

Effect of Gum Arabic on Coagulation System of Albino Rats

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Abstract: Gum arabic(GA) is the dried gummy exudates obtained from stems and branches of *Acacia senegal* trees, *Willdenow* or other related African species of *Acacia* (*Leguminosae*) and it is widely used in many pharmaceutical preparations as emulsifying agent and suspending agent and as food additive in food manufacturing. It is a beneficial adjunct to chronic renal failure patient because it reduces serum urea nitrogen level and may increase hemoglobin 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 acetaminophen-induced hepatotoxicity and doxorubicin-induced cardiotoxicity in rats. This study was conducted to investigate the effect of gum arabic on the coagulation system of Wistar rats. Twenty four male Wistar albino rats were assigned randomly to four groups (I, II, III and IV), each with six rats. Group (I), the rats were drinking water free from GA as control while rats in groups: II, III and IV were drinking water containing 3, 6 and 10g/100ml of gum arabic respectively for four consecutive weeks. Blood samples were collected and coagulation parameters were determined. The BT of rats treated with 6gGA/100ml was significantly prolonged and other rats not significantly affected compared with control. The PT of rats treated with 10gGA/100ml were significantly prolonged and not significant different from control for rats treated with 3 and 6gGA/100ml The APTT of all rats were not significantly different from the control. This study showed marked effect of gum arabic on the coagulation system that it prolongs the BT and PT.

Key words: Gum Arabic, PT, APTT, Bleeding Time, Rats.

Introduction

Gum arabic (GA) is the dried gummy exudates obtained from the stems and branches of *Acacia senegal* (Linne') *Willdenow* 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).

The main constituent of gum arabic is very high molecular weight acidic heteropolysaccharide-protein complex present in mixture with calcium, magnesium, potassium and sodium form (2&3).

It is commonly used in the pharmaceutical industry as emulsifying agent and as suspending agent for insoluble drugs (4) also it is used as food additive(5).

Many literatures classified gum arabic as water-soluble low-viscosity fermentable dietary fiber (6&7) which it is degraded in the caecum and it is associated with increased methane excretion and changes in the proportions of various volatile fatty acids in the faeces (8) with no significant effect on wet and dry stool weight, faecal constituent or intestinal transit time (9),

but the proportion of the faecal flora able to degrade the gum arabic polymer rose during ingestion (7).

Alasdair et al (9) found that gum arabic was decreased the serum cholesterol level and had little effect on glucose tolerance when gum arabic administrated to men for three weeks.

Tulung (10) studied the effect of gums on the digestion in rats, and found that gum arabic elicited a marked enlargement of the caecum of rats also they found the volatile fatty acid, K, Mg and Ca absorption was higher. It was enhanced water, electrolyte and glucose absorption from Oral Rehydration Salts in jejunal perfusion of healthy rats and animals with chronic diarrhea (11).

In chronic renal failure (CRF) patients, fecal bacterial mass and fecal nitrogen content were significantly increased during supplementation with gum arabic. Also Serum urea nitrogen was significantly decreased during supplementation with gum arabic but Nitrogen balance did not change significantly (12). Another study showed improvement in the quality of life and reduces or eliminates the need for dialysis in children with end stage renal disease which it is alternative to renal replacement therapy in developing countries (13).

Gum arabic is a potent superoxide scavenger (14) so that it give protective effect against both acetaminophen-induced hepatotoxicity (15) and doxorubicin-induced cardiotoxicity in mice (14) but it fails to protect the kidney from damage effect of cisplatin (16) and little effect against gentamicin-induced nephrotoxicity (17).

Coagulation studies are of great importance considering the role of blood in life (18). These studies are important to investigate the arrest bleeding from small blood vessels by drugs (19) and also to study the anti-thrombotic effect of drug which it is applied to prevent atherosclerotic diseases, including myocardial and cerebral infarction. This therapy includes the suppression of primary hemostasis using anti-platelet drugs such as aspirin and ticlopidine hydrochloride, and suppression of the coagulation system using anticoagulants such as warfarin and heparin (20). In the present study, we examined the effect of GA on the coagulation system of normal rats by measuring the following parameters: bleeding time (BT), Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT).

Experimental

Experimental Animal Care and Diet:

Twenty four male Wistar albino rats 4-6 weeks weighing 180 to 250 g were obtained from animal house of Omar Al-Mokhtar University and they were housed in plastic cages protected with stainless steel

mesh under standard controlled conditions; humidity (68%), temperature ($21 \pm 2^\circ\text{C}$) and with lights on from 6:00 to 18:00 hr.

The animals were fed with Fiber-free diet. The daily amount of diet 10 g per rat and the daily drinking water was 100ml per rat.

Experimental Design:

The rats were assigned randomly to four groups (I, II, III and IV). Each with Six rats. Group (I), the rats were drinking water free from gum arabic as control while groups (II, III and IV) were drinking water containing 3, 6 and 10g/100ml of gum arabic respectively for four consecutive weeks.

Blood Sample Collection:

At the end of experiment, the rats were anesthetized with diethyl ether (using bell jar) and blood is obtained from the orbital sinus under anesthesia using a heparinized capillary tubes and collected into EDTA tubes.

Preparation of Gum Arabic:

Crude gum arabic obtained from the local market as spheroidal tears were milled and sieved to obtain fine pure powder.

Three different concentrations were prepared freshly 3, 6 and 10 g/ 100ml. They were dissolved into warmed water and given for the animals orally.

Determination of Blood Coagulation Parameters:

The (BT) was determined at the end of experiment and 4-ml blood samples were collected from each rat at the end of week four for determination of (PT) and (APTT).

Determination Activated Partial Thromboplastin Time (APTT):

A mixture of 0.1 ml of plasma with 0.1 ml of APTT reagent (BIOMAGREB, Morocco) containing Cephalin-Kaolin suspension was incubated at 37°C for 5 min, followed by the addition of 0.1 ml of 0.025 M CaCl_2 solution. APTT was taken as the interval between the addition of CaCl_2 and the moment when the fibrin clot was visually detected.

Determination Prothrombin Time (PT):

According to Quick's one stage method, 0.1 ml of plasma was mixed with 0.2 ml of PT reagent (BIOMAGHREB, Morocco) containing calcium thromboplastin in a water bath maintained at 37°C . Observation of the sample was then continued until formation of the fibrin clot.

Determination of Bleeding Time (BT):

It was measured by cutting the tail-tip as described by Tschopp and Zucker, 1972(21). The base

of each rat tail is cleansed and pricked with a sterile lancet. A stop clock is started immediately. Blood is blotted every 15 sec using Whatman filter paper until bleeding ceased. The time taken for the blood to stop flowing is recorded as the bleeding time.

Statistical Analysis:

All data obtained were analysed by one way analysis of variance (ANOVA) test using the Statistical Package for the Social Sciences (SPSS) version 12 at a statistical significance level of $P < 0.05$ and 95% confidence interval. All results were expressed as mean \pm standard error of mean (SE)

Results and Discussion

The effect of GA on the coagulation parameters in rats were summarized in Table 1. The BT of rats treated with 6gGA/100ml was significantly prolonged and other rats not significantly affected compared with control. (Fig. 1).

The PT of rats treated with 100gGA/100ml were significantly prolonged and not significant different from control for rats treated with 3 and 6gGA/100ml. (Fig. 2)

The APTT of all rats were not significantly different from the control. (Fig. 3).

Blood coagulation is a host defense mechanism that assists in maintaining the integrity of the closed, high-pressure mammalian circulatory system after blood vessel injury. In the abnormal conditions, it is also involved in the thrombosis, atherosclerosis, inflammation and metastasis by the activation of enzymes in the coagulation cascade and the platelets. The key enzyme, thrombin, and platelets, play an important role in the initiation of the coagulation process and involve in the formation of the fibrin clot and platelet plug in the vascular system. Thus, safe and effective inhibitors of thrombin and platelets should be useful tools in the treatment of venous thrombosis, arterial fibrillation, restenosis,

arterial thrombosis, and in the prevention of myocardial infarction. Because of this, the modulation of thrombin by direct inhibitors and antiplatelet agents are widely sought goals in the development of anticoagulant agents. A number of previous studies have tried to screened out potential candidates of anticoagulant from plants, and some herbs identified with potent anticoagulation activities (22). In the present study, we examined the effect of GA on the coagulation system of normal rats by measuring the following parameters: BT, PT and APTT.

Bleeding time is affected by many factors including vasoconstrictive effect of blood vessels, the formation of hemostatic plug and platelet activity. In general, fatty acids, palm oil and aspirin have been reported to increase BT in animals and humans, whereas saturated fatty acids and cholesterol decrease BT (23,24,25 & 26). The present work shows that GA prolongs BT significantly at concentration 3g/100ml and 6g/100ml compared with control. In order to further evaluate the ability of GA to cause prolongation of tail-BT, we measured the PT of the plasma.

Tests of PT and APTT are used to monitor abnormalities in blood coagulation, an extended PT or APTT indicates a decreased level of one or more of the factors (27).

PT is test used to measure the clotting time of plasma in the presence of tissue extract (thromboplastin) and indicates the overall efficiency of extrinsic system (28) our study showed that 10g of GA /100 water prolong the PT significantly.

APTT is used to assess the intrinsic pathway which depends on substances normally present in blood for its activity (28). In this study, GA prolong PT and not significantly change APTT at all concentrations which indicates GA suppress the intrinsic pathway of coagulation and failure to suppress extrinsic pathway

Table 1: Effect of gum arabic on coagulation parameters in rats

Groups p.o.	BT min	PT sec	APTT sec
I control	3.26 \pm 0.08	20.50 \pm 0.29	54.5 \pm 3.33
II GA 3g/100ml	6.12 \pm 0.66	19.50 \pm 0.50	60.33 \pm 2.67
III GA 6g/100ml	5.56 \pm 0.82*	21.50 \pm 0.29	57.67 \pm 4.28
IV GA 10g/100ml	3.63 \pm 0.32	27.75 \pm 1.80*	55.00 \pm 2.15

Mean \pm SE.

* Significant difference, $P < 0.05$

Fig. 1: Effect of GA on the BT in Wistar rats

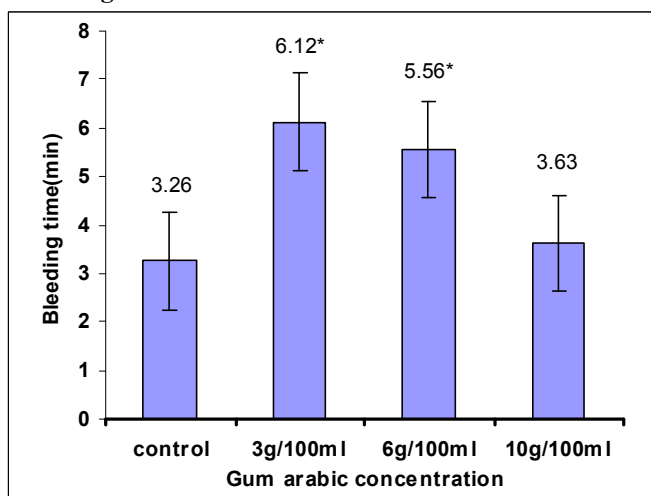
* Significant difference, $P < 0.05$

Fig. 2: Effect of GA on the PT in Wistar rats

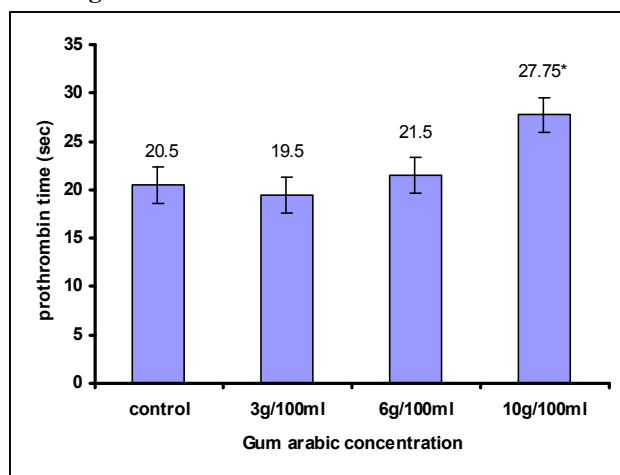
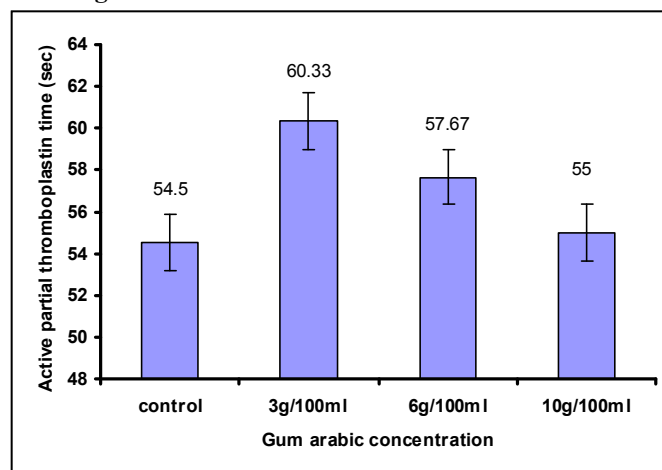
* Significant difference, $P < 0.05$

Fig. 3: Effect of GA on the APTT in Wistar rats



Conclusion

This study showed marked effect of gum arabic on the coagulation system that it prolongs the BT and PT. More studies are needed for explanation the anticoagulant mechanism and the effect of GA on other coagulation parameters such as the platelet aggregation.

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