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Protection by Liquorice in Alcohol Induced Gastric Mucosa Damage

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Abstract: Gastric diseases are widespread among the inhabitants of many countries and alcohol consumption is a known precipitating factor. This study investigates the protective effect of Liquorice, an indigenous plant in tropical and sub-tropical areas and belongs to the Fabaceae family on 80% alcohol-induce gastric mucosa lesions and morphological changes in rats. The rats were divided into five groups of five rats per group. Gastric damage was induced with 80% alcohol. The treated group received the crude extract of 200 mg/kg.oral prior to alcohol gastric mucosa damage induction. Histological studies, ulcer index, Alkaline Phosphotase (ALP), lipid peroxidation product (TBARS) which is an index of lipid peroxidation were studied. Liquorice pre-treatment showed protection against alcohol mucosa damage; a significant reduction in the ulcer index of 1.94±0.05 against 5.24±0.07 of positive control. The ALP and TBARS were also significantly reduced. The results suggest that Liquorice seed extracts have significant mucosal protective and antioxidative effects on the gastric mucosa in rats.

Key words: Alcohol, gastric lesion, protection

INTRODUCTION

Gastric diseases are widespread among the inhabitants of many countries. Reports suggest that reactive oxygen free radical species (ROS) play an important role in the pathophysiological processes of acute gastric lesions (Parks, 1989; Vaananen *et al*., 1991). Increasing evidence indicates that chronic alcoholism is associated with increased oxidative stress and free radical-associated injury (Nanji *et al*., 1994; Manso, 1997). The break down of alcohol in the liver results in the formation of molecules whose further metabolism in the cell leads to reactive oxygen species (Toykuni, 1999). Alcohol stimulates the activity of enzymes called cytochrome P450s which contributes to free radical and also, alcohol reduces the levels of agents such as selenium, zinc and vitamin E that can eliminate free radicals.

Ulceration of the stomach is due to the imbalance between the mucosal defensive and offensive factors. The antiulcerogenic activity of many plant products is reported due to an increase in mucosal defensive factors rather than decrease in the offensive factors. (Goel *et al*., 1985). A number of antiulcer drugs like gastric antisecretory drugs-H_2 receptor antagonists, antimuscarinic agents, proton pump inhibitors, mucosal protective agents-carbenoxolone sodium, sucralfate and prostaglandin analogues are available which are shown to have side effects and limitations. (Barrowman and Pfeiffer, 1989). There are several herbal ayurvedic preparations which have a protective effect against drug-induced gastric mucosal injury. (Shetty *et al*., 2000).

Liquorice is a wild plant, grows best in fairly dry regions of low elevation. It grows in tropical and subtropical areas such as Nigeria, India, Sri-lanka, West indies, South China. In fact it is now naturalized in all tropical countries (Dwivedi, 2004). Liquorice belongs to the fabaceae family. Other common names include; jequirity, Crab's eye, Glycyrrhizin glabra. The seed contains abrine, abriline, glycyrrhizin, gallic-acid, protein, trigonelline, calcium, lypolytic enzymes, pectin, lectin and precatorine. Other active principles include; Glucides (3-14%) mainly composed of glucose, saccharose and starch (20-30%), coumestrans, proteins, fat (0.5-1%), resin (5%), asparagine (2-4%), sterols (β-sitosterol, stigmasterol, di-hydrostigmasterol), polysaccharides, licobenzofuran, gums and lignan (Rajaram and Janardhanam, 1992; Ivan, 2003).

Liquorice is very stable in the gastrointestinal tract, from where it is slowly absorbed. Its Leaves, roots and seeds are used for medicinal purposes. The medicinal use of liquorice dates back to 3000 years ago. It is mentioned in Assyrian tablets, Egyptian papyruses and Chinese herbaria. Hippocrates prescribed it to treat cough, asthma and other respiratory diseases. It is also used for the treatment of conjunctivitis, epilepsy and externally, it is applied to treat abscesses and stomatitis (Hhabra *et al*., 1990). It is also traditionally used against leucoderma, wounds, alopecia, asthma, tubercular glands, fever, ulcer and tumor (Khare, 2004; Vaidyarathnam and Varier 1995). The saponin components of liquorice root, such as liquiritoside, has shown *in-vitro* anti-inflammatory activity (Anam, 2001). The lectin component of Liquorice has also shown properties of bactericidal and non-specific immune response *in-vitro*.

This study is aimed at determining the antiulcerogenic and anti oxidative properties of seed extract of liquorice.

MATERIALS AND METHODS

Plant material: The plant material, Liquorice seeds were obtained from a local market in Lagos and were authenticated in the Department of Botany, University of Lagos.

The seeds were ground into powder and soxhlet extracted with distilled water in the Department of Pharmacognocy, University of Lagos. The yield was concentrated into a solid paste *in vacuo* at 50°C using a rotary evaporator. It was then stored at 0°C until ready for use. 200 mg/kg of the extract was administered to rats and this was chosen because higher and lower doses have been used by other researchers to achieve desired effects. (Rao, 1990; Sinha and Mathur, 1990).

Alcohol: 80% Ethanol (NAAFCO, London) was obtained from the Department of Biochemistry, University of Lagos, Nigeria.

Sources and maintenace of rats: Male Sprague-Dawley rats used in this study were obtained from the animal house, College of Medicine, University of Lagos. The total number of rats used were 25 ranging in age from 12-14 weeks and weighing between 216-234 g, kept in well ventilated metal cage at room temperature of 29-30°C in the Department of Anatomy, University of Lagos. The rats were divided into 5 groups of five rats per group. They were fed on rat pellet obtained from the animal house and water was made available *adlibitum*. The animals were kept for at least two weeks to acclimatize to the laboratory condition before experimentation.

The experimental protocol is as follows;

Group A: (Negative control): This group contained a total of 5 Sprague-Dawley rats and were administered pelleted feeds and water.

Group B: This group contained a total of 5 rats and were not pre-treated before induction of gastric ulceration.

Group C: This group contained a total of 5 rats and was pre-treated with Liquorice extract, a daily dose of 200 mg/kg/body weight for two weeks prior to induction of gastric lesions.

Group D: A total of 5 male Sprague-Dawley rats were included in this group and were pre-treated with vitamin E, 400 mg/kg/rat for two weeks before induction of gastric lesions.

Group E: This group contained 5 male rats and were pre-treated with same dose of vitamin E and Liquorice as above for two weeks.

Induction of gastric ulcers: One ml of 80% ethanol was used orally to induce gastric ulcer as described by Nadkarni (1976). 1 ml of 80% ethanol was administered orally. One hour after the ethanol administration, the animals were sacrificed.

Retrieval of tissue: At termination, rats were anaesthetized with ketamin 1 mg/kg [intramuscularly (i.m.)], the chest was opened and blood samples collected by heart puncture. Plasma was separated and stored at 0°C. Stomach pieces were collected in buffered formalin solution for histology and rapidly frozen for biochemical assays and malonildialdehide (MDA) estimation.

Analytical and pathological evaluation

Estimation of Alkaline Phosphatase (ALP): The tissue immersed in 4 ml of buffer solution was ground in a mortar and centrifuged for 10 min. 3 ml of the supernatant solution was pipetted out and the marker enzyme ALP was measured using the method of Kind and King (1954) and expressed as IU/L.

Determination of malonildialdehide (MDA): MDA level was determined in the supernatant of the gastric homogenates by the modified method of Buege and Aust (1978). Concentration was calculated using the molar absorptivity of malondialdehyde which is 1.56 × 100000 M. It is an index of the degree of oxidative damage in biological tissues.

The inhibition of the rate of peroxidation is calculated by using the formula: rate of inhibition (%) = (1- mean × value of treatment group/mean × value of control) × 100.

Tissue preparation: The tissues were fixed in 10% formalin for 48 h and then removed from the solution. It was then dehydrated through ascending grades of alcohol (70%, 80%, 90%, absolute). When dehydration was completed the tissues were cleared in xylene, infiltrated and embedded in paraffin wax for light microscopic studies, then sections of 5 micron thickness were cut on Reichert ultra microtome, mounted on slides and stained with Haematoxylin and Eosin (H and E) according to routine procedures for light microscopy. Tissue prepared was examined for qualitative differences in comparison with group B by an anatomical pathologist who does not know the nature of the experiment.

Determination of ulcer index: After sacrifice, the abdomen was incised and irrigated with normal saline. Subsequently, the stomach was incised along the greater curvature and washed gently in running tap water. It was placed on the watch glass and examined for severity of ulceration using the method described by Pihan *et al.* (1987) according to the following scale: 0 =

normal gray colored stomach, 0.5 = pink to red coloration of stomach, 1 = spot ulcer, 1.5 = hemorrhagic streak, 2 = number of ulcers <5, 3 = number of ulcers >5, 4 = ulcers with bleeding. Ulcer index was calculated by adding the total number of ulcers plus the severity of ulcer.

Statistical analysis: Data are reported as means±SEM and were analyzed statistically by one-way analysis of variance and the Student-Neumann- Keuls test, with the level of significance set at $p<0.05$.

RESULTS

Alkaline phosphatase: The activities of Alkaline Phosphatase (ALP) increased significantly in group B in comparison to the group A (negative control). Groups C, D and E showed a significant reduction in the ALP activities compared to group B (positive control) $p<0.05$. In group E animals, the activities of the ALP was maintained at near normal hence did not show any significant increase in comparison to the control group ($p>0.05$) (Fig. 1).

Malonildialdehide levels in control and treated rats: Malonildialdehide concentration, an index of lipid peroxidation was significantly increased in group B compared to the group A ($p<0.05$). Groups C, D and E showed a significant reduction in the MDA levels compared to group A ($p<0.05$) as shown in Table 1.

Ulcer index: Figure 2 shows the effect of Liquorice on ulcer index. It revealed a significant reduction in the ulcer index in all the groups except groups ($p<0.05$).

Results of histopathological study of control and treated rats: Table 2 shows the degree of the lesions of the gastric mucosa. Group B showed a total mucosa ulceration, very severe mucosa necrosis and haemorrhage as against the mild and near maintenance of normal architectures noticed in groups C, D, and E.

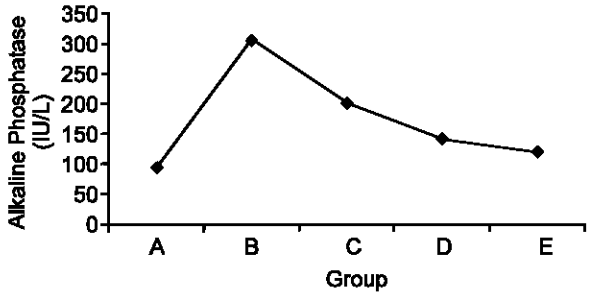

Fig. 1: Alkaline phosphatase in control and treated rats, All values are expressed as Mean±SEM (n = 5). Groups C, D and E showed a significant reduction in alkaline phosphatase level ($p<0.05$ significant)

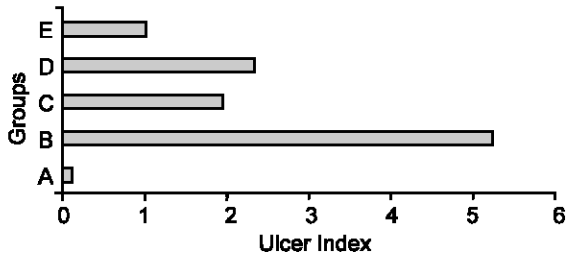

Fig. 2: Ulcer index of control and treated rats, All values are expressed as Mean±SEM (n = 5). Groups C, D and E showed a significant reduction in the value of ulcer index ($p<0.05$ significant). A; negative control, B; not pretreated, C; pretreated with Liquorice, D; pretreated with Vit. E, E; preated with vit E and liquorice

DISCUSSION

Ethanol serves as the most common ulcerogenic agent and when given intragastrically to rats it produces severe gastric hemorrhagic erosions (Shetty *et al*., 2000). This is in line with this study. The genesis of ethanol-induced gastric lesions is multifactorial with the depletion of gastric wall mucus content as one of the involved factors (Martin *et al*., 1994). Oral administration of absolute ethanol in rats is in fact noxious for the stomach, affecting the gastric mucosa topically by disrupting its barrier and provoking pronounced microvascular changes in few minutes after its application. Thus, rapid and strong vasoconstriction is accompanied by rapid and vigorous arteriolar dilation and this combination of microvascular events induces damage in mucosal capillaries (Ko *et al*., 1994; Glavin and Szabo, 1992). Not only alcohol causes direct mucosa damage, its abuse is also associated with development of gastric diseases such as gastritis and even gastric cancer (Ko *et al*., 1994). Acute gastritis caused by direct mucosal damages are usually further aggravated by other important risk factors such as Nonsteroidal Anti-inflammatory Drugs (NSAIDs), acid, Helicobacter pylori infection and physiological stress (Goel *et al*., 1985).

The histopathlogical observations in this study showed that, upon liquorice pretreatment, the mucosal epithelium had near normal architecture and it had less hemorrhage as against the ethanol-induced damages in the mucosal epithelium of the positive control. These observations on the cytoprotective nature of liquorice against ethanol-induced gastric ulcers prove its antiulcer activity.

Also ethanol treatment caused a significant increase in the ulcer index whereas liquorice and vitamin E pretreated rats showed a significant reduction in the ethanol effect. This further supports Glavin and Szabo

Table 1: Malonildehyde levels of control and treated rats

Groups	MDA (umol/mg)	Inhibition rate (%)
A	0.52±0.015	-
B	0.91±0.05	-
C	0.64±0.05*	30
D	0.68±0.07*	26
E	0.61±0.05*	33

All values are expressed as Mean±SEM (n = 5). Groups C, D and E showed a significant reduction in MDA level ($p<0.05$ significant)

Table 2: Histopathological findings of control and treated rats

Group	Mucosa Ulceration	Segmental Mucosa Negrosis	Haemorrage
A	-	-	-
B	++++	+++	+++
C	++	+	+
D	+	+	++
E	-	+	+

- = absence, + = mild, ++ = moderate, +++ = Severe, ++++ = very severe

(1992) study which revealed that free radical scavengers protect the gastric mucosa.

Increased Alkaline phosphatase activity results from damage to tissues and the release of this enzyme has been suggested to have a role in tissue necrosis associated with various models of gastrointestinal ulceration (Ebadi, 2002). The present result which revealed a significant elevation of this enzyme following the acute gastric damage concur with the Ebadi (2002) study, as ALP activity was significantly increased following mucosal damage in groups B compared to groups C, D and E. The decrease in the activity of ALP after liquorice pretreatment implicates its biochemical basis as an antiulcerogenic.

There is consensus that the deleterious effects of ethanol on gastric mucosa are consequence of enhanced lipid peroxidation. The presence of oxygen free radicals that cause lipid peroxidation have been reported in the pathogenesis of gastric mucosal lesions induced by ulcer inducing agents such indomethacin, alcohol and aspirin in rats (Takeuchi *et al*., 1986). Experimental evidence supporting this possibility comes from several studies on the protection from injury by some antioxidants, Prostaglandins (PGs) and sulfhydryl-containing compounds (Soldato *et al*., 1985; Szabo *et al*., 1992; Pearson *et al*., 1996). The antioxidant, vitamin E in this study supports the fact that antioxidants protect the gastric mucosa from injury.

Our findings demonstrated that ethanol increases lipid peroxidation with respect to non-treated control rats, but no significant differences were found in liquorice treated with respect to the control.

The reduction in the malonildialdehide concentrations of the stomach in the ethanol-induced rats might be due to the accumulation of free radicals, as free radicals induce lipid peroxidation damage to the tissues. This supports the fact that antioxidants reduce oxidative damage in tissues. Valenzuela *et al*. (1985) found that antioxidants, given prior to ethanol, abolished both hepatic oxidized glutathione accumulation and the increase in lipid breakdown products. In the current study, liquorice reduced lipid peroxidation in the stomach of ethanol-treated rats. Statistical analysis indicated that the liquorice inhibition of ethanol-induced lipid peroxidation was significant in the stomach.

liquorice pre-treatment offered protection against the action of ethanol on lipid peroxidation showing that the presence of some antioxidant phytoconstituents might have protected the gastric mucosa from free radical-induced damage. Some of which include; gallic acid (Lakshmi *et al*., 2006), glycyrrhizin (Zenei *et al*., 2004) ,trigonelline (Yen *et al*., 2005), pectin (Khasina *et al*., 2003), lignan (Kitts *et al*., 1999) and asparagines (Abad *et al*., 2002).

It is possible that the mechanism by which liquorice prevents its gastric mucosa damage may be due to increased mucus production or prevention of mucus depletion on exposure to a noxious agent. Alternatively, it may possibly exert its gastroprotective effects by its ability to inhibit lipid peroxidation.

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