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Anti-inflammatory effects of ethanolic extract of *Amaranthus viridis* against carrageenan induced paw oedema in Albino Rats

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ABSTRACT

The anti-inflammatory effects of ethanolic crude extracts of *Amaranthus viridis* whole plant was investigated in carrageenan induced rat paw oedema. Albino Rats were classified into four groups, each of 5. Group 1 received (0.1 ml of 1% W/V carrageenan in 0.9 ml normal saline in a dose of 2 ml/kg body weight) at sub-plantar region and served as negative control , group 2 received indomethacin (10 mg/kg bwt) intraperitoneally and served as standard reference (positive control) , group 3 and group 4 received crude ethanolic extract intraperitoneally at 250 mg/kg and 500 mg/kg bwt respectively. The oedema was quantified by measuring the hind paws thickness at 0, 1, 2 and 3 hour after the carrageenan injection. Administration of ethanolic extract (250 mg/kg and 500 mg/kg) significantly reduced the oedema thickness in a time and dose dependent manner. The inhibition percentage of inflammation was 40.30 % and 70.4 % at dose of 250 and 500 mg/kg respectively. The ethanolic extract at dose of 500 mg/kg shows a potent activity at the last hour of following up. The present study concluded that *Amaranthus viridis* extract displays remarkable anti-inflammatory activity and recommended for the possible use as anti-inflammatory remedy.

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INTRODUCTION

The plant kingdom is known to provide a rich source of botanical anti-inflammatories (Nadkarni, 1954). A number of medicinal plants have been used to treat inflammations in man and animals (Nadkarni, 1954). Numerous natural products have been tested as various therapeutics (Sannigrahi *et al.*, 2010).

Some medicinal plant considered as potential source of anti-microbial agents. Seventy-six extracts of thirty-one Sudanese medicinal plants belonging to twenty-one families were investigated for their anti-bacterial activity against four bacteria by (El Tohami *et al.*, 1997). Out of the seventy-six extracts tested, sixty-four exhibited inhibitory effects against at least one of the tested micro-organisms. Of these, seven plants showed significant activity against the four tested organisms, namely *Bacillus subtilis*, *Staphylococcus auerus*, *Escherichia coli* and *Pseudomonas aueroginosa*.

Acute inflammation is a rapid, short–lived physiological response, characterized by accumulation of fluid, plasma proteins and the leukocytes which release a large number of soluble mediators which modulate and maintain the inflammation (Rosenberg and Gallin, 1999).

Carrageenan induced rat paw oedema has been used for assessment of the anti-inflammatory activity of many plant extracts and essential oils (Hajhashemi *et al.*, 2003; Khalil *et al.*, 2006; Orhan *et al., 2006*) .

The *Amaranthus viridis* plant locally called Lissan elTair Kabir, is a member of the Amaranthaceae family, has been used in traditional medicine in many parts of the world. Traditionally is an edible plant which is grown in all regions of India, stem used as antidote for snake bites (Obi *et al.*, 2006). Leaves used for scorpion stings, constipation, inflammation, eczema, bronchitis, anemia, leprosy, an infusion of powdered used for stomach problems (Sena, 1998). Seeds also used in pregnant women to lessen labor pain. Infusion of plant has been used as a diuretic and anti-inflammatory agent of the urinary tract, venereal diseases, vermifuge, anti-emetic and laxative (Quershi *et al.*, 2008). Poultice and boils of leaves are used for abscesses and skin cleansing, anti-diabetic (Kesari *et al.*, 2005), anti-histaminic (Yamamura *et al.*, 1998) and anticarcinogenic (Yen *et al.*, 2001). In Sudan, the plant is used in Western Darfur and Wad Madani as anti-helminthic and a fodder for grazing animals. The present study aimed to investigate the anti-inflammatory effects of ethanolic extract of *Amaranthus viridis* in carrageenan induced rat paw oedema model.

MATERIALS AND METHODS

Plant material

Amaranthus viridis plant was obtained from Nile river banks (November 2012) in Khartoum, Sudan. The plant dried under sun-rays. The sample was kept in the Department of Pharmacology and Toxicology at the Medicinal and Aromatic Plants Research Institute (MAPRI) – National Center for Researches (Khartoum). Plant was authenticated by Dr.Haider AbdelGader (MAPRI) .

Preparation of extract

The plant dried under sun-rays, after complete dryness removed for extraction. Specific weight of the plant sample (260 gram) was soaked in 2500 ml of 80 % ethanol for about 3 days with daily filtration and evaporation of the solvent under reduced pressure using rotary evaporator apparatus. Final extract residues allowed to air in petri-dishes till complete dryness (Harborne, 1984).

All drugs used were of the highest commercially available purity. Indomethacin (powerful anti-inflammatory, time dependent, non-selective inhibitor for cycloxygenase enzymes), carrageenan (standard inflammatory inducer) and normal saline were purchased from Sigma – Aldrich, Germany.

Experimental animals

Albino rats were obtained from the Faculty of Pharmacy, University of Khartoum. No informed consent was obtained.

Experimental design

Carrageenan-induced rat paw oedema has been used for assessment of the anti-inflammatory activity of many plant extracts. The method used was carried out according to Winter *et al.* (1962). Twenty Albino rats were housed within the premises of the Medicinal and Aromatic Plant Research Institute, National Center for Research, Khartoum, with feed and water provided *ad libitum*. The rats were allotted into four groups each of 5 rats.

Group 1: received the vehicle carrageenan (2 ml/kg) at sub-plantar region and served as negative control.

Group 2: received indomethacin (10 mg/kg) intraperitoneally, and served as standard reference and act as positive control.

Group 3: received the crude ethanolic extract intraperitoneally at 250 mg/kg.

Group 4: received the crude ethanolic extract intraperitoneally at 500 mg/kg.

One hour following the previously mentioned treatments, paw swelling was induced by sub-plantar injection of 0.1 ml of 1 % w/v carrageenan in normal saline 0.9 ml into the right hind paw of all groups. The oedema was quantified by measuring the hind paw thickness at 0, 1, 2 and 3 hours after injection, with a micrometer screw gauge. The increase with linear diameter of the right hind paws were taken as an indication of the paw oedema. The percentage inhibition of the inflammation was calculated from the formula described by (Hajhashemi *et al.*, 2003).

$$Percent\ inhibition = D_0 - D_T / D_0 \times 100$$

D_0 is the the average inflammation (hind paw oedema) of the control group of rats at a given time.

Dt is the average inflammation of the drug treated (that is, reference indomethacin or extracts) in rats.

Statistical analysis

The collected data were statistically analyzed using Mann-Whitney test. P value < 0.05 was considered statistically significant.

RESULTS

The results of the effects of *Amaranthus viridis* plant extracts and indomethacin on oedema in carrageenan model are summarized in Table 1.

Negative inhibition percentage (-0.51 %) in the table at 250 mg/kg, means there was no reduction for oedema at Hour 1 according to the equation described earlier. The crude ethanolic extract of *Amaranthus viridis (*250 mg/kg) when compared with the control and the reference drug, produced significant inhibition ($P \leq 0.05$) in rat paw oedema of 40.34% at 3 hours post treatment. The crude ethanolic extract of *Amaranthus viridis (*500 mg/kg) when compared with the control and the reference drug, produced significant inhibition ($P \leq 0.05$) in rat paw oedema of 70.45 % at 3 hours post treatment.

DISCUSSION

The present study was conducted to investigate the possible anti-inflammatory effects of Amaranthus viridis extract in carrageenan model. Paw swelling is one of the major factors in assessing the degree of inflammation and efficacy of the tested drugs (Begum and Sadique, 1988) and (Mizushima *et al.*, 1972). The crude ethanolic extract of Amaranthus viridis at two doses level were investigated for their anti-inflammatory activity. The two doses significantly inhibited or decreased oedema in a time and dose dependent manner and the maximum inhibition percentage were recorded with the dose of 500 mg/kg as 70.45% at the end of the follow up period.

The current study results might be considered as first report for the anti-inflammatory activity by both dose levels of ethanolic extract at that time of Amaranthus viridis whole plant. Phytochemical screening of the medicinal plants showed good anti-inflammatory activity as it may contain secondary metabolites like alkaloids, triterpenoids, polyphenolics and flavonoids. These classes of plant, which possess secondary metabolites, are considered the sources of chemicals, which are responsible for wide therapeutic activities of several plants (Debella, 2002). Phytochemical investigation on Amaranthus viridis yielded several classes of secondary metabolites such as flavonoids, steroids, saponins and phenolic compounds many of which express biological activities (Mayer *et al.*, 1982; Emam, 1999; Khan *et al.*, 1982). These compounds are known to be potent cyclo-oxygenase-1 (COX-1) inhibitors, through their binding nature with proteins. (Reddy and Reddy, 2009). Since the carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agent acting by the mediators of acute inflammation. (Sawadogo *et al.*, 2006). The extract may have exhibited its anti-inflammatory actions by means of either inhibiting the synthesis, release or action of inflammatory mediators such as histamine, serotonin and prostaglandins. Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin act by the reduction of sensitization of pain receptors caused by prostaglandins at the

Table 1: Effects of treatment of crude ethanolic extract of *Amaranthus viridis* on oedema in carrageenan model.

Group	Hour 1	Hour 2	Hour 3
Control negative (Carrageenan)	1.95	1.89	1.76
2 mg/kg B.Wt			
Control positive (Indomethacin)	1.16 ± 0.37	0.47 ± 0.24	0.10 ± 0.06
10 mg/kg B.Wt	(40.51 %)	(75.13%)	(94.32%)
Ethanolic extract of *Amaranthus viridis*	1.96 ± 0.11	1.66 ± 0.35	1.05± 0.32
250 mg/kg	(-0.51 %)	(12.17%)	(40.34%)
P *value*	0.009*	0.009*	0.009*
Ethanolic extract of *Amaranthus viridis*	1.25± 0.41	1.10 ± 0.28	0.52± 0.22
500 mg/kg	(35.89%)	(41.79%)	(70.45%)
P value	0.117	0.009*	0.012*

B.Wt: Body weight
Data were expressed as mean ± SD and percentage
*P < 0.05 was considered statistically significant

inflammation site (Dhara *et al.*, 2000). The different triterpenoids, polyphenolics and other chemical constituents of the plant extract may be involved in the observed anti-inflammatory effects of the plant extract and may be having actions similar to NSAIDs.

CONCLUSION

The present study concluded that the ethanolic extract of Amaranthus viridis possessed potent anti-inflammatory activity at 250 and 500 mg/kg.

Recommendation: The present study recommended the possible use of Amaranthus viridis as a remedy for treatment of inflammation.

Further studies are needed to investigate the phytochemical/s responsible for the anti-inflammatory effect and toxicological studies are needed to evaluate the safety of the plant constituents in the different animal species.

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