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POTENTIAL PROTECTIVE EFFECT OF GUM ARABIC AGAINST DOXORUBICIN-INDUCED CARDIOTOXICITY IN WISTAR ALBINO RATS

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ABSTRACT

Gum Arabic (GA) is a water-soluble polysaccharide, obtained from stems of *Acacia senegal* trees as gummy exudates and it is metabolized in colon by normal flora into volatile fatty acids. It is a beneficial adjunct to the low-protein diet for chronic renal failure patient because it reduces serum urea nitrogen level. It increases water and electrolyte absorption, so that it could be a good additive to the oral rehydration solutions, in patient suffering from diarrhea. It is also have a good protective activity against CCl₄ and acetaminophen-induced hepatotoxicity in rats but it failed protect kidney from gentamicin-induced nephrotoxicity. The goal of current study was to investigate the cardioprotective of gum arabic aqueous extract against cardiotoxicity induced by doxorubicin in rats. Wistar albino rats were divided into four groups, 5 rats in each group: Group A (distilled water only, as a control), group B (Doxorubicin (DOX)-only treated rats, 15mg/Kg, IP as a single loading dose), group C (GA 10g/Kg body weight, orally for 4weeks prior DOX treatment) and Group D (GA-only treated group). Histopathological examination and serum biomarker enzymes like Creatine Kinase (CK), Lactate Dehydrogenase (LDH), serum aspartate transaminase (AST) and serum alanine transaminase (ALT) level were monitored at the end of study to evaluate cardiotoxicity. The study showed that doxorubicin-treated animals increased the levels of CK, LDH and ALT significantly and gum arabic-pretreated rats decreased the level of LDH significantly. In gum arabic-only treated animals, they showed no significant changes in all the serum enzymes compared with the control group. Histopathological studies of the heart showed marked cardiac muscle damage in doxorubicin treated rats and the damage was less in gum arabic treated rats prior doxorubicin administration. The results indicate that gum arabic administration have potential protective effect against doxorubicin-induced cardiotoxicity.

Keywords: Gum Arabic (GA), Doxorubicin (DOX), Cardiotoxicity.

INTRODUCTION

Gum Arabic [GA] is an edible dried gummy exudates obtained from the stems and branches of *Acacia senegal* or of other related African species of *Acacia* [Fam. *Leguminosae*]. These trees are abundant in the central Sudan, central Africa and in West Africa [1,2]

Chemically, gum arabic is a branched-chain, complex polysaccharide, either neutral or slightly acidic, found as calcium, magnesium and potassium salt [3].

GA is widely used in the pharmaceutical industries as an emulsifier and suspending agent for insoluble drugs as well as in food industries [4].

Pharmacologically, It has been reported that GA undergoes complete fermentation within the cecum of rats and humans. Such fermentation promotes bacterial proliferation. The resulting large bacterial mass induces increased production of short chain fatty acids [SCFAs] [3]. The major SCFAs produced are acetate, propionate and butyrate [5]

The incorporation of GA into fiber-free diets resulted in increased weight of the cecal wall or increased proliferation of cecal epithelial cells [6]. Hypertrophy of the cecum enlarges the cecal absorptive mucosa and increases cecal blood flow [7]. This phenomenon increases the absorption of volatile fatty acids, K^+ , Mg^{++} and Ca^{++} . The results of other studies indicate that GA enhance water, electrolyte and glucose absorption from oral rehydration salts [8].

The effects of GA on lipid metabolism were variable, Alasdair *et al* found that gum arabic was decreased the serum cholesterol level [9] and Ross *et al.* and Sharma reported reductions in total serum cholesterol level. In contrast, other studies found that the consumption of GA by normal or hypercholesterolemic subjects had no significant effect on plasma lipids [10,11]. In rats, the results were as contradictory [3]. Topping *et al.* found that plasma cholesterol concentrations were unaffected by feeding GA, but plasma triacylglycerols were significantly reduced [12]. GA had little effect on glucose tolerance when gum arabic administrated to men [9].

In chronic renal failure [CRF] patients, fecal bacterial mass and fecal nitrogen content were significantly increased and serum urea nitrogen was significantly decreased during supplementation with GA but nitrogen balance did not change significantly [13]. Another study showed improvement in the quality of life and reduces or eliminates the need for dialysis in children with end stage renal disease [14]. In an experimental model of CRF, Ali *et al.* [2004] showed that treatment of rats with GA was not effective in either reversing the decrease in body weight or the increases in creatinine and urea observed two weeks after induction of the CRF [15]. These results do not support the notion of a beneficial effect of GA in experimental CRF, as was reported in patients in the USA [13].

Using a lipid model system, Trommer and Neubert were found that GA protected against lipid peroxidation in skin in a dose-dependent manner [16]. More recently, however, Cindoruk *et al.* reported that GA was ineffective in ameliorating hepatocellular damage in cholestasis induced by fenofibrate in rats [17].

Gum arabic was reported by Gamal el-din *et al.* as a potent superoxide scavenger so that it give protective effect against acetaminophen-induced hepatotoxicity [18] but it fails to protect the kidney from damage effect of cisplatin¹⁹ and little effect against gentamicin-induced nephrotoxicity [20]. Co-administration of gum arabic in combination with aspirin in rats indicated protective effect

against intestinal mucosal toxicity compared with that of the control [21]. Therefore, the aim of the present study was to investigate the potential protective effect of gum arabic against doxorubicin-induced cardiotoxicity in rats

MATERIALS AND METHODS

Chemicals and Drugs

DOX (Adriamycin) 10mg injection was obtained from Pharmacia Co., Italy and the kits used for biochemical analysis were obtained from BioSystem, Spain.

All other chemicals and solvents were of analytical grades and were obtained from Omar Al-Moukhtar university store.

Gum arabic Preparation

Crude gum arabic purchased from the local market in Benghazi, Libya which obtained from state of Kordofan, Sudan, it was obtained as tears with different size and color, so that they were milled and sieved to obtain a fine clean powder free from any debrises. The GA solution was prepared freshly by dissolving 100 g of GA in 1L of drinking water to obtain GA concentration 10g/100ml.

Experimental Animals

Male Albino rats of Wistar strain ($250 \pm 50g$) used for the study were housed under standard hygienic conditions maintaining at 12hr light and 12hr dark cycle and fed with standard pelleted diet and water *ad libitum*. The experiment was approved in accordance with the International Animal Ethics.

Experimental Protocol

Twenty rats were divided randomly into the following four groups of five rats each:

Group A: normal control rats which received only water.

Group B: negative control rats receiving single injection of DOX (15mg/kg).

Group C: GA 10g/Kg body weight water given daily orally for four weeks prior administration of DOX.

Group D: the rats were given GA only for four weeks

At the end of experiment, all the rats were anaesthetized with chloroform and sacrificed. Blood sample was withdrawn from orbital sinus then serum was separated by centrifugation. Heart was dissected out and immediately preserved in 10% formalin and subjected to histopathological examination.

Biochemical Analysis

Diagnostic markers enzymes include creatine kinase (CK), lactate dehydrogenase (LDH) alanine transaminase (ALT) and aspartate transaminase (AST) were estimated in serum which they act as enzymatic indices of cardiac cellular damage.

In the biochemical analysis, the serum transaminases (ALT and AST) levels were measured using

the methods of Reitman and Frankel (22). LDH was assayed spectrophotometrically according to the method of Gay (23). CK was assayed spectrophotometrically as described by the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (24).

Histopathological Examination

Hearts from rats of all the groups were fixed in 10% buffered formalin and embedded in paraffin. Sections (5 μ m) were stained with hematoxylin and eosin and were examined under a light microscope by a histopathologist who did not know the details of the treatment groups.

Statistical Analysis

Values were expressed as Mean \pm SEM. the results were statistically evaluated using one-way ANOVA using computerized program SPSS version 12 with $P < 0.05$ considered significant.

RESULTS

Biochemical changes

Table 1 summarized the changes in serum enzymes for different experimental groups.

Table 1. Biochemical parameters of heart function(CK, LDH, AST and ALT) for different groups

Groups.	CK IU/dl	LDH IU/dl	AST IU/dl	ALT IU/dl
A Control	49.75 \pm 8.73	144.4 \pm 14.98	37.5 \pm 8.00	28.60 \pm 1.80
B DOX only (15mg/Kg)	91.25 \pm 12.60*	208.2 \pm 12.67*	52.25 \pm 7.82	43.20 \pm 6.24*
C GA + DOX	55.60 \pm 16.46	193.2 \pm 6.49*	32.00 \pm 1.87	34.50 \pm 4.09
D GA only(10g/Kg)	40.75 \pm 9.64	146.0 \pm 18.79	36.00 \pm 7.37	36.00 \pm 4.00

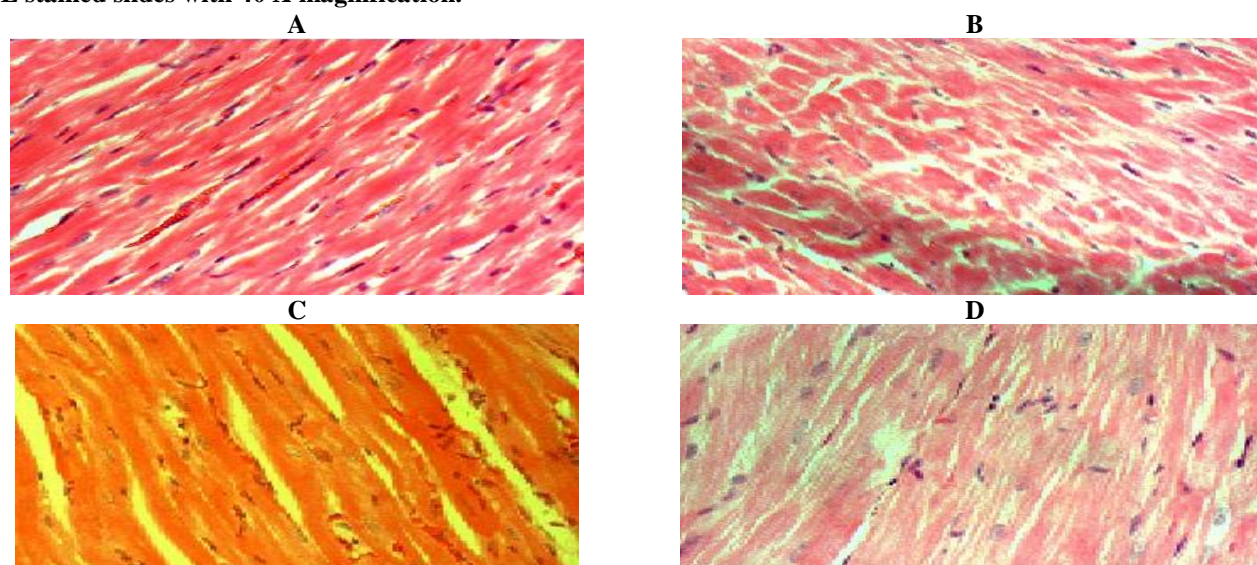
Mean \pm SE.* Significant difference, $P < 0.05$ comparison with control

In DOX only treated rats (group B), The results showed significance increase in CK, LDH and ALT significantly to 91.25 \pm 12.60 IU/dl, 208.2 \pm 12.67IU/dl and 43.20 \pm 6.24IU/dl respectively. The rats treated with GA and before DOX administration (group C), the level of CK, LDH, AST and ALT were reduced to 55.60 \pm 16.46, 193.2 \pm 6.49, 32.00 \pm 1.87 and 34.50 \pm 4.09 IU/dl respectively compared with group (B) and the level of CK, AST and ALT were not significant different compared with the control. In gum arabic treated-only rats (group D), the level of all enzymes showed no significant change compared with the control.

Histopathological changes

Histopathological observation of the H&E stained transverse sections through the cardiac muscles, which showed normal tissues in section (Fig. 1A) but there was a suggested massive damage to the cardiac muscles and loss of some nucleus of these groups of rats (Fig. 1B) . the cardiac muscles section in rats treated with GA before administration of DOX (Fig. 1C) showed less damage than the control(Fig. 1A). Rats treated with GA only showed normal cardiac muscles(Fig. 1D).

Fig 1. Representative photograph of sections of cardiac muscles under light-microscope of rats treated with either distilled water (A) showed regular cross-striations, DOX (15 mg/kg/day for one days (B) showed necrotic myocytes, GA treated animals (10 g/Kg/day for 4weeks) prior DOX administration(C) and the animals treated with GA only(D). H&E stained slides with 40 X magnification.



DISCUSSION AND CONCLUSION

Doxorubicin (DOX), an anthracycline antibiotic, is used primarily in the treatment of a variety of solid tumors including hemopoietic malignancies in children and adults. However, its use has been limited primarily due to cardiotoxicity after an acute dose as well as cumulative doses. The DOX-induced cardiotoxicity has been shown to be mediated through different mechanisms including: free radical generation, membrane lipid peroxidation, mitochondrial damage and iron-dependent oxidative damage to macromolecules. Antioxidants such as vitamin E have been reported to have beneficial effects against DOX-induced cardiotoxicity in mice and rats. Free radical scavengers such as melatonin and alpha-lipoic acid have been shown to ameliorate myocardial toxicity induced by doxorubicin. Natural products such as Ginkgo biloba extract, containing potent antioxidant activity, significantly protected the mice from doxorubicin-induced cardiotoxicity (25). Some drugs such as probucol and vitamin A reduce DOX-induced cardiotoxicity in mice and rats (26&30). Experimental studies in animals using DOX aid in the understanding of the drug's unfavorable cardiac toxicities, and several therapeutic strategies have been evaluated to counter the adverse effects which aim to limit free radicals mediated cardiac injury. Myocardial markers enzymes: ALT, AST, CK and LDH serve as sensitive indices to assess the extent of cardiotoxicity due to DOX (31).

In all recent organ protective studies of GA, the mechanism of protection was based on that GA has strong antioxidant properties and a major mechanism for the

induction of these toxicities is the generation of free radicals (32&33).

Results of the present study indicate that the high dose administration of DOX increased the serum enzyme content of these marker enzymes. This increase in enzymes in serum may be due to an increase in their release following DOX induced lipid peroxidation of cardiac membranes. It has been reported that as a result of lipid peroxidation, inflammation cells accumulate in cardiac myocytes (34). Compared to rats treated with DOX only, rats pretreated with GA restored these levels of enzymes indicating an ameliorative effect of GA on myocardium. In GA pretreated rats, there was restoration of the levels of these enzymes. These biochemical analyses showed a cardioprotective effect of GA and it is supported by histopathological examinations in which DOX showed confluent necrosis and separation of muscle fibers. This type of myocardial damage including myofibrillar degeneration, mitochondrial dilatation and cellular vacuolization is specific to DOX. These lesions were significantly reduced in GA treated rats prior to doxorubicin administration.

This effect of GA could be due to its ability to scavenge free radicals thus acting as an antioxidant but it is difficult to explain this protective effect depending on this assumption because another study was found that GA has no antioxidant effect (15) so that more studies are needed to explain the cardioprotective effect of GA.

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