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Gastroprotective Effect of *Conocarpus Erectus* Plus Omega-3 on Experimentally Induced Ulcer in Rats

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ABSTRACT

There has been a dearth of research on the gastroprotective effect of *Conocarpus erectus* in the literature so the current study was designed to estimate the ability of *Conocarpus erectus* (*C. erectus*) leaves extract alone and in combination with omega-3 regarding gastroprotective effects. A total of 30 male rats were divided into five groups (n = 6). All animals induced gastric ulcer by 80 mg/kg of naproxen orally twice a day for three consecutive days. At the same time, the animals treated orally with 175 mg/kg omega-3, 250 mg/kg *C. erectus*, 80 mg omega-3 + 150 mg *C. erectus*, 10 mg/kg of lansoprazole, and 2 ml/kg of DMSO were named T1, T2, T3, T4, and TC, respectively. The obtained results of the present study indicated the presence of flavonoids, saponin, and tannin as active ingredients in *C. erectus* leaves extract. Consequently, *C. erectus* seemed to have the potential of chelating metals in a concentration-dependent manner. Gross and histopathology findings showed the highly protective capability of *C. erectus* and omega-3 against ulcerative lesion, compared to the time each was used alone. The outcomes of the current study indicated that using *C. erectus* alone or plus omega-3 can protect the gastric mucosa from the ulceration induced by naproxen, and the chelating properties of *C. erectus*.

Keywords: *Conocarpus erictus*, Naproxen, Omega-3, Rat, Ulcer

INTRODUCTION

Gastric ulcer (GU) is one of the most popular gastrointestinal diseases affecting individuals of all ages. The simplest definition is small sores that appear on the lining mucosa of the stomach mainly caused by *Hylicobacter pylori* and prolonged swallowing of non-steroidal anti-inflammatory drugs (NSAIDs). Approximately, 4.1-10% of people are exposed to peptic ulcer disease during their lifetime (Ko et al., 2016). The broad-spectrum indications of NSAIDs contribute to extensively increased incidence of GU as used for relieving pain, reducing fever (analgesic properties), and alleviating anti-platelet aggregation (Wongrakpanich et al., 2018).

Indeed, the ulcers are affected when NSAIDs strongly inhibited COX1 that is responsible for the biosynthesis of prostaglandin leading to the inhibition of major protective defense of the stomach (Xiao et al., 2017). Clearly, NSAIDs extended to suppress the COX2 enzyme (Kuna et al., 2019). *Conocarpus erectus* (*C. erectus*) widely spread tree belongs to the family Combretaceae and it abundantly exists in tropical regions all around the world. Furthermore, many studies have shown that all parts of the plant are beneficial due to antioxidant, anticancer, and antimicrobial properties (Al-ameedi and Nahi, 2019). Docosahexaenoic acid (DHA) is an omega-3 long-chain polyunsaturated fatty acid found in fish oil with anti-inflammatory, neuroprotective, and cardioprotective effects (Dagai et al., 2009). Overall, Bradbury (2011) reported that the use of DHA as pure oil could remarkably reduce inflammation and oxidative stress, so it plays an important role in the inhibition of the pathogenesis in gastric ulceration.

In this regard, the current study aimed to assess the gastro-protective effects of *Conocarpus erectus* (*C. erectus*) leaves extract alone and in combination with omega-3 on an induced ulcer in rats.

MATERIALS AND METHODS

Ethical approval

The study was carried out after approval of the Scientific Committee in the Department of Physiology, Biochemistry, and Pharmacology, College of Veterinary Medicine, Al-Qasim Green University in accordance with ethical standards of animal welfare.

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Chemicals

Omega-3, naproxen, and lansoprazole were purchased from Bioactive® pharmaceutical company, UK. Naproxen dissolved in distilled water and orally administered at once at 80 mg/kg twice a day for 3 consecutive days (Naproxen was given to animals at fasting state or randomly administrated through a day).

Plant preparation

Conocarpus erectus

Fresh leaves of *C. erectus* were collected from an agricultural field in the Al-Najaf province, Iraq, during September 2019. After washing and shadow drying the leaves, they were crushed carefully by a blinder.

Animals

A total of 30 male rats (200-250 g, 8 weeks old) were obtained from the animal lab of College of Veterinary Medicine Al-Qasim Green University, Iraq. Animals fasted for 15 hours before the study. Following the first dose of naproxen, food and water were provided *ad libitum* up to the end of the experiment. Al-Qasim Green University committee of animal care performed the animal experiment.

Plant extraction

Hydro-alcoholic extraction of leaves of *C.erectus* powder was carried out according to Harborne (1984) and Al-ameedi and Nahi (2019). As can be seen in Table 1, the phytochemical ingredients were identified using a chemical reagent according to Adegoke et al. (2010).

Table 1. Phytochemical screening of *Conocarpus erectus* profile (identified chemically)

	Ingredients extract	Principle and reagent	Positivity result	Reference
1	Flavonoids (0.2g)	Add to 2% sodium hydroxide solution	Intense yellow color	Adegoke et al. (2010)
2	Alkaloids (0.5 g)	Dissolved in 5 ml of 1% dilute hydrochloric acid	Yellow-colored precipitate	Adegoke et al. (2010)
3	Terpenoids (0.1g)	0.5 ml of chloroform followed by 1 ml of sulphuric acid	Reddish-brown precipitate	Evans et al. (2002)
4	Tannins (0.2g)	Dissolve in D.W, three drops of ferric chloride	Brownish blue or dark color	Adegoke et al. (2010)
5	Steroids (0.5g)	2 ml of chloroform + 2 ml of concentrated sulfuric acid	Red color	Adegoke et al. (2010)
6	Saponnins (0.2g)	6 ml of DW and shaken by fortex	Foam formation	Evans et al. (2002)

DW: Distilled water

Metal chelating capacity of *Conocarpus Erectus*

Chelation is the bonding of molecules to metal ions. Chelating agents are organic or inorganic compounds that can bind to toxic metal ions to form complex structures for easily excreting toxicity from the body intracellularly or extracellularly (Swaran and Pachauri, 2010). The chelation of ferrous ion by extract was estimated according to Dinis et al. (1994). Briefly, the mixture of 0.1 mM FeSO4 with 0.25 mM of ferrozine led to the formation of a Fe2þ-ferrozine complex, then his produced complex was added into 1 mL of the extract. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine complex solution. The mixture was shaken and left to stand at 37°C for 10 minutes. EDTA was considered as a positive control.

Metal chelating activity = {(**A** control – **A** sample)/**A** control ×100}

Where, $\mathbf{A}_{\mathbf{control}}$ is the absorbance without plant extract and $\mathbf{A}_{\mathbf{sample}}$ signifies the absorbance in the presence of a plant extract.

Experimental design

After inducing ulcer, the animals were divided into five groups (n= 6 rats per group). The experimental groups of T1, T2, T3, and T4 received 175mg/kg.bw omega-3, 300 mg/kb.bw *C. erectus*, omega-3 + *C. erectus* (1:1), and 10 mg/kg.bw lansoprazole, respectively. The control group was named TC which received Dimethyl sulfoxide (DMSO).

Histopathology

The rats fasted for 22 hours. At the end of treatments, the animals were euthanized in a diethyl ether euthanasia chamber. The current protocol was accepted by the Ethics Committee of the College of Veterinary Medicine, Al-Qasim Green University. A longitudinal incision of **s**tomach tissue was done and a 5g sample was fixed in 10% neutral

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formalin, then send to the histology lab to make the slides according to standard procedures as described by Luna (1968).

RESULTS

Phytochemical analysis

The hydro-alcoholic extract of *C. erectus* showed the presence of flavonoids, tannins, and saponins and the absence of alkaloids, steroids, and terpenoids (Table 2)

Table 2. Specific phytochemical compounds of *Conocarpus erectus*

Phytochemical composition	Hydro-alcoholic leave extract of *Conocarpus erectus*
Flavonoids	+
Alkaloids	-
Terpenoids	-
Tannins	+
Steroids	-
Saponins	+

Chelating capacity

The results of the study indicated that *C. erectus* extract contained a significant amount of Fe2+ (11 mg/ml). Moreover, the Fe2+ chelating potential *C. erectus* was concentration dependent manner (Diagram 1).

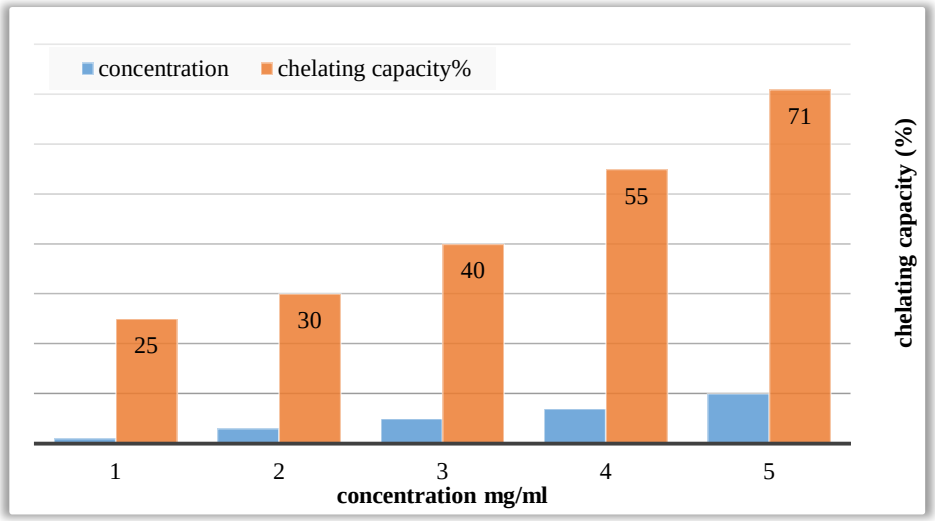

Diagram 1. Metal chelating capacity of *Conocarpus erectus* leaves

Grossly inspection

The gross lesion showed in Figure 1. There is an obvious gastric lesion in the mucosal layer represented by extensive mucosal hemorrhage and gastric hyperemia (Figure 1D), while the animals in the group pretreated with a combination of *C. erectus* + omega-3 indicated no ulcerative lesion leading to high protection against gastric ulcer (Figure 1B). The same protection against gastric lesions was observed in the group pretreated with lansoprazole (Figure 1E). Furthermore, moderate protection against gastric mucosal ulcer was determined in group T2 as pretreated with the *C. erectus* extract only (Figure 1C). Finally, the rat pretreated with omega-3 alone showed notable protection against gastric lesion induced by naproxen but less protection than compared groups T3 and T4 (Figure 1B, 2E).

Histopathological results

The presented gastric section showed complete loss of gastric mucosa leaving a few partial cells with an irregular appearance in the control group (DMSO, Figure 2A). Moreover, animals that received omega-3 at a dose of 175mg/kg revealed the irregular appearance of gastric pits with various types of mononuclear cells (MNCS) noticed with subnuclear tissue with mild degenerations (Figure 2B, C, D). On the other hand, there was focal ulceration of gastric mucosa associated with necrotic debris in the lumen with squamous epithelial covering non-glandular stomach portion in animals received 300mg/kg *C. erectus* as protective (Figure 2 C). Normal columnar mucosal epithelium covers the surrounding muscle with the prominence of parietal cells that appeared as fried eggs (*C. erectus* + omega-3).

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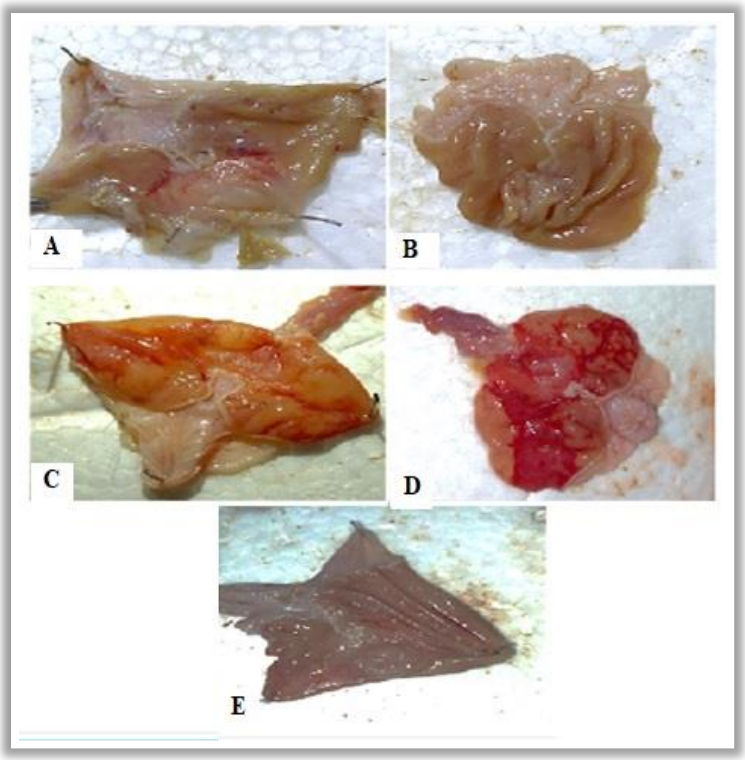

Figure 1. Gastric ulcer area induced by naproxen in rat. **A:** 175 mg/kg.bw omega-3**, B:** combination *C.erectus* (150 mg) + omega-3 (85 mg), **C:** *C.erectus* 300 mg/kg.bw, **D:** Control Dimethyl sulfoxide, **E:** 10 mg/kg.bw Lansoprazole

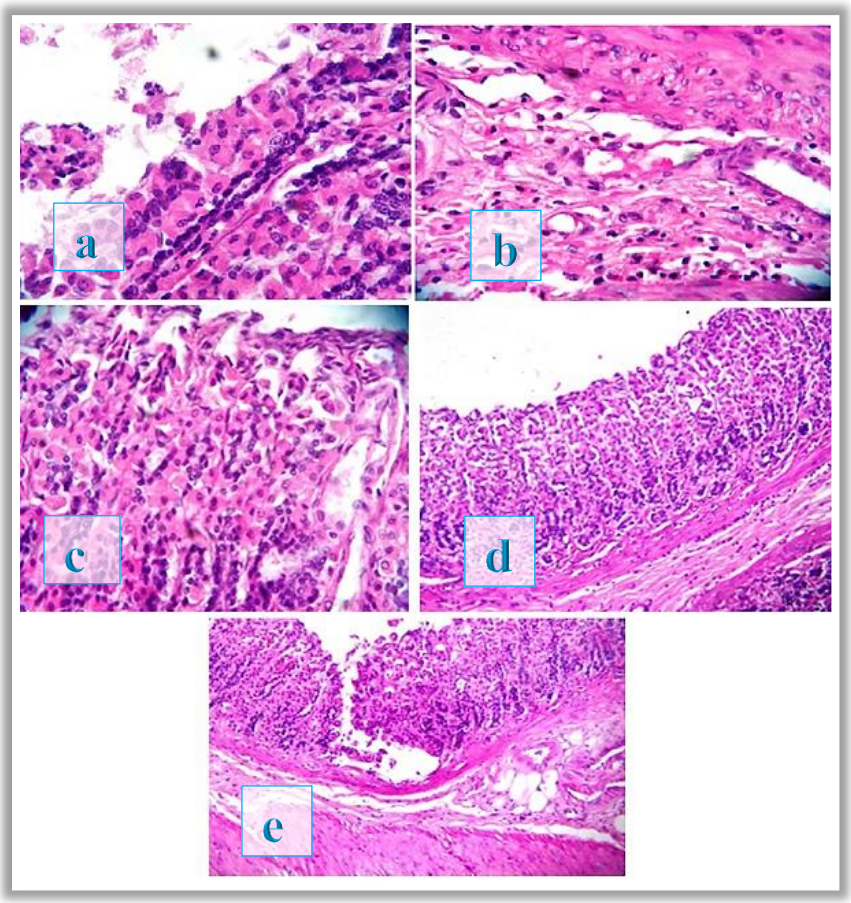

Figure 2. Histopathological changes of gastric tissue in rats with experimentally induced ulcer. **a:** Complete loss of gastric mucosa leaving a few partial cells with irregular finding (control group), **b:** Various types of mononuclear cells (MNCS) indicated subnuclear tissue (omega-3), **c:** Normal columnar mucosal epithelium covers the surrounding muscle with the prominence of parietal cells appeared as fried eggs (*C. erectus* +omega-3), **d:** Normal clear alteration of gastric mucosa and sub-mucosa with mild cellular infiltration (lansoprazole). **e:** Focal ulceration lesion observed with gastric mucus including a deeper sub-mucosal layer with the partial presence of parietal cells and peptic cells (*C. erectus*).

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DISCUSSION

The presence of many phytochemicals was detected in a hydro-alcoholic extract of *C. erectus* leaves as flavonoids, tannin, and saponin. The recorded components differed according to the location, weather, season, and water source (Nascimento et al., 2016). Studies on *C. erectus* leaves showed antimicrobial, antioxidant, anticancer, hepato-protective, and astringent properties of this plant (Shohayeb et al., 2013). All these pharmacological properties are directly related to phytochemical compositions, such as flavonoids, which have antioxidant, anti-inflammatory, and hepatoprotective effects (Lopes et al., 2000). Furthermore, saponin provides excellent anti-inflammatory action (Müller et al., 2006). The metal chelating capacity of *C.erectus* leaves extract revealed good chelating capabilities and concentration-dependent manner. The chelating potential of *C.erectus* created the impression that this activity was linked to the presence of flavonoids in the herbal plant, and consequently its antioxidant effect and scavenging of radicals, which was previously confirmed by (Wong et al., 2014). One of the main risk factors to inducing gastric ulcer are NSAIDs since they are known to cause erosion, antral ulceration, hemorrhage, and petechial bleeding on the gastric mucosa due to anti-inflammatory, antipyretic and pain-relieving properties (Mohod and Bodhankar, 2013). The mechanism of naproxen in induced ulcers can be summarized by non-selectivity inhibition of COX1 and COX2 leading to a decrease in prostaglandin synthesis and mucous secretion.

Fallone et al. (2016) affirmed that when they used a combination of drugs for gastric ulcer therapy like (Amoxicilline, clarithromycine), the healing and recovery results were more promising, compared to the time each was used alone. Many studies discussed and confirmed that flavonoids revealed gastric cytoprotective activities by regulating prostaglandins biosynthesis pathways (Zhang et al., 2020). Furthermore, current results were consistent with those reported by Sharifi-Rad et al. (2018) and Khan et al. (2018) indicating that tannins and saponin showed a gastroprotective effect in animal studies.

The number of trials addressing the combined drug-flavonoid-containing plant in peptic ulcer therapy is fairly limited. Moreover, a combination of medicines and flavonoids exhibited better efficacy in the treatment of peptic ulcers, establishing a unique peptic ulcer therapy method. Alpha-linolenic acid, eicosapentaenoic acid (EPA), docosapentaenoic acid, and docosahexaenoic acid are omega-3 fatty acids. The EPA and DHA are important precursors for lipid-derived modulators that are known to contribute to anti-inflammatory effects.

Omega-3 is the major substrate for eicosanoid synthesis (Ahmed et al., 2020). Ozgocmen et al. (2000) and Wall et al. (2010) briefly suggested protective mechanisms of omega-3 as blocking the metabolism pathway of arachidonic acids, suppressing the inflammatory eicosanoids, adhesion molecules, and cytokines production; and increasing the levels of catalase enzyme within the peroxisome and in the cytoplasm resulting in enhanced defense against reactive oxygen species. Omega-3 increases the production of glutathione in gastric mucosa when glutathione act as a co-factor in some steps of prostaglandin E2 (PGE2) synthesis, so it will be assessed to convert PGG2 to PGH2 followed by conversion to PGE2, prostaglandins (PGs), leading to the protection of gastric mucosa against different types of gastric lesions (Sakr, 2016). The findings of the current study were supported by Ahmed et al. (2020) and Sakr (2016) when reported the role of omega-3 in the reduction of hyperacidity in rats. Moreover, the results of gross lesions were further confirmed with histopathological findings although the improving and protecting effects of *C.erectus* and omega-3 should gain more attention. While the animals received omega-3 at a dose of 175 mg/kg, the irregular gastric pits appeared with various types of mononuclear cells (MNCS) with mild degenerations. On the other hand, focal ulceration of gastric mucosa was associated with necrotic debris in lumen observed in animals that received 300mg/kg of *C.erectus.* Finally, normal columnar mucosal epithelium covers appeared as fried eggs in animals receiving combination therapy (*C.erectus* 150 mg/kg + omega-3 80 mg/kg), which was strongly similar to that of the lansoprazole group. Throughout the current observation of the phytochemical detection of the *C. erectus*, the protective role of *C. erectus* leaves and the omega-3 combination can be well observed, which was more efficient compared to the time each was used alone. In addition, the obtained results showed the positivity effect of flavonoids, tannin, and saponins and their significant role in scavenging the ROS produced by naproxen. Many studies focused on the role of omega-3 and flavonoids as antioxidants (Sakr, 2016; Zhang et al., 2020). It became clear the role of *C. erectus* and omega-3 in scavenging ROS can be resulted from naproxen; *C. erectus* and omega-3 were also confirmed to increase the production of PGE2 as protective to gastric mucosa. It can also play an important role in reducing the aggressive effects of the acidity of the stomach and increasing the mucous property.

CONCLUSION

According to the outcomes of the study, it can be concluded that *C.erectus* extract could have gastroprotective activity when used either alone or in combination with omega-3 that arising synergistic role in protection against ulcers. The use of natural medication as herbal and or omega-3 can provide efficient therapeutic effects against gastric ulcers.

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DECLARATIONS

Authors' contribution

All authors contributed equally to this work.

Competing interests

The authors declare that they have no conflict of interest.

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, double publication and/or submission, and redundancy) have been checked by the authors.

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