STUDY THE BIOCHEMICAL EFFECT OF GUM ARABIC IN LIVER INJURY AND BLOOD SERUM OF MICE INDUCE BY GENTAMICIN

Ghassan F. Alubaidy
Department of Pharmacy, Institute of Technical, Mosul, Iraq.
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ABSTRACT

Forty adult male Bulb/c mice weighing between 25-30 GM reared in wire cages were randomly divided into four groups (10 mice each group). Group (G1) received the regular mice diet and (1 ml/kg Saline p.o.) for 8 days maintained as a control group. Group (G2) treated daily intra peritoneal (i.p) injection of gentamicin (40mg/kg bwt/day) for 8 days. In group (G3) received Gum Arabic (GA) (10 gm/kg bwt/day) for 8 days orally by using stomach tube. Group (G4) is received gentamicin (40 mg/kg bwt/day) (i.p) plus (10gm/kg bwt/day) orally of Gum Arabic for 8 days. Blood samples were collected after overnight fasting 12-24 hours for determination some biochemical marker including AST, ALT and ALP activities, creatinin, uric acid, urea and total bilirubin in serum. Assay the total protein, Deoxyribonucleic acid DNA, ribonucleic acid RNA in liver tissue of mice.

The results revealed that treatment with gentamicin (G2) increased in AST, ALT and ALP activities in serum, creatinin, uric acid, urea and total bilirubin value in serum also there are elevation the level of DNA, RNA and total protein in liver tissue when a compared with the control group (G1). Treatment of GA along with gentamicin (G4) and administration of GA alone (G3) decreased in the level of serum creatinine, serum uric acid, serum urea and serum total bilirubin, the level of DNA, RNA and total protein in liver tissue also decrease, as compared to the gentamicin treated group (G2) but not reach in both groups (G3,G4) to normal level as in control group.

In conclusion Gum Arabic exhibit hepatoprotective activities probably through free radical scavenging properties. Altered level of hepatic markers such as AST, ALT, and ALP with gentamicin exposure was reversed towards normalization with Gum Arabic. Similarly, biochemical parameters were also restored by Gum Arabic. Bioactive of Gum Arabic ameliorated the oxidative damage and had increased the regenerative and reparative capacity of liver.

INTRODUCTION

Gum Arabic 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. Legumenosae). These trees are abundant in the central Sudan, central Africa and in West Africa, it is a complex of very high molecular weight acidic hetero-polysaccharides,
which is widely used in pharmaceutical preparations as a suspending, demulcent, soothing and emulsifying agent (1). Gum Arabic has wide industrial uses as a stabilizer, thickening agent and emulsifier, mainly in the food industry (e.g. in soft drinks syrup, gummy candies and marshmallows), but also in the textile, pottery, lithography, cosmetics and pharmaceutical industries. It has a complex chemical composition (2). The Gum Arabic is built upon a backbone of D-galactose units with side chains of D-glucuronic acid with L-rhamnose or L-arabinose terminal units, the molecular weight of the gum is large and estimates suggest the weight lies in the range of 200,000 to 600,000 Daltons (3).

The liver is one organ of gastrointestinal tract and drug targets through the body is control to the metabolism of virtually every foreign substance, most drugs cause liver injury infrequently (4), one of these drugs are gentamicin, is bactericidal antibiotic with wide clinical use but disturbing toxicity. Nephrotoxicity and ototoxicity are the most common adverse reaction (5). While hepatotoxicity is almost always related to nephrotoxicity by hepatic renal syndrome. Many attempts take attention to controlling gentamicin toxicity including the use of pyridoxal phosphate, ascorbic acid and calcium loading, calcium channel blockers; vitamin E (6). Gentamicin generates free oxygen radicals, leading to tissue injury such as nephrotoxicity and ototoxicity (7). Gentamicin is one of the amino glycosides that also cause liver toxicity (8). Toxicity of amino glycosides has been widely studied (9) it has been reported that renal damage can in turn leads to liver injury due to amino glycosides (10). Amino glycosides (Gentamicin) are potentially toxic at levels only slightly above therapeutic range (11, 12).

**MATERIAL AND METHODS**

**Animal:**

Forty adult male *mus musculus* Bulb/c mice weighing 25–30 g were used in the experiment. They were obtained as 40-50 days old from the experimental animal Care Center, Mosul medical college. They were housed under conventional laboratory conditions in a room temperature maintained at 25 ± 1 °C. With a regular 14 h light: 10 h dark cycle. The mice were fed a standard animal pellet diet and allowed free access water unless otherwise indicated.

**Gentamicin:** 80mg/2mL was obtained from megental, Italy.

**Gum Arabic:** In powder form was provided from Dar Savanna Ltd., Khartoum, Sudan.

**Experimental design:**

Forty adult Male mice weighting between 25-30 gm reared wire cages were randomly divided into four groups of mice (10 mice each group). Group 1(G1) received the regular mice diet and (1 ml/kg Saline p.o.) for 8 days maintained as a control group. Group 2 (G2) mice were received gentamicin daily intra peritoneal (i.p) injection of (40m g/kg bwt/day) for 8 days (13). In group 3(G3) mice were received Gum Arabic (10 gm/kg bwt /day) for 8 days orally by using stomach tube. Group 4(G4) are received
gentamicin (40mg/kg bwt/day) (i.p) plus (10gm/kg bwt / day) orally of Gum Arabic for 8 days.

Autopsy procedures:
Animal were anesthetized with ether inhalation, and then killed by cervical dislocation.

Sample collection and biochemical assay:
Blood samples were collected after overnight fasting 12-24 hours at the end of the experiment, the mice were anesthetized with diethyl ether (using bell jar) and blood is obtained from the ocular sinus by non-heparinized capillary tubes, the blood was centrifuged at 2500 rpm for 20 minutes. The obtained serum was stored at 4°C for the estimation of serum. Biochemical marker including AST, ALT and ALP activities, creatinin, uric acid , urea and total bilirubin in serum .Assay the total protein, Deoxyribonucleic acid DNA, ribonucleic acid RNA in liver tissue of mice. Biochemical factors of serum were measured spectrophotometrically using auto analyzer and respective kits (biomerix).

Estimation of liver protein and nucleic acid:
A weighed quantity of liver tissue (2gm) was homogenized with distilled water and was treated with 10% cold trichloroacetic acid to precipitate the proteins. The residue was washed twice with 10% TCA to remove all acid soluble compounds. The resultant residue was treated with 95% ethanol twice and with solvent ether twice. The residual dried fat free sample was used for estimating protein and nucleic acid content. Total protein was estimated by the method (14) using bovine serum albumin as the standard. RNA was extracted and estimated by the method (15). DNA was extracted by the method (16).

Statistical analysis:
The data were expressed as means ± standard errors (S.E.M.), differences between groups means were estimated by using ANOVA followed by Turkeys Test. Differences of (p≤ 0.05) were considered significant.

RESULTS

Effect of GA on liver marker enzymes:
Gentamicin (40mg/kg bwt/day) increased the activity of AST,ALT and ALP in serum of (G2) as compared to the control group(G1) (Table 1). (G4) treatment of GA(10gm/kg bwt/day) in combination with gentamicin (40mg/kg bwt/day) and administration of GA (10gm/kg bwt/day) alone significantly (P≤0.05) reduce the level of AST,ALT and ALP in serum dose dependently, as compared to the gentamicin treated group (G2) but not reach to normal level as in control group.
Table 1: Effect of Gum Arabic on SAST, SALT, and SALP activities of mice treated with gentamicin

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>SAST U/L</th>
<th>SALT U/L</th>
<th>SALP U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Control</td>
<td>206 ± 13.3c</td>
<td>159 ± 9.3c</td>
<td>1281 ± 21.2c</td>
</tr>
<tr>
<td>G2</td>
<td>Gentamicin 40mg/kg bwt/day (i.p)</td>
<td>492 ± 17.2a</td>
<td>301 ± 16.6a</td>
<td>1498 ± 54.3a</td>
</tr>
<tr>
<td>G3</td>
<td>Gum Arabic 10gm/kg bwt/day (orally)</td>
<td>189 ± 11.4d</td>
<td>138 ± 8.3d</td>
<td>1206 ± 23.4d</td>
</tr>
<tr>
<td>G4</td>
<td>Gentamicin 40mg/kg bwt/day (i.p) with G.A 10gm/kg bwt/day (orally)</td>
<td>245 ± 12.6b</td>
<td>187 ± 9.1b</td>
<td>1304 ± 9.8b</td>
</tr>
</tbody>
</table>

P< (0.05) Values are mean ± standard error of mean

Effect of GA on serum biochemistry:

Injection of gentamicin (40mg/kg bwt/day) to mice for 8 days (G2) significantly (P ≤ 0.05) increased the serum level of creatinine, uric acid, urea and total bilirubin than that of the control group (G1) (Table 2). Treatment of GA (10gm/kg bwt/day) along with gentamicin (40mg/kg bwt/day) (G4) and administration of GA (10gm/kg bwt/day) alone (G3) significantly (P≤0.05) decreased the level of serum creatinine, serum uric acid, serum urea and serum total bilirubin dose dependently, as compared to the gentamicin treated group (G2) but not reach to normal level as in control group (Table 2).

Table 2: Effect of Gum Arabic on serum creatinine, serum uric acid, serum urea and serum total bilirubin of mice treated with gentamicin

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum creatinine mg/dl</th>
<th>Serum uric acid mg/dl</th>
<th>Serum urea mg/dl</th>
<th>Serum Total bilirubin mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Control</td>
<td>0.521 ± 0.03c</td>
<td>210 ± 8.0c</td>
<td>44 ± 1.71c</td>
<td>0.62 ± 0.01c</td>
</tr>
<tr>
<td>G2</td>
<td>Gentamicin 40 mg/kg bwt/day (i.p)</td>
<td>1.92 ± 1.01a</td>
<td>285 ±11.9a</td>
<td>59 ± 4.01a</td>
<td>4.02 ± 1.01a</td>
</tr>
</tbody>
</table>
Effect of GA on the Total Protein and DNA, RNA in the liver tissue:
Administration of gentamicin (40mg/kg bwt/day) (G2) significantly (P < 0.05) increased the level of (DNA, RNA) and significantly (P< 0.05) increase in total protein as compared to the control group(G1).Administration of GA (10gm/kg bwt/day) in combination with gentamicin (40mg/kg bwt/day) (G4) and administration of GA (10gm/kg bwt/day) alone (G3) significantly (P<0.05) reversed the level of (DNA, RNA) and total protein injuries as compared to gentamicin treated group (G2) in a dose dependent manner in both the liver samples.

Table 3: Effect of Gum Arabic on DNA, RNA and total protein of liver tissue of mice treated with gentamicin

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>DNA mg/100 mg of tissue</th>
<th>RNA mg/100 mg of tissue</th>
<th>Total protein mg/100 mg of tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Control</td>
<td>4.251±0.12c</td>
<td>5.895±0.58c</td>
<td>26.26±0.69c</td>
</tr>
<tr>
<td>G2</td>
<td>Gentamicin 40mg/kg bwt/day (i.p)</td>
<td>7.621±0.25a</td>
<td>9.121±0.52a</td>
<td>41.05±1.01a</td>
</tr>
<tr>
<td>G3</td>
<td>Gum Arabic 10gm/kg bwt/day (orally)</td>
<td>3.695±0.6d</td>
<td>4.195±0.98d</td>
<td>22.15±0.96d</td>
</tr>
<tr>
<td>G4</td>
<td>Gentamicin 40mg/kg bwt/day with(i.p) Gum Arabic 10gm/kg/day (orally)</td>
<td>5.157±0.125 b</td>
<td>7.051±0.24b</td>
<td>29.65±0.92b</td>
</tr>
</tbody>
</table>

P< (0.05) Values are mean ± standard error of mean

DISCUSSION

Treatment with gentamicin (80 mg/kg/day) for five days induced moderate to severe histological damage (17). Gentamicin induced free radical generation and alteration in antioxidant enzyme activities may be the cause of tissue injury (18). Free radicals may be designated as molecular sharks that damage molecules in cell membranes, mitochondria, DNA, and are very unstable, tends to rob electrons from the molecules in the immediate
surroundings in order to replace their own losses (19). Gentamicin treatment causes hepatotoxicity as clearly indicated by the significant increase in serum enzyme level of ALT, AST, ALP, is generally considered as sensitive markers of liver function and their concentrations are increased in the serum because of their cytoplasmic nature and are thus released in blood by changing in the permeability of hepatocyte membranes. Treatment of gentamicin causes significant increase in the serum level of liver and kidney function markers such as creatinine, BUN, total cholesterol, triglycerides, total bilirubin, and direct bilirubin as compared to respective control indicating hepatorenal dysfunction. These injuries could be due to the production of free radicals and involvement of oxidative stress to hepatorenal toxicity caused by gentamicin treatment (20). Several authors recorded the damage effects of gentamicin on the biological and physiological functions in both man and animals. Gentamycin toxicity was proved as suppression of reticuloendothelial system, ribosomal disturbance as well as synthesis of nucleic acids (DNA, RNA) (20-21). Treatment of the rats with gentamicin for 10 days induce significantly increased DNA of hepatorenal tissues and causes depletion in total protein and albumin, useful markers of liver function, might be depressed as a result of defective protein synthesis (22). Results of this study confirmed that gentamicin at a dose of (40 mg/kg b.wt) produces significant hepatotoxicity evidenced by increase in serum total bilirubin, SAST activity, SALT activity, SALP, creatinine, uric acid, urea and elevated levels of liver biochemical markers (DNA, RNA) and total protein in the liver tissue as compared to the control group (G1).

Oral administration of Gum Arabic (10gm/kg b.wt) produces significant hepatoprotective effects in gentamicin treated mice our results are in agreement with (23). Who suggest that Gum Arabic may find clinical application in a variety of condition where cellular damage is a consequence of oxidative stress. Gum Arabic modestly ameliorated histological and biochemical parameters, an effect attributed to a decreased production of free oxygen radicals (24, 25). Gum Arabic protects mice from acetaminophen-induced liver injury as evident from significant decrease in serum alanine transaminase (ALT), aspartate transaminase (AST) and hepatic lipid peroxides (26). Gum Arabic increases fecal nitrogen excretion and decreases production of free oxygen radicals (27). The effect of Gum Arabic on the concentration of certain metabolites of patients with CRF on a low-protein diet, when Gum Arabic was given orally (50 gm/day) for 3 months, serum creatinine, urea and uric acid concentrations were reported to be significantly reduced (28). Gum Arabic has strong anti-oxidant properties, and a major mechanism for the induction of these toxicities is the generation of free radicals (29). Antioxidants neutralize toxin and volatile free radicals that are defined as atoms or groups of atoms having an unpaired electron. These also include related reactive oxygen species (ROS) that leads to free radical generation, causes the cascading chain reaction in biological system. In a normal, healthy organism or human body, the generation of pro-oxidants in the form of (ROS) is effectively kept in check by various levels of antioxidant defense. Antioxidants present in various dietary supplements offered their beneficial effects by neutralizing these (ROS) during various disease conditions. Lipids, proteins and DNA are all susceptible to attack by free radicals and cellular damage induced by oxidative stress has been implicated in the etiology of numerous diseases (30). It is believed that antioxidants exert their protective effect by decreasing oxidative damage to DNA and by decreasing abnormal increases in cell division (31). Reactive oxygen species
and free radicals have received a lot of attention in recent years as associated with cellular injury and aging process (32). In conclusion Gum Arabic exhibit hepatoprotective activities probably through free radical scavenging properties. Altered level of hepatic markers such as AST, ALT, and ALP with gentamicin exposure was reversed towards normalization with Gum Arabic. Similarly, biochemical parameters were also restored by Gum Arabic. Bioactive of Gum Arabic ameliorated the oxidative damage and had increased the regenerative and reparative capacity of liver.

On the other hand, in a study conducted by Mohamad Ayoub Fathah and Mansour Alhadeef, the effect of kermesic acid on the liver and kidney of male Syrian albino rats was studied. The study was conducted on 40 rats, each weighing between 25-30g, and were divided into 4 groups. The first group received kermesic acid at 10 mg/kg, the second group received kermesic acid at 20 mg/kg, the third group received kermesic acid at 30 mg/kg, and the fourth group received kermesic acid at 40 mg/kg. The rats were treated with kermesic acid for 8 days.

The results showed that the kermesic acid at 20 mg/kg and 30 mg/kg had a significant effect (P ≤ 0.05) on the level of AST, ALT, and ALP enzymes in the blood, as well as on the level of DNA and RNA. The treatment with kermesic acid at 40 mg/kg did not have a significant effect on the level of AST, ALT, and ALP enzymes in the blood, as well as on the level of DNA and RNA.
REFERENCE


