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Anti-inflammatory and gastro-protective effects of *Apodytes dimidiata*

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ABSTRACT

Apodytes dimidiata E. Mey. Ex. Arn (Icacinaceae), a well-known plant in Zulu traditional medicine is used for the treatment of various inflammatory and gastrointestinal ailments. The plant is reported to have antioxidant activity. The present study evaluated the anti-inflammatory and gastro-protective effects of *A. dimidiata* in various experimental models. Oral administration of leaf methanolic fraction (AMF) at 100 and 250 mg/kg, b. wt. doses inhibited the carrageenan induced acute paw oedema in mice by 30.8 and 55.17%, respectively. Similarly, 23.94 and 50% reduction was observed in formalin induced chronic oedema at the same doses. In an *in vitro* assay, lipoxygenase enzyme activity was inhibited by AMF with an IC_{50} value of 48.9 μg/ml. In rats, the gastritis was induced by the administration of 70% ethanol orally. The pre-treatment of mice with AMF (250 mg/kg b. wt.) attenuated the ethanol mediated reduction in SOD ($p < 0.001$), GPx ($p < 0.001$), catalase ($p < 0.001$) and GSH ($p < 0.001$) levels and the rise of lipid peroxidation ($p < 0.001$) in gastric tissue compared to control. On histopathological examination, a significant reduction in gastric lesions was observed ($p < 0.001$) and the tissue architecture appeared nearly normal in AMF treated group of animals. Altogether, the results of the present study reveal the anti-inflammatory and gastro-protective effects of *A. dimidiata* leaf extract. The study provides a scientific support for the traditional use of *A. dimidiata* as medicine.

1. Introduction

Peptic ulcer, the sore on the inner lining of the stomach, duodenum or occasionally on the lower esophagus is a serious concern in gastroenterology (Najim, 2011). Globally, around 4% of the population is reported to have peptic ulcers occasionally and 10% of it develops a diseased condition as chronic or acute (Snowden, 2008). Etiological studies have revealed that various factors such as stress, alcoholism, smoking, regular use of medications and infection of *Helicobacter pylori* are involved in developing gastric ulcers. However, an inappropriate proportion of digestive fluid in the stomach is considered to be the definitive reason for the ulceration. In addition, associated reactive oxygen species (ROS) mediated infiltration of neutrophils (Wallace, 1997) and related inflammatory changes (Leirisalo-Repo et al., 1993, Jainu and Devi, 2006, Wallace, 2011) are also known to augment the processes of gastritis. The prolonged gastric lesion and ulcers are reported to play a pivotal role in developing gastric cancer (Anand et al., 2012; Oyagi et al., 2010). The products currently been used for treating gastric ulcers are steroidal (SAID) and non-steroidal anti-inflammatory (NSAID) drugs and reciprocal antiulcer drugs such as the antacids, anti-cholinergics and proton pump inhibitors. Although these drugs have brought about remarkable changes in ulcer therapy, their efficacy is still debatable and some are out of success in certain clinical trials due to their side effects that limit their usage (Ji et al., 2012). Therefore, it is imperative to find out natural remedies with low side effects (Boakye-Gyasi et al., 2008).

Today, a number of modern drugs are derived from the plants which are used as traditional medicines (de Souza Almeida et al., 2011). *Apodytes dimidiata*, belonging to the family Icacinaceae is a prominent and common tree in South African forests. The plant is commonly known as *umDakane* to the rural communities of KwaZulu-Natal (Watt and Breyer-Brandwijk, 1962) and used as molluscicide for schistosomiasis control. The plant is also widely used in Zulu traditional medicines for treating gastrointestinal ailments, helminthes (Gestner, 1938; Bryant, 1966; Hutchings et al., 1996) and the leaves are used as a remedy for ear inflammation (Watt and Breyer-Brandwijk, 1962). In a study conducted to validate the anti-mycobacterial plants used by traditional healers in three districts of the Limpopo Province (South Africa), it was evidenced that *A. dimidiata* acetone leaf extract showed anti-bacterial and antioxidant properties. Moreover, the phytochemical screening of the extract showed the presence of saponins, tannins, terpenes, steroids and flavonoids (Masoko and Nxumalo, 2013). An iridoid glycoside, genipin was isolated from the bark of *A. dimidiata* in relation to its molluscicidal activity (Drewes and Kayonga, 1996). In a previous study, we have detected an iridoid glycoside, genipin from the methanolic leaf extract of *A. dimidiata* (Divya et al., 2015). Genipin isolated from *Gardenia jasminoides* is reported to inhibit the growth of *H. pylori* and reduce HCl/ethanol induced gastric lesions (Lee et al., 2009) due to its acid-neutralizing and antioxidant properties. Considering various reports, it is presumed that the substances with antioxidant

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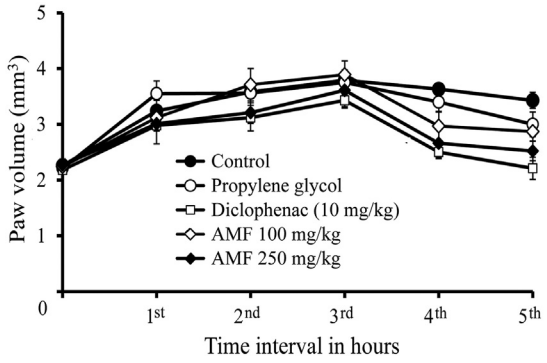

Fig. 1. Effect of AMF on carrageenan induced acute paw oedema.

and anti-inflammatory properties may inhibit the gastritis on mucosa (Dekanski et al., 2009). Considering the ethnopharmacological significance of the plant, the present study is aimed to evaluate the anti-inflammatory and gastro-protective efficacy of *A. dimidiata*. Since ethanol induces petechial lesions quickly (Ramirez and Roa, 2003), the study was performed in ethanol-induced gastric ulcer model in rats (Santos and Rao, 2001).

2. Materials and methods

2.1. Plant material

Leaves of *A. dimidiata* E. Mey. Ex Arn, used for the study was collected from Periya, Wayanad District of Kerala, India (Altitude: 810 m, Geographical location: Lat. N 11° 51′ 03.19″, Long. 75° 48′ 05.54″) during the month of January, 2014 and identified by Dr. Sujanapal P, Taxonomist, Kerala Forest Research Institute (KFRI) Peechi, Thrissur, Kerala. A voucher specimen (No. KFRI 28024) was lodged in the Herbarium of KFRI.

2.2. Preparation of extract

Leaves of *A. dimidiata* were dried under shade and powdered using a mixer grinder. Approximately, 30 g of the powder was extracted separately with 250 ml of petroleum benzene, chloroform, acetone and methanol using Soxhlet apparatus for 24 h. The extracts obtained were concentrated to dryness. Crude methanolic extract (4 g) was loaded on to a 600 × 30 mm silica gel (60–120 mesh) column and eluted successively by passing various solvents (150 ml each) of increasing polarities such as petroleum benzene, chloroform, acetone and methanol. The active methanolic fraction (AMF) which was found most effective in scavenging free radicals was used for further studies.

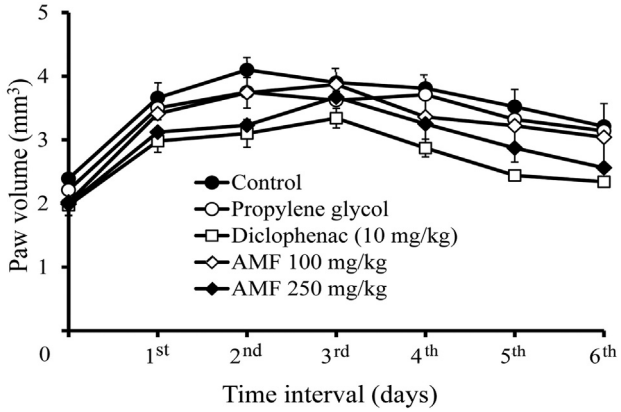

Fig. 2. Effect of AMF on formalin induced chronic paw oedema.

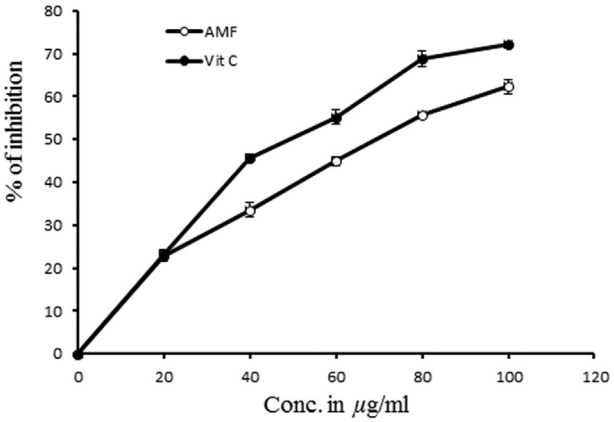

Fig. 3. Anti-lipoxygenase activity of AMF.

2.3. Animals

Experiments were conducted using male BALB/c mice used for the study (25–30 g size) and male Wistar rats (180–200 g), purchased from the Small Animal Breeding Station, Kerala Veterinary and Animal Sciences University (KVASU), Mannuthy, Kerala, India. The animals were maintained under standardized environmental conditions (22–28 °C, 60–70% relative humidity, 12 h dark/light cycle) and fed with standard rat feed (Lipton, India) and water *ad libitum*. All the animal experiments were carried out with the prior permission of Institutional Animal Ethics Committee (IAEC) and by following the ethical guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by Animal Welfare Division, Government of India.

2.4. Carrageenan-induced paw oedema

Acute inflammation was induced by carrageenan (Winter et al., 1962). Male BALB/c mice were divided in to 5 groups comprising six animals each. One group served as positive control by giving carrageenan alone. Group II which served as standard reference drug diclophenac (10 mg/kg b. wt.) was given intraperitoneally. Group III and IV were treated with different concentrations of AMF (100 and 250 mg/kg b. wt.) orally for five consecutive days and group V were treated with propylene glycol (vehicle control). On fifth day, acute inflammation was induced by the sub-plantar injection of 0.02 ml freshly prepared 1% suspension of carrageenan in 0.1% CMC (carboxymethyl cellulose) on right hind paw in all groups of animals. Following administration, the footpad thickness was measured using Vernier calipers every hour for 5 h. The percentage of inhibition in paw volume swelling was calculated according to the following formula $[(V_t - V_o)$ control $- (V_t - V_o)$ treated group / $(V_t - V_o)$ control$] \times 100$, where V_t is the paw oedema at various time intervals and V_o is the initial paw oedema.

2.5. Formalin induced paw oedema

Male BALB/c mice ($n = 6$) were divided as group I — control, group II served with standard drug diclophenac (10 mg/kg b. wt.), group III and group IV — AMF (100 and 250 mg/kg b. wt.), respectively and group V treated with propylene glycol (vehicle control). Chronic inflammation was induced by sub-plantar injection of freshly prepared formalin (0.02 ml of 2% formalin) on the right hind paw in all groups of animals (Chau and Alan, 1989). The drug was given orally, 1 h before the formalin injection and continued for six consecutive days. The percentage of inhibition in paw volume was calculated according to the following formula $[(V_t - V_o)$ control $- (V_t - V_o)$ treated group / $(V_t - V_o)$ control$] \times 100$, where V_t is the paw oedema at various days and V_o is the initial paw oedema.