorders but, their prolonged use may cause severe side effects (Yesilada *et al.*, 1997), the most common being gastrointestinal bleeding and peptic ulcer (Corley *et al.*, 2003). Consequently, there is a need to develop new anti-inflammatory agents with minimal side effects. Plant derived drugs are known to play a vital role in management of inflammatory diseases.

Brassica oleraceae Linn Var. DC belongs to the family of plants known as Brassicaceae, they are popularly known as the mustards, mustard flowers, the crucifers or the cabbage family. It is a leafy green biennial plant, grown as an annual vegetable crop. Its seedlings have a thin taproot and cordate (heart-shaped) cotyledons. The first leaves produced are

ovate (egg-shaped) with a lobed petiole. Plants are 40–60 cm (16–24 in) tall in their first year at the mature vegetative stage, and 1.5–2.0 m (4.9–6.6 ft) tall when flowering in the second year (Dixon, 2010). It is widely used in traditional medicine as anti-inflammatory and anti-nociceptive agents. These properties have not been scientifically evaluated. Therefore, the present study is an attempt to investigate the anti-inflammatory property of the aqueous leaf extract of *Brassica oleraceae* in experimental animals.

MATERIALS AND METHODS

Animals

Healthy albino mice (20-25 g) and rats (120- 200 g) of both sexes obtained from the Laboratory Animal Centre of the College of Medicine of the University of Lagos, Idi-Araba, Lagos, Nigeria were used. The animals were maintained under standard environmental conditions, fed with the standard laboratory animal's diet purchased from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria and were given clean drinking water. The animals were kept in a room with controlled 12 hours light and dark cycle, in clean plastic cages while they acclimatized for a period of one week and were fasted for 12 hours prior to the experiment. All experimental procedures were carried out in compliance with the United States National Institute of Health (NIH) guide for care and use of laboratory animals and recommendation of International Association for the Study of Pain (IASP) (Zimmermann, 1983).

Plant Collection And Extraction

Fresh leaves of *Brassica oleraceae* were purchased from vegetable garden in Idi-Araba, Lagos, Nigeria. The plant was identified and authenticated by Mr. T.K. Odewo of the Department of Botany and Microbiology, University of Lagos, Lagos, Nigeria. The plant was given a voucher number LUH 5575. Fresh leaves *Brassica oleraceae* were rinsed with distilled water to remove dirt. The leaves weighed 1825 g and were chopped into small pieces with a knife, blended and macerated with 400 mls of distilled water. It was left at room temperature for two hours and refrigerated for 24 hours. After refrigerating, it was left for another one hour at room temperature. It was filtered using a clean white handkerchief to remove the chaff and was filtered with filter (size 125 mm) paper. The filtrate was separated into different beakers and was evaporated to dryness in an oven (40 °C). The percentage yield was 1.87%

Phytochemical Screening

The phytochemical analysis of the aqueous crude leaf extract of *Brassica oleracea* was carried out using standard chemical processes outlined by Odebiyi and Sofowora (1978) and Trease and Evans (2005).

Anti-Inflammatory Models

Carrageenan-Induced Rat Paw Oedema

Adult rats (120-150 g) of both sexes fasted overnight were divided into five groups (n=5). Group 1 (Distilled water 10 ml/kg), Group 2 (75 mg/kg Extract), Group 3 (150

mg/kg Extract), Group 4 (300 mg/kg Extract), and Group 5 (Indomethacin 10mg/kg). All treatments were given through oral cannulation.

One hour post-treatment, oedema was induced by injection of carrageenan (0.1 ml, 1% $^{w}/_{v}$ in saline) into the sub plantar tissue of the right hind paw. The linear circumference was measured using cotton thread method. Measurements were made immediately before injection of carrageenan and at 30 minute intervals for 3 hours. The mean increase in paw swelling was measured and the percentage inhibition was calculated (Okpo *et al.,* 2001, Agbaje and Fageyinbo, 2012).

Xylene-induced Ear Oedema

Adult mice (18-30 g) fasted overnight were divided into five groups (n=5). Group 1 (Distilled water 10 ml/kg), Group 2 (75 mg/kg Extract), Group 3 (150 mg/kg Extract), Group 4 (300 mg/kg Extract), and Group 5 (Dexamethasone 10mg/kg). All treatments were given through oral cannulation. One hour post-treatment, oedema was induced by applying 30 µL (0.03ml) of xylene to the inner surface of the right ear. After 15 minutes the animals were sacrificed under ether anesthesia and both ears were cut off to approximately equal size and weighed. The mean difference between the right and left ears was determined for each group (Agbaje and Fageyinbo 2012).

Formalin-induced Oedema

Adult rats (120-150 g) were randomly divided into five groups of five animals each and separately given distilled water (10ml/kg, *per oral*), *Brassica oleraceae* (75, 150 and 300 mg/kg. *per oral*) and aspirin (10 mg/kg). Inflammation was induced in all the animals by sub-plantar injection of 20µL of freshly prepared 2% formalin in the rat hind paw. Paw thickness was measured 1 hour prior to and also after formalin injection. The drug treatments were continued for 7 consecutive days and paw oedema measured 1 hour after drug treatment each day. (Turner, 1965; Agbaje and Fageyinbo, 2012). The percentage or inhibition was calculated using % inhibition= 100 (Vc-Vt/Vc).Where Vc-mean edema in control and Vt- mean control in treated (standard and extract).

Inflammatory Mediators-Induced Oedema

Serotonin-induced Rat Paw Oedema

Adult rats (100-200 g) fasted overnight were divided into three groups of five animals each and were treated as follows: Group 1 (Distilled water 10 ml/kg), Group 2 (300 mg/kg Extract), and Group 3 (Indomethacin 10mg/kg). All treatments were done orally.

One hour post treatment, oedema was induced by injection of 0.1 ml serotonin (10^{-3} mg/ml) into the sub-plantar tissue of the right hind paw. The linear circumferences were measured using cotton thread method (Agbaje *et al.,* 2008; Agbaje and Fageyinbo, 2012). Measurements were made before injection of serotonin and 30 minutes after serotonin injection thereafter at 30 minutes interval for 3 hours. The mean of the paw size were computed and percentage inhibition was calculated

Histamine induced Rat Paw Oedema

The same procedure for serotonin-induced oedema was repeated except that histamine 10^{-3} mg/ml was injected into the sub-plantar tissue of the right hind paw (Agbaje and Fageyinbo, 2012).

Castor Oil induced Diarrhoea

This method (Awounters *et al.,* 1978; Agbaje and Fageyinbo, 2012) was to elucidate the involvement of prostaglandins in the anti-inflammatory activity of *Brassica oleraceae.* Aspirin 100 mg/kg, distilled water 10 ml/kg and *Brassica oleraceae* 300 mg/kg were separately given through oral intubation to groups of mice followed by 20 ml/kg castor oil 1 h thereafter. Animals were examined for presence or absence of characteristic diarrhoea droppings, onset of diarrhea and number of wet stools, semi-solid and solid stools on a white paper on the floor of their cages every hour for 4 h. Absence of diarrhoeal droppings as well as delay in onset of diarrhoea, were recorded as a positive result, indicating possible inhibition of prostaglandin biosynthesis.

Statistical Analysis

Results obtained were expressed as mean ± SEM. The data were analyzed using one way ANOVA followed by Tukey's multiple comparison test or by two-way ANOVA followed by Bonferroni posttest using Graph Pad Prism 5 (Graph Pad Software Inc., CA, USA). Results were considered significant when $p<0.05$.

RESULTS

Carrageenan-induced Rat Paw Oedema

The subplantar injection of carrageenan (1% w/v, 0.1 ml) produced oedema, which increased progressively with time in the control group. The extract of *Brassica oleraceae* produced a significant ($p<0.05$, $p<0.01$) dose dependent inhibition of oedema with the peak inhibitory effect (51.16 %) produced at the dose of 300 mg/kg at 120 min this effect was sustained for 180 min. Oral administration of indomethacin (10 mg/kg) also significantly ($p < 0.01$) reduced the oedema with 74.42 % inhibition at 150 min and the effect was also sustained till 180 min (Table 1).

Xylene-induced Ear Oedema

Brassica oleraceae produced a significant ($p<0.01$) dose dependent inhibition of ear oedema development with a peak inhibitory effect (65.33 %) observed at the highest dose (300 mg/kg). Dexamethasone (10 mg/kg) also produced a significant ($p<0.05$, $P<0.01$) inhibition of edema development with 74.67 % inhibition (Table 2).

Formalin-induced Rat Paw Oedema

Brassica oleraceae (75, 150, and 300 mg/kg) produced an appreciable significant ($P < 0.05$) daily inhibition of oedema (anti-arthritis) in a dose dependent manner. Peak inhibitory effect (89.47%) was obtained with a dose of 300 mg/kg at day 7. Comparatively, acetylsalicylic acid (aspirin) gave 94.74% inhibition on day 7 (Table 3).

Serotonin And Histamine-induced Rat Paw Oedema

A significant ($p < 0.001$) reduction in the oedema of rat treated with *Brassica oleraceae* was observed in the serotonin-induced paw oedema model (Table 4). The maximum inhibition was observed at 120 min (60.87 %).

In histamine-induced oedema, *Brassica oleraceae* produced a more pronounced inhibition (83.33 %) of histamine observed at 180 min (Table 5).

Castor Oil-induced Diarrhoea

The extract of *Brassica oleraceae* (300 mg/kg) showed a significant inhibition of prostaglandin synthesis as a result of delayed onset of diarrhoea and decreased number of wet stools coupled with increased number of solid stool (Table 6)

Phytochemical Analysis

The phytochemical analysis showed the presence of alkaloids, tannins, cardiac glycosides, flavonoids, phlobatanins, anthraquinones and saponins (Table 7).

Table 1: Effect of aqueous leaf extract of *B. oleraceae* on carrageenan induced rat paw oedema.

Grp	Dose (mg/kg)	0 Min.	30 Min.	60 Min.	90 Min.	120 Min.	150 Min.	180 Min.
Cont		1.52±0.03	2.62±0.03	2.80±0.08	2.68±0.03	2.52±0.03	2.38±0.03	2.138±0.03
B.o^{+}	75	1.70±0.06	2.48±0.07	2.62±0.07	2.56±0.10	2.44±0.02	2.32±0.05*	2.32±0.05*
	% inhibition		29.09	28.13	25.86	26.00	27.91	27.91
	150	1.64±0.07	2.32±0.04	2.60±0.10	2.52±0.14	2.34±0.10*	2.26±0.09**	2.26±0.09**
	% inhibition		38.18	25.00	24.14	30.00	29.07	29.07
	300	1.76±0.06	2.58±0.07	2.72±0.09	2.70±0.14	2.28±0.03**	2.18±0.07*	2.18±0.07
	% inhibition		25.46	25.00	18.96	48.00	51.16	51.16
Indn	10	1.66±0.09	2.42±0.10	2.34±0.09	2.34±0.05	2.02±0.11*	1.88±0.07**	1.88±0.07**
	% inhibition		30.91	46.88	46.88	64.00	74.42	74.42

Mean±S.E.M - Standard Error of Mean, (n=5)

Indn - indomethacin, Grp - Groups, Cont- Control

B.o^{+} - *Brassica oleraceae*, $^{*}p < 0.05$, $^{**}p < 0.01$ statistically significant compared to control (Two Way ANOVA followed by Bonferronni post tests).

Table 2: Effect of aqueous leaf extract of *B. oleraceae* on xylene induced ear oedema in mice.

Groups	Dose (mg/kg)	RE wt (g)	LE wt (g)	RE-LE wt (g)	% Inhibition
Control		0.086±0.004	0.071±0.002	0.015±0.004	
B.oleracea	75	0.068±0.004*	0.060±0.002*	0.008±0.003	46.67
B.oleracea	150	0.079±0.007*	0.073±0.009**	0.006±0.002*	60.00
B.oleracea	300	0.070±0.003*	0.065±0.003*	0.005±0.000	65.33
Dexamethasone	10	0.076±0.006*	0.072±0.007**	0.004±0.001*	74.67

RE wt - Right Ear Weight;
LE wt - Left Ear Weight,
Mean± S.E.M - Standard Error of mean, (n=5)
$^{*}p<0.05$, $^{**}p<0.01$ statistically significant compared to control (Two Way ANOVA followed by Bonferronni post tests)

Table 3: Effect of aqueous leaf extract of *B.oleraceae* on formalin induced rat paw oedema.

Grp	Dose (mg/kg)	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Cont		2.04±0.02	2.58±0.02	2.60±0.05	2.52±0.03	2.52±0.03	2.48±0.04	2.46±0.05	2.42±0.03
B.o+	75	2.00±0.03	2.42±0.04	2.42±0.02	2.34±0.02	2.30±0.00*	2.22±0.02*	2.18±0.03**	2.14±0.04**
	% inhibition		22.22	25.00	29.17	37.50	50.00	57.14	63.16
	150	2.14±0.06	2.58±0.12	2.38±0.04*	2.34±0.06	2.30±0.07*	2.28±0.05*	2.24±0.07**	2.20±0.07***
	% inhibition		18.52	57.14	58.33	66.67	68.18	76.20	84.21
	300	2.04±0.05	2.38±0.10*	2.34±0.08*	2.26±0.09**	2.20±0.07**	2.16±0.09***	2.12±0.07***	2.08±0.05***
	% inhibition		37.04	46.43	54.17	66.67	72.73	80.95	89.47
ASA	10	2.12±0.03	2.46±0.14*	2.44±0.11*	2.32±0.08**	2.26±0.06**	2.20±0.07**	2.18±0.05***	2.14±0.04***
	% inhibition		37.04	42.86	58.33	70.83	81.82	85.71	94.74

ASA - Acetyl salicylic acid (Aspirin)
B.o+ - *Brassica oleracea*, Grp: Groups, Cont: Control, Mean ±S.E.M: Standard Error of Mean
$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ statistically significant compared to control (Two Way ANOVA followed by Bonferronni post tests).

Table 4: Effect of aqueous leaf extract of *B. oleraceae* on serotonin-induced rat paw oedema

Groups	Dose (mg/kg)	0 Min.	30 Min.	60 Min.	90 Min.	120 Min.	150 Min.	180 Min.
Control		1.68±0.08	2.46±0.04	2.26±0.05	2.14±0.05	2.14±0.05	2.10±0.05	2.12±0.08
B.oleracea	300	1.66±0.05	2.44±0.04	2.22±0.03	1.90±0.06**	1.84±0.05**	1.84±0.05**	1.88±0.05**
% inhibition			0.00	3.45	47.83	60.87	57.14	50.00
Indomethacin	10	2.02±0.03	2.26±0.02	2.14±0.04*	2.14±0.04*	2.22±0.02	2.20±0.00	2.20±0.00
% inhibition			69.23	79.31	73.91	56.52	57.14	59.09

Mean ±S.E.M - Standard Error of Mean, (n=5)
*$p<0.05$, **$p<0.01$ statistically significant compared to control (Two Way ANOVA followed by Bonferronni post tests)

Table 5: Effect of aqueous leaf extract of *B. oleraceae* on histamine-induced rat paw oedema

Groups	Dose (mg/kg)	0 Min.	30 Min.	60 Min.	90 Min.	120 Min.	150 Min.	180 Min.
Control		1.78±0.08	2.30±0.07	2.20±0.05	2.14±0.06	2.10±0.04	2.04±0.05	2.02±0.03
B.oleracea	300	1.70±0.08	2.20±0.05	2.10±0.08	1.92±0.04*	1.84±0.02**	1.78±0.03***	1.74±0.04***
% inhibition			3.85	13.64	38.89	56.25	70.37	83.33
Indomethacin	10	1.78±0.02	1.96±0.02	1.88±0.02**	1.90±0.03*	1.92±0.03	1.88±0.02***	1.90±0.03***
% inhibition			65.39	78.26	66.67	56.25	62.96	50.00

Mean ±S.E.M - Standard Error of Mean, (n=5)
*$p<0.05$, **$p<0.01$, ***$p< 0.001$ statistically significant compared to control. (Two Way ANOVA followed by Bonferronni post tests)

Table 6: Effect of aqueous leaf extract of *B. oleracea* on castor-oil induced diarrhoea

Groups	Dose (mg/kg)	Onset of Diarrhoea (Minutes)	Number of Wet Stool	Number of Semi-solid Stool	Number of Solid Stool	Weight of Wet Stool (g)	Weight of Semi-solid Stool(g)	Weight of Solid Stool (g)
Control		20.20±0.66	8.40±0.92	5.40±0.67	0.60±0.40	6.06±0.57	0.28±0.15	0.01±0.01
B.oleracea	300	88.60±1.24*	1.20±0.20*	0.40±0.24*	2.80±0.37	0.32±0.02	0.16±0.10**	0.10±0.04
ASA	100	109.60±3.24*	0.80±0.37**	1.00±0.44*	2.00±0.94	0.25±0.11	0.27±0.12*	0.20±0.08

ASA - Acetylsalicylic Acid (Aspirin)
Mean ±S.E.M - Standard Error of Mean, (n=5)
*$p<0.05$, **$p<0.01$ statistically significant compared to control. (Two Way ANOVA followed by Bonferronni post tests).

Table 7: Phytochemical analysis of *Brassica oleraceae.*

TEST	INFERENCE
Alkaloids	Present
Tannins	Present
Cardiac glycosides	Present
Flavonoids	Present
Phlobatanins	Present
Anthraquinones	Present
Saponins	Present

DISCUSSION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of these were based on the uses of the agents in traditional medicine (Cordell, 2000). In this study, the anti-inflammatory activity of *Brassica oleraceae* was investigated using the carrageenan, histamine, serotonin, xylene, formaldehyde-induced edema (anti-arthritis), and castor oil induced diarrhea (prostaglandin synthesis) tests.

Carrageenan-induced rat paw oedema model is a suitable test for evaluating anti-inflammatory drugs, which has frequently been used to assess the antiedematous effect of the drug. It is also used to study non-steroidal anti-inflammatory drugs (Mazzanti and Braghiroli, 1994; Sowemimo *et al.,* 2013). It is believed to be triphasic. The first phase (0– 1.5h) of the carrageenan model is mainly mediated by histamine and serotonin; the second phase (1.5–2.5 h) mediated by kinin and the last phase (2.5–6 h) which begins after the kinin phase and is consequence to the liberation of prostaglandins (Suba *et al.,* 2005; Sowemimo *et al.,* 2013). *Brassica oleraceae* showed significant dose dependent

inhibitory effect on the rat paw edema development. The effect peaked at 90 minutes and was sustained till 180 minutes. This suggests the extract possibly acts by inhibiting the action of histamine, serotonin, kinin and prostaglandin.

To investigate the action of the extract on the inflammatory mediators, the effective dose of the extract (300 mg/kg) was used in the histamine and serotonin edema tests. The results showed that the extract effectively inhibited the edema produced by histamine and serotonin, indicating that the extract exhibits its anti-inflammatory action by inhibiting the synthesis, release or action of histamine and serotonin.

To elucidate the mechanism that might account for the inflammatory action of *B. oleracea*, castor oil induced diarrhea, which results in biosynthesis of prostaglandin in rats was used. The aqueous leaf extract of *B. oleracea* (300 mg/kg) produced a significant ($P < 0.05$) delayed onset of diarrhea (88 minutes) when compared to the control (20 minutes). Moreover, there was a marked reduction in the production of wet stools. This showed an inhibition of prostaglandin synthesis which accounts for characteristics of diarrhea seen in control group with its rapid onset (20 minutes), an increased number of wet stools and a decrease in the number of solid stool. The delayed onset of diarrhea (88 minutes) observed in *B. oleracea* treated animals are comparable to that of aspirin (109 minutes) which indicates the inhibition of prostaglandin that is released during diarrhea episode.

Xylene induced ear oedema model is partially associated with substance P which is an undecapeptide that is widely distributed in the central and peripheral nervous system and functions as a neurotransmitter or neuromodulator in a variety of physiological processes (Junping *et al.*, 2005; Agbaje and Fageyinbo, 2011). Release of substance P from the sensory neurons causes vasodilation and plasma extravasations suggesting its role in neurogenous inflammation. Thus, it causes the swelling of ear in mice. This test distinguishes NSAIDs from steroidal anti-inflammatory drugs (Agbaje and Fageyinbo, 2011). The effectiveness of *B. oleraceae* in this model may suggest the inhibition of phospholipase A_2 which is involved in the pathophysiology of inflammation due to xylene (Lin *et al.*, 1992).

Formalin evokes biphasic events: an early neurogenic component followed by a later tissue-mediated response. The second phase is a chronic inflammatory pain (Gheibi *et al.*, 2010) mediated by release of inflammatory mediators. This model is used to screen anti-arthritic and anti- inflammatory agents (Greenwald, 1991). The effectiveness of the extract on formalin induced paw edema in rats revealed its anti-arthritic activity.

The result of phytochemical analysis revealed the presence of flavonoids, tannins, alkaloids, cardiac glycosides, phlobatanin, anthraquinone and saponin. Flavonoids are known to prevent the synthesis of prostaglandins. Biochemical investigations on the mechanism of action of flavonoids have shown that these compounds can inhibit a wide variety of enzymes. The release of arachidonic acid is closely related to the

cyclooxygenase and 5-lipoxygenase enzyme systems (Williams *et al.,* 1995; Middleton *et al.,* 2000). Glycosides, the active component in willow bark have also been found to exhibit anti-inflammatory effects (Ma *et al.,* 1998; Agbaje *et al.,* 2008). Ahmadiani *et al.,*(2000) also reported that flavonoids and tannins were found to have anti-inflammatory and anti-nociceptive activity therefore; flavonoids and tannins found in *B. oleraceae* could have contributed to its anti-inflammatory property. The low acidity of the extract (pH 8.5) makes it more tolerable than aspirin which is highly acidic. *B. oleraceae* may serve as a suitable substitute to NSAIDs with high incidence of gastric ulceration.

CONCLUSION

The present study confirmed that *B. oleraceae* possesses significant anti-inflammatory activity. The results also support the use of the plant in the treatment of inflammation-related diseases traditionally.

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