Global Veterinaria 14 (6): 848-852, 2015
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2015.14.06.9428

# Effect of Methanol Extract of *Tetracarpidium conophorum* Seed on Indomethacin-Induced Ulcer in Rats

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**Abstract:** This work assessed the antiulcer activity of *Tetracarpidum conophorum* (African walnut) seed against indomethacin induced ulcer in rats. The seeds of the plant were dried, ground and extracted with methanol. To evaluate the antiulcer activity of the extract, five groups of four rats each were used. Group one (control group), two, three, four and five (reference group) were administered with 2 ml/kg b.w of vehicle, 100 mg/kg b.w, 200 mg/kg b.w, 400 mg/kg b.w of extract and 50 mg/kg b.w of ranitidine - a standard ulcer drug respectively. The mean ulcer score for the control, 100, 200, 400 mg/kg b.w of extract and 50 mg/kg bw of ranitidine after administration of indomethacin (40 mg/kg b.w) were 3.3, 0.35, 0.425, 0.45 and 0.2 respectively. The percentage ulcer inhibition of the 100, 200, 400 mg/kg bw of extract and 50 mg/kg b.w of ranitidine were 89.40%, 87.12%, 86.36% and 93.93% respectively. The result of this study showed that methanol extract of *Tetracarpidium conophorum* (African walnut) possesses good antiulcer activity.

**Key words:** *Tetracarpidum conophorum* · Ulcer · Ranitidine And Rats

## INTRODUCTION

*Pluckennetia conophora* Mull, formerly known as *Tetracarpidium conophorum* is from the family *Euphorbiaceae* and commonly called the African walnut [1]. African walnut is a perennial climber found in the moist forest zones of sub-Sahara Africa [2]. It is cultivated principally for the nuts that are cooked and consumed as snacks, along with boiled corn [3, 4]. Apart from consuming as snacks, some studies on the plant have revealed that there is good nutritive value in the nuts [5- 8]. The nuts have been shown to cure male infertility problems and the leaves are used for the treatment of dysentery [9]. The husk has anti microbial activity [10] and it is also useful for its anti proliferative activity [11].

Compared to certain other nuts, such as almonds, peanuts and hazelnuts, walnuts (especially in their raw form) have a relatively higher total level of antioxidants, including both free antioxidants and antioxidants bound to fibre [12].

Walnuts are one of the best plant sources of protein. They are rich in vitamins, magnesium and antioxidants such as Vitamin E [13, 14].

Entry of dietary protein into the stomach stimulates the gastric mucosa to secrete the hormone gastrin, which in turn stimulates the production of hydrochloric acid by the parietal cells and pepsinogen by the chief cells of the gastric gland [15]. The acidic gastric juice (pH 1- 2.5) is both antiseptic, killing most bacteria and foreign cells and a denaturing agent, unfolding globular proteins and rendering their internal peptide bond more accessible to enzymatic hydrolysis. Pepsinogen, an inactive precursor, or zygmogen, is converted to active pepsin by enzymatic action of pepsin itself.

The major causative factor (60% of gastric and up to 90% of duodenal ulcers) is chronic inflammation due to *Helicobacter pylori* that colonizes the antral mucosa. Although some studies have found correlations between smoking and ulcer formation [16], others have been more specific in exploring the risks involved and have found that smoking by itself may not be much of a risk factor unless associated with *H. pylori* infection [17]. Another major cause is the use of non-steroidal anti-inflammatory drugs (NSAIDs). The gastric mucosa protects itself from gastric acid with a layer of mucus, the secretion of which is stimulated by certain prostaglandins such as leukotrienes [15, 18]. Non-steroidal anti-inflammatory

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drugs block the function of cyclooxygenase 1 (COX-1), which is essential for the production of these prostaglandins. Cyclooxygenase 2 (COX-2) selective anti-inflammatory agents such as celecoxib or the since withdrawn rofecoxib, preferentially inhibit COX-2, which is less essential in the gastric mucosa and roughly halve the risk of NSAIDs-related gastric ulceration. Researchers also continue to look at stress as a possible cause, or at least complication, in the development of ulcers [19].

Hence the need to assess the possible antiulcer activity of methanol extract of walnut (*Tetracarpidium conophorum*) seed in order to alleviate these problems associated with peptic ulcer.

## MATERIALS AND METHODS

**Animals:** Twenty male rats were obtained from the department of Veterinary medicine in the University of Nigeria Nsukka. They were housed in cages covered with wire gauze from within. The animals were placed on standard pellet feed (Niger feed, Nigeria) and were given free access to water. They were kept in a well ventilated room with a 12hr/12hr light/dark condition and temperature ranging between 23-27°C. Animals were handled according o the Helsinki declaration in relation to ethics (1964).

The rats were acclimatized for 2weeks. Then 5 groups of 4 rats each were created with each group kept separately in cages labeled I-V.

**Ulcer Model (Indomethacin Model):** The vehicle for administration of the plant extract was 3% tween 80 in normal saline. Group 1 received the vehicle only. Groups two to four received 100, 200 and 400 mg/kg b.w of the extract, while group five was given 50 mg/kg b.w of Ranitidine (standard ulcer drug). Thirty minutes after drug and extract administration, the animals were treated with indomethacin (40 mg/kg b.w) and then allowed eight (8) hours. The animals were sacrificed using chloroform anesthesia, the stomach removed along the greater curvature and cleaned. The erosions formed on the glandular portion of the stomach were counted and ulcer index calculated. The ulcer was counted and scored as 0 = no ulcer, 1 = faint spots, 2 = lesions and 3 = deep spots. The values gotten were used to calculate the mean ulcer index and the % ulcer inhibition was calculated relative to control as follows

Percentage inhibition (% UI) = $(1-^{Ut}/Uc)$ where
Ut = ulcer index of treated group.
Uc = ulcer index of control.

## RESULTS AND DISCUSSION

Walnut (*Tetracapidum conophorum*) as a plant has been researched on by a good number of individuals and it is very obvious that the plant is a multipurpose one since every part of the plant serves an advantageous purpose.

Brown dyes are extracted from the husks [11]. The leaf extracts is use to reduce hiccups [16], has anti microbial activity [4] and so is used for the treatment of dysentery. The seeds have been used to improve male fertility [4]. The oil from the seed has found use in the production of wood vanishes, vulcanized oil for leather and rubber substitute. Proximate analysis carried on this plant showed that *Tracapidum conophorum* has; moisture content (41.5%), crude fat (4.28%), crude protein (2165%), crude fiber (7.34%), ash content (5.27%), carbohydrate (19.96%) [6]. The nut is highly nutritious and contains important components of food in relatively sufficient amounts. The fiber contained in the seed bulks stool, improve bowel movement and prevent constipation. Mineral analysis results show that *Tetracapidum conophorum* has potassium (6250 mg/kg), sodium (4830 mg/kg), magnesium (1711.25mg/kg), calcium (4337.5mg/kg), iron (110.00mg/kg), zinc (40mg/kg), manganese (22.00mg/kg), copper (16.45mg/kg) [6]. The phytochemical analysis of *Tetracapidum conophorum* shows that it contains; alkaloids (2.380mg/kg), tannins (0.080 mg/kg) [6]. Their alkaloid content is responsible for the bitter taste when consumed before drinking water. They are also used for many therapeutic purposes. Saponins and tannins they contain act as anti inflammatory agents [3]. They also contain oxalates, phytates and tannins [8].

The results from these research show that there is significant difference between the ulcer scores from the control group and from the groups that were administered with varying concentrations (100mg/kg, 200mg/kg, 400 mg/kg) of the extracts($P \leq 0.001$), with the later groups having lower ulcer scores, signifying that methanol extract of walnut seed has anti ulcer activity.

The groups administered with the walnut seed extract significantly ($P \leq 0.001$) reduced ulcerations when compared with the control group.

Table 1: Effect of methanol extract of walnut seed on indomethacin induced ulcer

| Treatment | Dose (mg/kg) | Quantal ulcer incidence | Mean ulcer index | (%) ulcer inhibitions |
|---|---|---|---|---|
| Vehicle | 2ml/kg | 4/4 | 3.30 ± 0.54 | |
| Extract | 100 | 4/4 | 0.35 ± 0.13 *** | 89.39 |
| Extract | 200 | 4/4 | 0.43 ± 0.19 *** | 87.12 |
| Extract | 400 | 4/4 | 0.45 ± 0.19 *** | 86.36 |
| Ranitidine | 100 | ¾ | 0.20 ± 0.13 *** | 93.93 |

***= $P \leq 0.001$, ***= $P \leq 0.001$, ***= $P \leq 0.001$, ***= $P \leq 0.001$

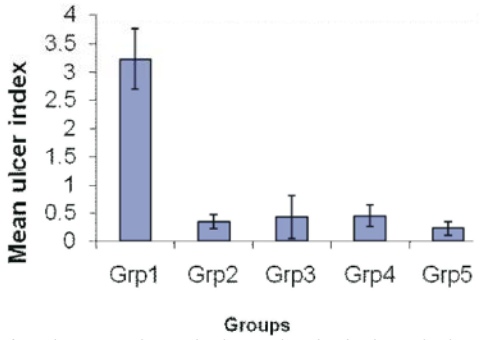


Fig. 1: Effect of methanol extract of walnut seeds on indomethacin-induced ulcer in rats

**Mean Plots**

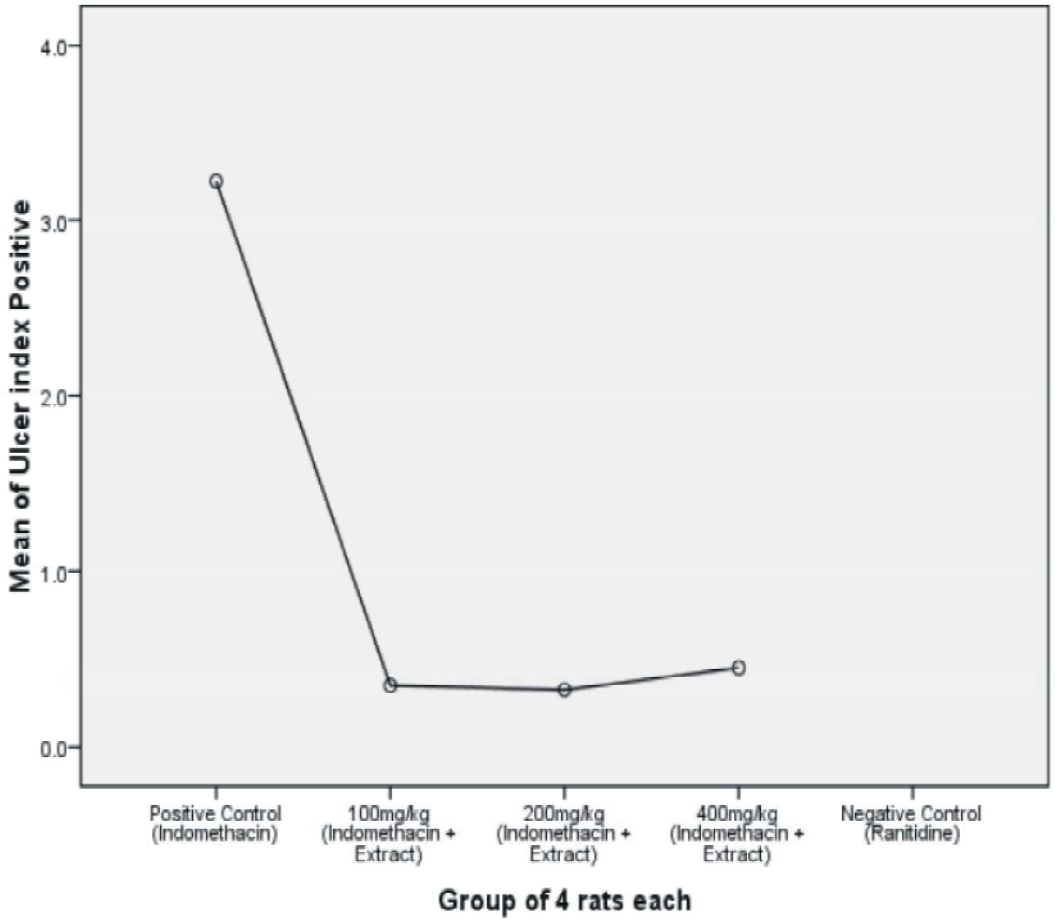


Fig. 2: Graph that Compares the Control with the Groups

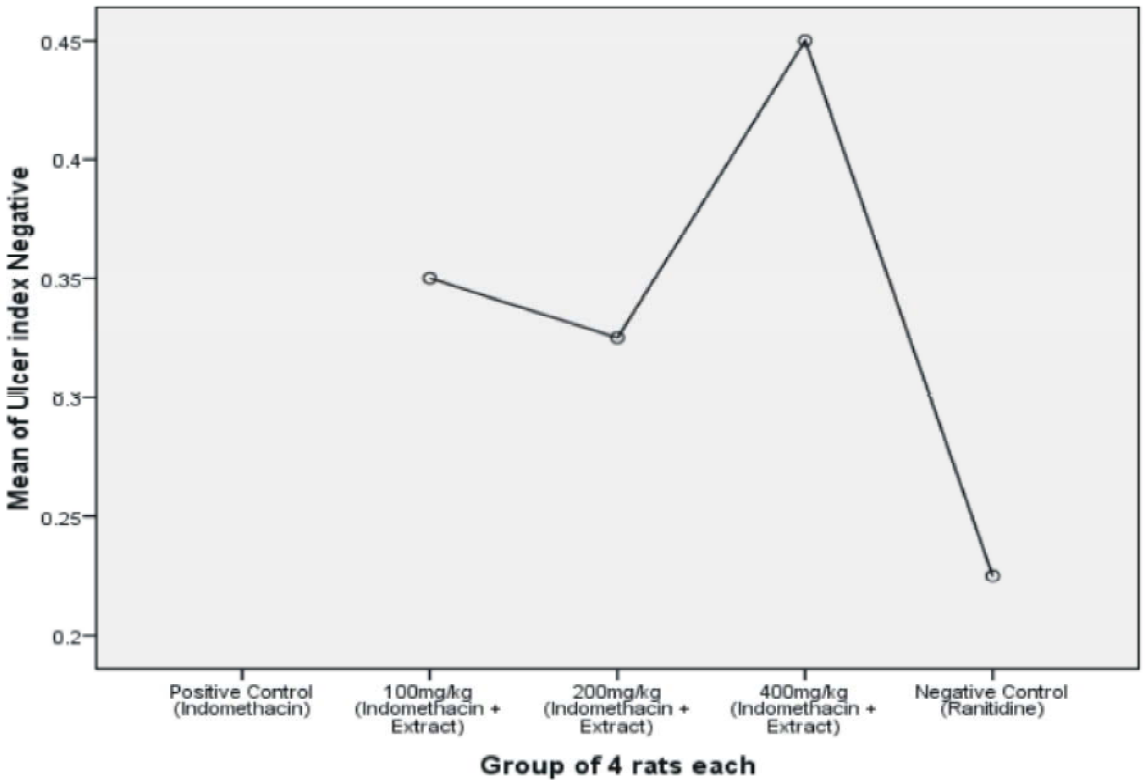


Fig. 3: Graph that Compares the Reference (Ranitidine) with the Groups

The significantly reduced ulcerations effected by the extract was not dose dependent and was comparable to that obtained with ranitidine, the standard antiulcer drug which showed maximum ulcer inhibition against indomethacin induced ulcer.

Ranitidine (ranitidine hydrochloride) is a non imidazole, small molecule that is a blocker of the histamine receptor ($H_2$) which is used to treat gastric ulcer and dyspepsia [9].

Ranitidine is similar to cimetidine and famotidine that are $H_2$ blockers, it blocks the histamine (released by ECL cells) from binding on the parietal $H_2$ receptors. This binding stimulates acid secretion. With the use of ranitidine, other substances that stimulate acid secretion (gastrin and acetylcholine) have a reduced effect on parietal cells. It has been noticed that the removal of the furan ring reduces the $H_2$ receptor blocking activity of ranitidine [12]. Biopsy report of rat stomach treated with ranitidine showed good healing of ulcers [19].

The extracts of *Tetracapidum conophorum* that has activity comparable to that of ranitidine could be due to the presence of a similar or even the same active ingredient.

## CONCLUSION

From the result of the research, it can be concluded that *Tetracapidum conophorum* has significant anti ulcer activity. It is therefore recommended in diet as it is a natural source of food and drug.

## REFERENCES

1. Oke, O.L. and M.A. Fafunso, 1995. Lesser known Oil Seeds *in vitro*. Nutrition report International, 12: 41-49.
2. Adebona, M.B., A.O. Ogunsua and M.O. Ologunde, 1988. Development of Conophor nut-based cereal snack food 1-Biscuits. J. Food and Agric., 2: 123-136.
3. Edem, C.A.A., M.I. Dosunmu and F.I. Bassey, 2009. *Determination of proximate Composition, ascorbic acid and heavy metal content of African walnut (Tetracarpidium conophorum*). Pakistan Journal of Nutrition, 8(3): 225-226.
4. Ajaiyeoba, E.O. and D.A. Fadare, 2006. Antimicrobial potential of extracts and fractions of the African walnut (Tetracarpidium conphorum). African Journal of Biotechnology, 22: 2322-2325.

5. Akpuaka, M.U. and E. Nwankwo, 2000. Extraction analysis and utilization of a drying oil from Tetracarpidium conophorum. Biores. Technol., 73: 195-196.
6. Ayoola, P.B., O.O. Onawuni and O.O. Taboya, 2011. Chemical evaluation of nutritive values of Telra carpidim comophiru. Nigerian Walnut Seeds, 11 (15).
7. Bardeley, W.B., L.M. Diane and B. Jetteny, 2010. The Phytochemical Composition and Antioxidant Actions of Tree Nuts.
8. Enujugha, V.N. and O.O. Ayodele, 2003. Evaluation of Nutrient and anti nutrients in lesser known under-utilized oil seeds. Int. J. Food Sci. and Technol., 38: 525-528.
9. Gadekar, R., P.K. Chaurasiya and R.S. Pawar, 2010. A potential of some medical plants as antiulcer. Pharmacognocy Review, 4(8): 136-146.
10. Nael, A.T. and A.A. Mohammed, 2011. Utility and importance of walnut Juglans regia. African Journal of Microbiology, 32: 5796-5805.
11. Hogue, P., 2000. "Real black walnut stain". Primitive Acher Magazine.
12. Institute of pharmacogcosy and pharmacology, 1988. Histamin H2-receptor blocking activity of ranitidine and Iamtidine analogues containing amino metlyl-substituted aliphatic systems. Pub Med. Guv., 25: 3-4.
13. Kim, Y.H and J.H. Lee, 2002."long-term stress and H. Pylori intection independently induce gastric mucosal lesions in C 5 7B4 6 mices. J. Gastro Enterol., 37(11): 1259-64.
14. Negi, A.S., S. Suaib, Krishna, Vinay, Clupta and P. Mahedra, 2011. "Anti proliterative and antioxidant activities, of Julgans rejia fruit extract. Pharmacological Biology, 49(6): 669-73.
15. Nelson, D.L. and M.M. Cox, 2005. Amino acid oxidation and production of urea. In: Lehninger Principles of Biochemistry 5th edition. W.H. Free Man and Company, New York, pp: 658.
16. Oyenoga, V.A., 1997. Nigerian food and teach shiff Ibadan. University Press, Ibadan.
17. Paul, F., 2011. Walnuts are excellent source of Omega - 3. Alignlife, 877: 688-7241.
18. Salih, B., A. Faith, M.B. Niza and S. Eisa, 2007. "Helicobacter pylori" Intection andother risk factors associated with peptic ulcers in Turkish pationts: A retrospective, study. World Journal of Gastro Enterology, 13(23): 3245-3248.
19. Somasundaram, R.C. and D. Dasaratha, 2010. Evaluation of gastric and duodenal antiulcer activity of ranitidine for mulation in experimental animals. Trad Science, 4: 3.