Comparison of gastric ulcerogenicity of percolated extract of *Anacardium occidentale* (Cashew Nut) with indomethacin in rats

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Abstract: In traditional Iranian medicine, the core of the fruit of *Anacardium occidentale* (cashew nut) has been used in the management of the pain. In this study gastric ulcerogenicity effect of the percolated extract of *A. occidentale* was investigated in rats. The extract or indomethacin (200, 300, 400 and 800 mg/kg) was administrated orally. In the control group normal saline (5 ml/kg) was used.

After getting extract, indomethacin or normal saline, animals were slaughtered. The stomachs were detached and 10ml of 2% formalin injected in to the stomach for fixing the internal coat of the gastric wall. The stomachs were then slitted open near the bigger curvature and lacerations in the glandular part were evaluated. The ulcer index was determined using j-score.

Data demonstrated that the oral dose of 200mg/kg of the extract did not provoke any ulcerogenic consequence in the rat's stomach. Gastric ulcerginicity of the extract at the doses of 300, 400 and 800 mg/kg was less than the similar doses of indomethacin ($p<0.01$).

Therefore, *A. occidentale* is an appropriate plant for ongoing search for establishing an analgesic agent with low gastro-intestinal side effects for clinical use.

Keywords: *Anacardium occidantale*, gastric ucerogenicity, medical plants, cashew nut, j-score.

INTRODUCTION

In developing countries, plants have been utilized for times as an origin of conventional medicine. There is also a new worldwide concern in the use of plants to treat different diseases (Heidari *et al.*, 2005; Heidari *et al.*, 2009; Konan *et al.*, 2007b; Razali *et al.*, 2008).

Chronic inflammatory diseases are one of the international health problems and non-steroid anti-inflammatory drugs (NSAIDs) are the most given drugs for handling of inflammatory diseases. Since the NSAIDs help patients as a symptomatic assistance, they cannot alter the source of inflammation or diminish the cartilage and bone damage (Ford-Hutchinson *et al.*, 1981). Consequently, it is necessary to look for new materials in the management of inflammation and pain.

Despite the advances in the analgesic drugs, NSAIDs; e.g. indomethacin, still show a broad variety of problems, such as effectiveness and unwanted side effects. Peptic ulcer, gastric dyspepsia and hemorrhagic damage of mucosal layer in the gastrointestinal system are the most important adverse effects of NSAIDs (Filaretova *et al.*, 2001; Heidari *et al.*, 2007; Ivey, 1986; Katzung, 2007; Murray *and Brater*, 1993).

Anacardium occidentale L.*,* commonly identified as the cashew (Barcelos *et al.*, 2007a; Barcelos *et al.*, 2007b). The cashew tree goes to the *Anacardiaceae* family (Barcelos *et al.*, 2007b; Santos *et al.*, 2007) type *Anacardium* L., class *Anacardium occidentale* L. (usual cashew tree) and *Anacardium occidentale* L. var. *nanum* (dwarf cashew tree)(Santos *et al.*, 2007). The plant is originated from Asia, Central America and Africa (Santos *et al.*, 2007). The medicinal parts of the plant are finely chopped bark, cashew nut, fresh leaves, and extracted cashew oil (Thomson, 2004). The seed and case contain alkyl phenols, anacardic acid, cardol and methyl cardol, fatty acids and proteins (Thomson, 2004). Analizing of the leave extract showed that the phenolic part is the basic component. Glycosylated quercetin, amentoflavone derivative and proanthocyanidin were recognized by liquid chromatography joined to mass spectrometry. The stage of whole phenolics in the extract was assessed at 35.5% and flavonoid substance was 2.58% (Konan *et al.*, 2007a).

In traditional medicine, *A. occidentale* L. has been used in fever, pain, swelling (Rajesh *et al.*, 2009; Thomson, 2004), asthma (Olajide *et al.*, 2004), and to control of the arthritis and other inflammatory conditions (Ojewole,

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2004). The infusion of cashew stem bark demonstrates pain-relieving effects (Almeida *et al.,* 2001), anti diabethic (Olatunji *et al.,* 2005) and aphrodisiac possessions. Also it has been used in the treatment of the peptic ulcer and gastro intestinal spasms (Barcelos *et al.,* 2007b) and some special disorders related to Kidny and brain (Ofusori *et al.,* 2008). The main product of *A. occidentale* L. is the cashew nut (Garruti *et al.,* 2006). The cashew nut of the plant creates an antibacterial, vesicant and anthelmintic property (Barcelos *et al.,* 2007b). It also reveals anti inflammatory and analgesic effects in our previous investigation (Heidari *et al.,* 2000).

The ulcerogenicity of *Semecarpus anacardium* (other member of this family) has been reported previousely (Ramprasath *et al.,* 2006), but to our knowledge there is not any report on ulcerogenicity of *Anacardium occidentale*.

This study was carried out to investigate the ulcerogenic effect of the analgesic dose and higher doses of percolated extract of *A. occidentale* (Heidari *et al*. 2000) in comparison with indomethacin. Our purpose is to find a logical basis for employing of this plant as an antinociceptive agent with lower gastric ulcerogenicity in traditional medicine.

MATERIALS AND METHODS

Animals

Male albino rats (200-250g) were gained from the central animal house of Kerman University of Medical Sciences. Animals were kept at room temperature (22±2°C) on a 12:12 h light: dark cycle. They had free access to food and water except 24 h prior to the tests that they were just allowed to access to the water. The animals were put in control and test groups and each groups contained of five. According to the international policies for animal's experiments, we tried to decrease the number of animals used and to minimize animal's pain (Zimmermann, 1983).

Plant material

Anacardium occidentale was purchased from Kandelos herbal medicine store and identified by botanist of the Department of Pharmacognosy, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran. (Voucher specimen No.1460).

Extract preparation

Cashew nut was dried and fifty grams of the powder was extracted by percolation with % 80 aqueous methanol (48h). The extract was filtered and the residue was concentrated by a rotary evaporator apparatus (45°C) and then was dried at room temperature (Heidari *et al.,* 2006a; Heidari *et al.,* 2006b; Heidari *et al.,* 2007). The dry residue was melted in normal saline to provide the concentrations of 200, 300, 400, 800 mg/5ml of the extract (Heidari *et al*., 2004; Mandegary *et al.*, 2004).

Gastric ulcerogenicity study in rats

Rats were kept in the net cages 24 h before the tests, to avoid them from consumption their feces and they had free access to water. We arranged nine groups of five rats: one group was treated with normal saline 5mL/kg orally as negative control. Other groups received percolated extract with experimental analgesic dose (200 mg/kg) and higher doses (300, 400 and 800 mg/kg) as previous experiment (Heidari *et al*., 2000) and indomethacin suspensions with the same doses as treated groups and positive controls respectively. Four hour after treatment, animals were slaughterd with ether. The stomachs were detached and 10 ml of 2% formalin was injected into the stomachs to fix the internal coatings of their walls. Stomachs were then opened beside the larger curvature and the lacerations in the glandular segment were estimated. The ulcer index was considered with the J. Score. The lesions were assessed in diameter's size order: 0–1 mm = a; 1–2 mm = b; bigger than 2 mm = c. The total points were defined as the ulcer index (Dehpour *et al.*, 1998; Dehpour *et al.*, 1999; Konan *et al.,* 2007a). UI=a+2b+3c

STATISTICAL ANALYSIS

Results are presented as mean ± SEM and the statistical significance between groups was considered by analysis of variance (ANOVA) with Newman-Keul's post test. $P < 0.05$ was considered significant (Dehpour *et al.,* 1998; Dehpour *et al.,* 1999; Heidari *et al.,* 2007).

RESULTS

The ulcer index of *A. occidentale* extract and indomethacin(200, 300, 400, 800 mg/kg) were compared. Treatment of the animals with the analgesic dose of 200 mg/kg of the extract did not induce any ulcer in rat´s stomachs, but indomethacin with the same dose, caused damage in the gastric mucosa. The higher doses of 300, 400 and 800 mg/kg of extract induced gastric ulcer, but the ulcer index of the percolated extracts at different doses were less than the similar doses of indomethacine significantly ($P < 0.01$) (fig).

DISCUSSION

Non-steroid anti-inflammatory drugs (NSAIDs) are still a large amount of prescribed drugs all around the world. They specially used for the treatment of long lasting inflammatory conditions like Reumathoid Arthritis. Due to unwanted effects of these drugs (Ford-Hutchinson *et al.,* 1981; Ivey, 1986; Murray *and Brater*, 1993), it is required to search for new enterprise in the management of pain and inflammation.

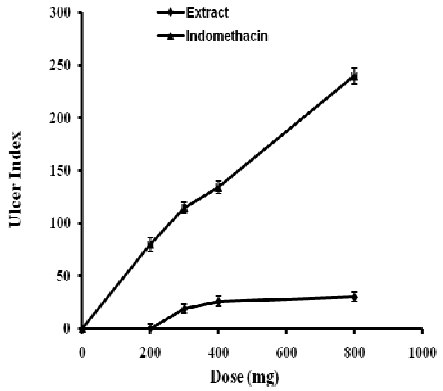

Fig. Comparison of The ulcer index of different doses of *A. occidentale* extract and indomethacin in rats. Different doses of extract or indomethacine were administered orally in rats. Each point indicates the mean ± SEM of J Score in seven animals. $P<0.01$ significant difference from control group in all points.

Photo shows the gastric ulcer.

In Iranian folk medicine, the fruit of *Anacardium occidentale* (cashew nut) were used as an analgesic (Heidari *et al.,* 2000). Considering that it could inhibit the pain in the second stage of formalin test completely, it seems that it acts like some drugs such as aspirin (Ahmadiyani *et al.,* 1997; Heidari *et al.,* 1996; Shibata *et al.,* 1989); therefore it could have ulcerogenic effect on stomach. Gastric ulcers and dyspepsia are important adverse effects of persistently used of medicines like non-steroidal anti-inflammatory drugs (NSAIDs) (Yang *et al.*, 2006, Ivey 1986, Heidari *et al.,* 2007).

This study was carried out to examine the ulcerogenic effect of percolated extract of *A. occidentale* on rats' stomach in comparison to indomethacin.

Previous research on this plant has shown (Heidari *et al.,* 2000) that the best antinociceptive effect was observed with the dose of 200 mg/kg. Therefore we compared 200 mg/kg of extract with the same dose of indomethacin. Treatment of animals with extract (200 mg/kg) did not show any ulcer in rat´s stomachs, but indomethacin at the same dose caused damage in the gastric mucosa ($p<0.01$). For further evaluation we increased the dosages and three doses of 300, 400 and 800 mg/kg were used. The ulcer index of the percolated extract at different doses was lower than the same dose of indomethacine significantly ($P < 0.01$). Our results showed that ulcerogenic side effect of the percolated extract of *A. occidentale* is significantly lower than indomethacin.

In a recent study, Anacardic acid showed a dose-dependent protection against the gastric damage induced by ethanol in mice. It also prevented the alterations in the amounts of SOD, GSH, MDA, catalase, and nitrate/nitrite at the doses of 10, 30 and 100 mg/kg. These results suggest that the mechanism of protection might be through an antioxidant pathway (Morais *et al.,* 2010). Hydroethanolic extract of the leaves of *Anacardium occidentale* L. can also inhibit HCl and ethanol induced peptic ulcers. Cashew leaves contain queretin glycosides and saponin. Quesretin is able to increase glycoproteins in the gastric mocusa (Konan *et al.,* 2007a) and prevents peptic ulcer induced by HCL.

In our previous study, therapeutic analgesic dose of this extract, did not induce any hepatotoxicity or renal toxicity effects and confirmed the safety of this plant (Heidari *et al.,* 2010).

Putting together all the results, *A. occidentale* could be a choice for more investigation. Though; the chemical ingredients responsible for the pharmacological activities of this plant remain to be investigated and it could be a suitable candidate for continuing toxicology and clinical researches.

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