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Effects of single and repeated doses of argan oil (*Argania Spinosa L*) on ethanol-induced gastric ulcers in rats

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ABSTRACT

The health advantages of argan oil (Argania spinosa L), which is derived from the kernels of Moroccan argan trees, include analgesic, anti-inflammatory, antihypertensive, antithrombotic, hypolipidemic, and antidiabetic effects. Male albino rats with ethanol-induced stomach ulcers were studied to determine the gastroprotective effects of oral argan oil doses, both once and repeatedly. In addition to lowering ulcer length, number, and total mucus production, the results demonstrated that argan oil dramatically decreased the number of ulcers and overall gastrointestinal acidity. For the first time, it has been found that argan oil protects rats' stomachs from developing ulcers caused by ethanol

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KEYWORDS: *Gastric ulcer, Argan oil (AO), Total gastric acidity, Gastroprotective.*

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INTRODUCTION

Peptic ulcer disease (PUD) is a serious ailment of the gastrointestinal tract (GIT) which includes both gastric and duodenal ulcer. It arises when there is an imbalance between the "aggressive" and "protective" factors at the luminal surface of the epithelial cells, and it affects a lot of people all over the world. Helicobacter pylori, hydrochloric acid (HCl), pepsins, bile acids, ischemia, hypoxia, smoking, and alcohol consumption are examples of aggressive factors. Conversely, growth factors, prostaglandins (PGs), mucus layer, mucosal blood flow, and bicarbonate are defensive factors (1, 2). PUD is pharmacologically treated with medications like antacids, acid suppressive drugs, cytoprotective agents, antimicrobials for the eradication of H. pylori (amoxicillin, clarithromycin); and triple therapy (a one-week triple therapy consisting of a proton pump inhibitor like omeprazole and the antibiotics clarithromycin and amoxicillin) (3).

Herbs, medicinal plants, spices, vegetables, and crude drug substances are valuable for controlling gastric ulcer and ulcerative colitis. Scientific literature investigates medicinal plants and their secondary metabolites for their gastroprotective activity using experimental models (4). Examples include turmeric (5), zataria multiflora (6), byrsonima crassa (7), fenugreek seeds (8), pomegrante peel extract (9), rhus tripartite (10),flaxseed oil (10, 11), trixis divaricate Spreng (12), clove (syzygium aromaticum L.) (13), the fruit of carica papaya L. (caricaceae) (14), Mentha arvensis L.(15), aloe vera L. (16), liquorice of glycerrhiza glabra L. (17), matricaria chamomilla (18), and nigella sativa (19). The anti-ulcerogenic and gastroprotective activity of these plants was attributed to the presence of tannins, flavonoids, terpenes, and to the ability to increase production of mucus and prostaglandin E2 (PGE2), to reduce acid secretion and also to their antioxidant free-radical scavenging activity (4). Argan oil, derived from the kernels of the Argan tree in Morocco, is a popular vegetable oil in the daily diet and folk medicine. Its biological properties include antidiabetic (21), antihypertensive, hypolipidemic, hypocholesterolemic, antithrombotic (22-25), analgesic, and anti-inflammatory activities (26).

MATERIALS AND METHODS

ANIMALS

The study involved 32 Albino Wistar rats, weighing 150-200 grams, housed in a 23°C, 12-hour light-dark cycle, with a balanced diet and 48-hour water access before experiments.

DRUGS AND CHEMICALS

Argan oil, ranitidine hydrochloride, Alcian blue, and absolute ethanol were obtained from Galiasrl, Sigma-Aldrich, Scharlab S.L, and other analytical grade chemicals from E. Merck and BDH Chemicals Ltd., Poole, England.

ETHANOL-INDUCED GASTRIC ULCERS

The experiment involved fasting rats for 48 hours (27), allowing them access to drinking water (28) before administering ethanol orally (1ml/200 g). After 60 minutes, they were killed with an ether overdose. The stomach was examined for ulcers. The ulcer index was calculated by dividing the total length of ulcers and petechial lesions in each group. Five petechial lesions were counted, and each one was interpreted as a 1 mm ulcer (29).

The curative ratio was determined according to the formula described by Khazaei et al . (30) as follows:

$$\text{Curative ratio} = \frac{(\text{Control ulcer index}) - (\text{test ulcer index}) \times 100}{(\text{Control ulcer index})}$$

DETERMINATION OF GASTRIC CONTENTS

The recovered solution was centrifuged at 3500 rpm for 10 minutes, then the supernatant was used for acid determination. with phenolphthalein as an indicator. One ml of the supernatant was completed to 20 ml with distilled water and titrated against 0.01N NaOH, using phenolphthalein as indicator. Acid conte[[nt was expressed as µEq per 100 g body weight (31).

ASSESSMENT OF GASTRIC MUCUS

The study used a modified procedure by Corne et al. (32) to determine gastric mucus content. The stomach's glandular portion was separated, stained with alcian blue, washed, and eluted. The eluate was extracted with diethyl ether, and the absorbance was measured using a spectrophotometer at 605 nm, and the alcian blue content was analyzed, expressed as µg alcian blue/g wet tissue.

EXPERIMENTAL PROTOCOL

Rats were divided into four groups of 8 rats each and treated as follows: Group 1: Control ulcer, given 1 ml/200g absolute ethanol. Group 2:Argan oil 1 ml/100g + 1 ml/200g absolute ethanol Group 3:Argan oil 1 ml/100g/ 7d + 1 ml/200g absolute ethanol. Group 4: Ranitidine 50 mg/kg + 1ml/200g absolute ethanol.

HISTOPATHOLOGICAL ANALYSIS

Rat stomachs were examined for ulcers, and specimens were fixed in 10% formalin, sliced, haematoxylin and eosin stained (34), and studied under light microscopy for histological changes. At least 3 slides were studied from each specimen in a blinded fashion for any histological changes.

STATISTICAL ANALYSIS

Data were analyzed using statistics software package (SPSS for windows V.22.0). Student's t test was used and results were presented as mean ± SEM and a P value less than 0.05 were considered statistically significant.

RESULTS

EFFECTS OF PRETREATMENT WITH SINGLE AND REPEATED DOSES OF ARGAN OIL ON ETHANOL-INDUCED GASTRIC LESIONS

Rats treated with absolute ethanol showed ulcerogenicity, with hemorrhagic lesions in the glandular part of the stomach (Figure 1A).

Pretreatment with single and repeated doses of AO and ranitidine significantly reduced the number of ulcers ($p<0.05$, $p<0.01$,$p<0.001$) respectively as compared to the ulcer control group (Figure 1, B,C, and D) and (Table 1). Both single and repeated doses of AO significantly reduced ($p<0.001$ and $p<0.01$) respectively the mean ulcer length,

with the single dose showing a more prominent reduction ($p<0.001$) (Table 1).The curative ratio of single and repeated doses of AO and ranitidine against ethanol-induced gastric ulcers was: found to be 46.48%, 31.75% and 54.77% respectively.

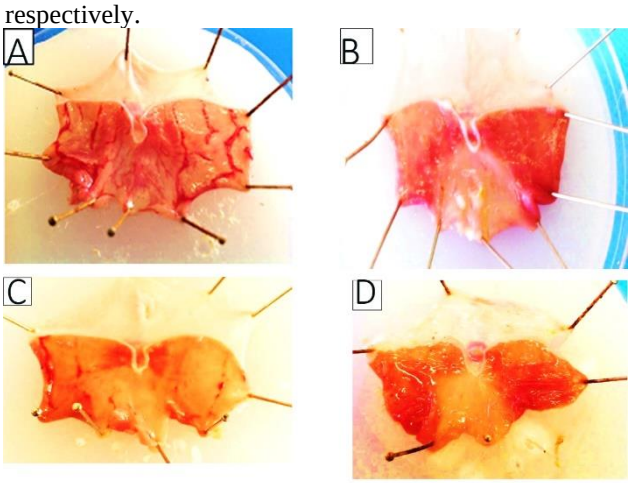

Figure 1. Shows representative stomachs from A: control ulcer (ethanol-treated) group; B: stomach from a rat pretreated with a single dose of AO (5ml/kg); C: stomach from a rat pretreated with repeated doses of AO (5ml/kg/7 days).

EFFECTS OF PRETREATMENT WITH SINGLE AND REPEATED DOSES OF ARGAN OIL ON TOTAL GASTRIC ACIDITY AND GASTRIC WALL MUCUS IN RATS TREATED WITH ABSOLUTE ETHANOL

Repeated but not the single dose of AO produced a significant reduction in total gastric acidity ($p<0.001$) compared to ulcer control group, ranitidine group and single dose AO administered group. Only the single dose of AO resulted in a significant reduction in total gastric acidity compared to control ulcer group. (Table 2).

Both single and repeated doses of AO resulted in a significant increase in mucus production ($p<0.05$) compared to the control ulcer group. (Table 2)

HISTOPATOLOGICAL RESULTS

The histopathological examination of rat stomachs showed ulcer-induced sections with mucosal discontinuity, blood vessel congestion, and edema (Figure 2) (A-1 and A-2). Argan oil treatment showed minimal distortion (C), mild inflammation, and normal epithelial layer definition. Repeated doses resulted in less protection against ulcer formation. Ranitidine treatment showed mild edema and hyperemia (E-1 and E-2).

Table 1. Effect of single and repeated doses of argan oil on the number and length of ethanol-induced gastric ulcers in rats

Group	Dose	Number of glandular ulcers	Cumulative length ulcers in (mm)
Control (Ethanol)	1ml/200g	6.63±0.75	7.96±0.59
AO (Single dose)	5ml/kg	3.86±0.74*,#	4.26±0.66***
AO (Repeated doses)	5ml/kg (7 days)	3.75±0.49**,##	5.43±0.92**
Ranitidine	50 mg/kg	1.88±0.35***	3.60±0.37***

Data are expressed as mean ±SEM (n=8) *, $p<0.05$; **,$p<0.01$; ***$p<0.001$ significantly less than control ulcer group. #,$p<0.05$; ##,$p<0.01$ significantly higher than ranitidine group.

Table 2. Effect of single and repeated doses of argan oil on total gastric acidity and gastric wall mucus production of ethanol-induced gastric ulcers

Group	Dose	Total gastric acidity (µEq/100 g BW)	Mucus (µg/g wet tissue)
Control (Ethanol)	1ml/200g	654.15±51.13	265±9.75
AO (Single dose)	5ml/1kg	680.56±25.38$^{###}$	306±6.30**
AO (Repeated doses)	5ml/kg (7 days)	302.14±10.71$^{***, ###, \$}$	335.88±18.36**
Ranitidine	50 mg/kg	495.75±24.75*	301.87±7.25**

Data are expressed as mean ±SEM (n=8) *, $p<0.05$; **,$p<0.01$; ***$p<0.001$ significantly different from control ulcer group. #,$p<0.05$, ##,$p<0.01$;###$p<0.001$ significantly different from ranitidine group

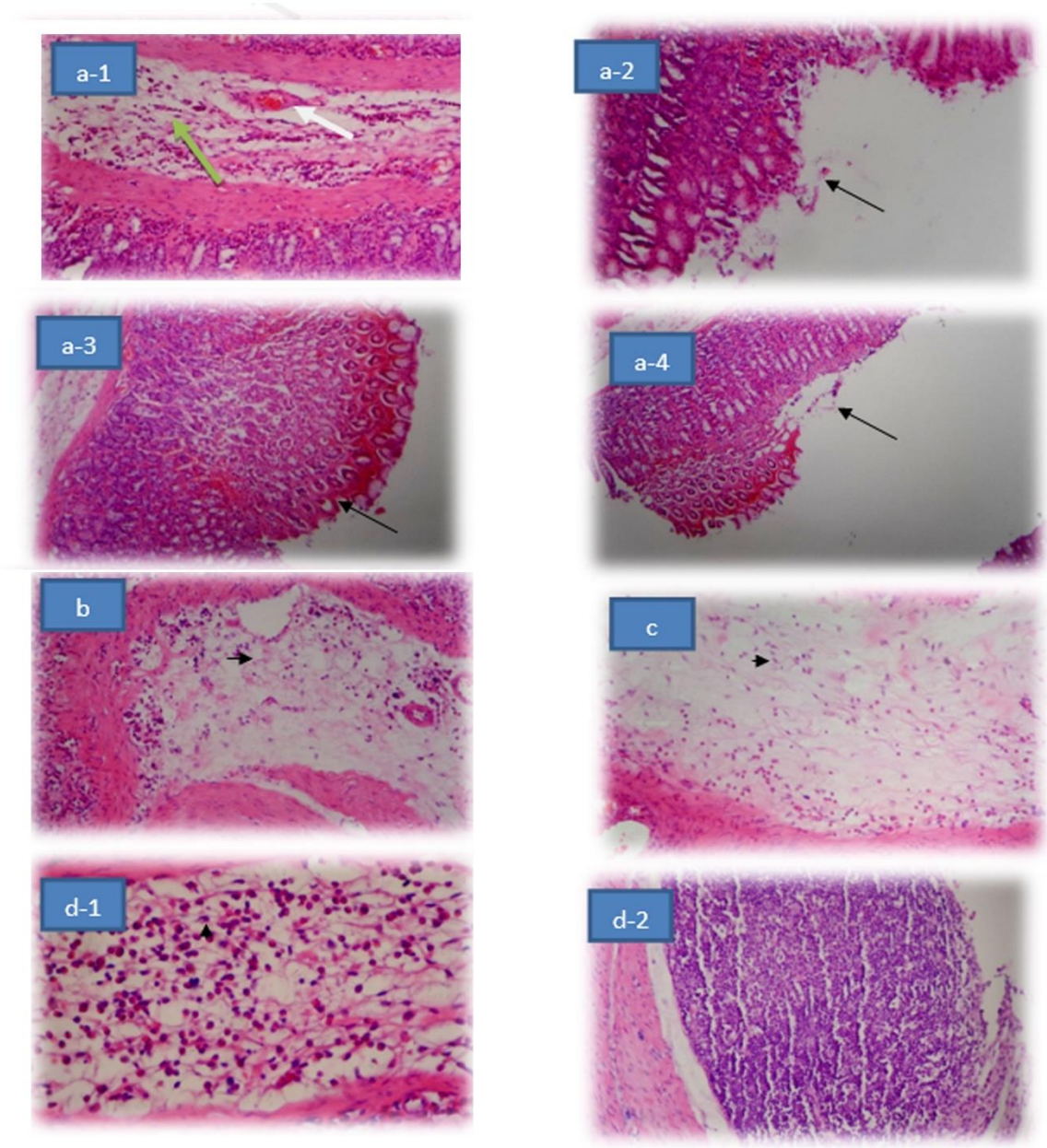

Figure 2. Light micrograph of rat stomach from:a1 and a2 (Control ulcer) showing erosion and disruption of epithelium (black arrow), congestion and dilation of blood vessels (white arrow) and submucosal edema (green arrow) acute inflammation mainly neutrophils (arrow head);b: Single dose AO showing epithelial replenishment (black arrow) with mild acute inflammation in submucosa; c:Repeated doses AO showing mild acute inflammations with few neutrophils. d1 and d2 ranitidine group showing massive infiltration of leukocytes with lymphoid aggregation. a1, a3,a4, b, c: H-E; x10, a2, d1, And d2: H-E; x40.

DISCUSSION AND CONCLUSION

Peptic ulcers, a common gastrointestinal disorder, is believed to be caused by an imbalance between aggressive and defensive elements, with ethanol potentially causing stomach damage through the production of oxygen-derived free radicals (35,36). Extremely reactive byproducts, free radicals cause oxidative damage by peroxiding lipids (37, 38).

The study found that a single dose of AO significantly reduced stomach mucosal lesions caused by 100% ethanol, but repeated treatment may increase acid production. The single dose's success may stem from enhanced mucus production and lower acid output.

The study explores the link between argan oil's main chemical components and its gastroprotective effects against ethanol-induced ulcers. Reviewing the existing literature revealed that AO primarily consists of mono-unsaturated fatty acids (up to 80%) and saturated fatty acids (up to 20%), along with minor components like polyphenols, tocopherols, sterols, squalene, and triterpene alcohols (39). Dahi et al. (40) discovered that vitamin E may offer potential therapeutic benefits for treating stress-induced gastric ulcers in rats, which were induced through the cold-restraint stress (CRS) method in fasted animals. The phenolic components in the hydroethanolic extracts of Acacia nilotica and Erythrina indica L. leaves have been found to have gastroprotective effects on various gastric ulcer models in rats (41, 42). Oliveira et al. (43) found that ethanol extracts from five plants showed anti-ulcer activity, possibly due to their antacid or cytoprotective properties, and their inhibitory effect may be due to tannins, terpenes, and fatty acids.

The study reveals that argania spinosa oil, a blend of monounsaturated and polyunsaturated fatty acids, has gastroprotective effects against ulcers caused by ethanol. Linoleic acid (C18:2n−6) is an essential fatty acid that helps produce arachidonic acid (C20:4n−6), which in turn is a precursor for prostaglandin E1 (PGE1) (44). Prostaglandin E1 analogues are known for their strong anti-ulcer properties.

In summary, this study provides a clear evidence of the gastroprotective effects of AO against ulcers caused by ethanol. It also supports the oil's traditional use in medicine for its anti-inflammatory properties, its ability to reduce skin irritation, and its role in promoting scar healing.

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