Protective Effect of Arabic Gum on liver Injury Experimentally Induced by Gentamycin in Mice

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Abstract:

This research was conducted to evaluate the effect of Arabic gum (Ag) on hepatotoxicity induced by gentamycin in mice.

Forty adult male Bulb/c mice were used. The animal were divided into four groups (Gs) each group 10 animals. G1 considered as control, G2 treated with gentamycin(40mg/kg/day) for 8 days (i.p.). G3 treated with Ag (10 gm/kg/day) for 8 days administrated orally. Then G4 treated with both gentamycin and Ag for 8 days.

Biochemically, the results showed a significant decrease in levels of serum ALT and AST in G4 as compared with G2 . Moreover, serum Glutathione (GSH) in G4 also elucidate a significant increasing in its level, as well as a significant decrease in levels of malondialdihyde (MDA). Histopathologically, liver sections of animals in G4 revealed an ameliorative effects of Ag as compared with its compatible tissue sections of G2. Liver of mice treated with Ag (G3) showed an apoptosis in hepatocytes.

In conclusion, treatment of hepatotoxicity by Arabic gum , showed melioration in histopathological changes in hepatic tissues in addition to have ability for induction of apoptosis and meliorating liver picture.

التأثير الوقائي للصمغ العربي على اذى الكبد المحدث تجريبيا بالجنتاميسين في الفئران

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الخلاصة:

ضم هـذا البحث تقييم آثار الصمغ العربي على سمية الكـبد المحدث بالجـنتاميـسين في الفئران. لذا استخدمت اربعون من ذكر الفئران نوع Bulb/c قسمت الى اربعة مجاميع كل مجموعة تضم 10

حيوانات، عدت المجموعة الاولى كمجموعة سيطرة اما المجموعة الثانية تم معاملتها بالجنتاميسين (40 ملغم/ كغم/ يوميا) لمدة ثمانية ايام بالتجويف الخلبي. وعوملت المجموعة الثالثة بالصمغ العربي (10 ملغم/ كغم يوميا) لمدة ثمانية ايام فمويا. اما المجموعة الرابعة فقد تم معاملتها بكل من الجنتاميسين والصمغ العربي معا لمدة ثمانية ايام.

كيموحيوي، اضهرت النتائج انخفاض معنوي في مستوى انزيمات الالنين امينو ترانسفيريز والاسبارتيت امينوترانسفيريز في مصل المجموعة الرابعة (المعاملة بالصمغ العربي والجنتامايسين) مقارنة مع المجموعة الثانية (المعاملة بالجنتاميسين فقط). اكثر من هذا، كلوتاثيون المصل في المجموعة الرابعة اظهر زيادة معنوية في مستواه، ايضا اظهرت النتائج انخفاض معنوي في مستوى المالوندايلديهايد.

المرضي النسجي، اظهرت حيوانات المجموعة الرابعة تحسنا في صورة الكبد مقارنة مع المجموعة الثانية. فئران المجموعة الثالثة المعاملة بالصمغ العربي اظهرت وجود موت خلوي مبرمج في خلايا الكبد.

تستنتج هذه الدراسة ان علاج سمية الكبد بالصمغ العربي اظهر تحسنا في التغيرات المرضية النسجية في نسيج الكبد فضلا عن قابليته في تحفيز الاستماتة وتحسن صورة الكبد.

Introduction:

The liver is one organ of gastrointestinal tract and drug targets through the body, is control to the metabolism of virtually every foreign substances. Most drugs cause liver injury infrequently (1). One of these drugs are Gentamycin, is abactercidal antibiotic with wide clinical use but disturbing toxicity. Nephrotoxicty and ototoxicity are the most common adverse reaction (2,3). While hepatotoxicity is almost always related to nephrotoxicity by hepatic renal syndrome. Many attempts take attention to controlling gentamycin toxicity including the use of pyridoxal phosphate, ascorbic acid and calcium loading ,calcium channel blockers, vitamin E (4).

Arabic gum (Ag), one a plant hydrocolloid water soluble, is extensively used in pharmaceutical and processed food industries as a stabilizing and texture enhancing agent (5). Ag a natural proteoglycan exudates from the stems of *Acacia Senegal*, is a branched-chain complex polysaccharide either neutral or slightly acidic found as a mixed calcium, magnesium and potassium salt of polysaccharide acid or that referred as Arabic gum acid. Also Ag has recognized the usefulness in regulating the inflammation intestinal mucosa and as external soothing agent (6). Also it considered as acytoprotetive agent have demonstrated its usefulness agent a number of drugs like cisplatin induced nephrotoxicity and ccl4 induced hepatoxicity in mice (7),and gentamycin mediated nephrotoxicity in rats(8). Hence, our studies were concluded to investigate the possible protective effects of Arabic gum on liver injury resulting from gentamycin in mice model system. Biochemical parameter and histologic changes have been studied to evaluate the protective effect of Arabic gum.

Material and Methods:

In this study adult male *mus musculus* Bulb/c mice were obtained as 40-50 days old, weighting between 25-30 gm. from Mosul medical college. The animals were housed under standard laboratory condition (14 hs :10 hs light and dark) in a room with controlled temperature during the experimental period. Water and food (standard commercial mice diet) were provided ad libitum.

Gentamycin was obtained from megental, Italy.

Arabic gum was obtained from Dar Savanna Ltd., Khartoum, Sudan.

Experimental design

Forty male mice reared in wire cages were randomly divided into four groups (10 mice each). Group 1 received the regular mice diet and maintained as a control group. Group 2 mice were received a daily intraperiotoneal (i.p.) injection of 40 mg/kg /day (9) for 8 days. In group 3 Arabic gum (Ag) 10 gm/kg/day for 8 days orally by using stomach tube. While in group 4 received gentamycin 40 mg/kg/day (i.p) plus Ag 10 gm/kg/day orally for 8 days. Blood samples were collected after overnight fasting 12-24hours and analysis performed in fresh heparin ,treated serum standard enzymatic assay Aspartate aminotrasferase (AST) and Alanine aminotrasferase (ALT) by using kits (biomerix).

Biochemical assay:

Measurement the levels of Alanine aminotransferase enzyme (ALT) and Aspartate aminotransferase enzyme (AST) by using kits (biomerix). GHS Measurment:

GHS concentration of animal in all groups serum was measured by the moron et al assay (10).

MDA Measurment:

The extent of lipid peroxidation in animal serum of all groups was measured by thiobarbituric acid (TBA) test was previously described (11).

Autopsy procedures:

Animal were anesthetized with ether inhalation, then killed by cervical dislocation and immediately after death, liver was fixed in 10% neutral buffer formalin, embedded in paraffin, sectioned at 5 Mm and stained with haematoxylin-eosin (H+E).light microscope was evaluate the lesions (12).

Statistical analysis

The data were expressed as means ± standard errors (S.E.M.), differences between groups means were estimated by using ANOVA followed by Tukeys Test. Differences of $p \leq 0.05$ were considered significant.

Results:

Biochemical assay

ALT +AST

In this result, the ALT activity in serum was significantly increased in groups (3and4) then in group 2. (Table-1) showed significant reduction in ALT activity in group 3as compared to group to groups (2and3) but not reach to normal levels control group. While AST

activity revealed reduction in level significant at $p \leq 0.05$ in group 3 and 4 as compared to group 2 but not reach to its normal level as in control. Also (Table-1) illustrates in general significance differences among the groups as comparing them with control group. The results revealed a significant decrease of AST level in serum in mice treated with AG in group 3 at 8 days post Treatment (45±1.22) as compared with group 2 (992.21±0.11) but not reach to normal level as in control group.

MDA+GSH

Serum MDA (TBA reactive substances) end product of lipid peroxidation, were significantly decreased in mice treated with Ag after 8 days as compared with control group (Table 2). The greatest increased in MDA content was seen in plasma group 2(gentamycin treatment)(943± 5.15). Significant reduction in MDA values were seen in group 4 (treated with Ag and gentamycin) reach to (503±1.02).

Table 2, also shown GSH contents after 8 days of Ag treatment they were significantly increased by (4.01±0.23) in group 2 and 3 as compared with group 4 which reduction the value of GSH to (1.01±0.22) as compared with control group.

Table -1: Effect of Ag on serum AST and ALT of mice treated with gentamycin +Ag

Group	**Treatment**	**ALT IU/L**	**AST IU/L**
G1	control	60.42±1.84	321±15
G2	Gentamycin 40mg/kg/day for 8 days	820± 0.33	425±11
G3	Arabic gum 10g/kg/day For 8 days	45 ±1.22	232±0.05
G4	Gentamycin 40mg/kg/day + Arabic gum10g/kg/day For 8 days	93±2.21	291±17

Table-2: effect of Ag on serum MDA and GSH concentration of mice treated with gentamycin .

Group	**treatment**	**MDA**	**GSH**
G 1	control	446±4.44 a	3.6 ± 0.55 a
G 2	Gentamycin 40mg/kg/day for 8 days	943± 5.15 b	1.01 ±0.22 b
G 3	Arabic gum 10g/ kg/ day for 8 days	422± 2.33 a	4.01 ± 0.23 a
G 4	Gentamycin 40mg/kg/day + Arabic gum 10g/kg/day For 8 days	503 ± 1.02 ab	3.5 ± 1.05 ab

Histopathological appearance:

Histological section of hepatic tissues in group 1(control) showed normal architecture, represented by the hepatic central vein surrounded by hepatic cord, which connected together by sinusoids. Each three lobule connected together by portal area (bile duct, hepatic artery and the portal vein). While in group 2 treated with gentamycin liver section of mice revealed histopathologic lesions represented by sever congestion dilation of sinusoid, hemorrhage. Also Centro lobules vacuolar degeneration has been seen associated with hemosiderin. Pigmentation deposition in cytoplasm of kupffer cells and hepatocytes. Apoptosis also observed in hepatocytes. Some sections revealed focal hemorrhagic necrosis, Centro lobular necrosis association with mononuclear infiltration of inflammatory cells(macrophage and lymphocytes) in addition to hypertrophy of kupffer cells and megalohepatocytes (figs 1-5).

In group 3, which treated with (Ag) showed congestion of sinusoid and central vein associated with perivascular infiltration of inflammatory cells. Also mild vacuolar degeneration have been seen, in addition to apoptosis of hepatocytes with presence of apoptosis bodies (figs 6and7).Group 4 treated with gentamycin and Ag, liver section elucidate the presence of sever apoptosis in hepatocytes association cell swelling. Vacuolar degeneration in cytoplasm and mild infiltration of mononuclear inflammatory cells, Also kupffer cells hypertrophied and dilation of central vein in addition to megalohepatocytes. has been seen (figs 8-12).

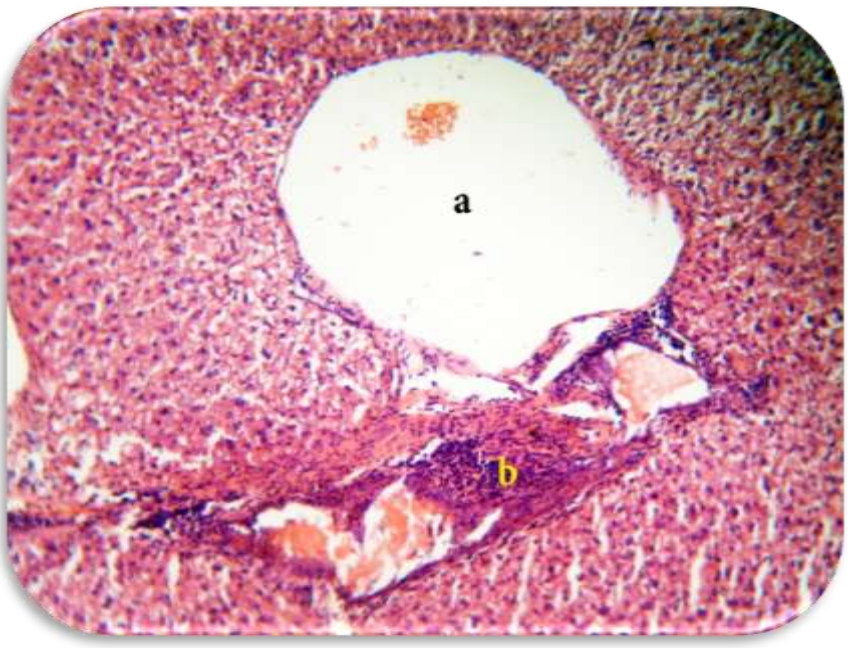

Fig. 1: Histological section of mice liver treated with gentamycin for 8 days, revealed, dilation of blood vessels (a) and perivascular infiltration of mononuclear inflammatory cells (b). H&E, 115X.

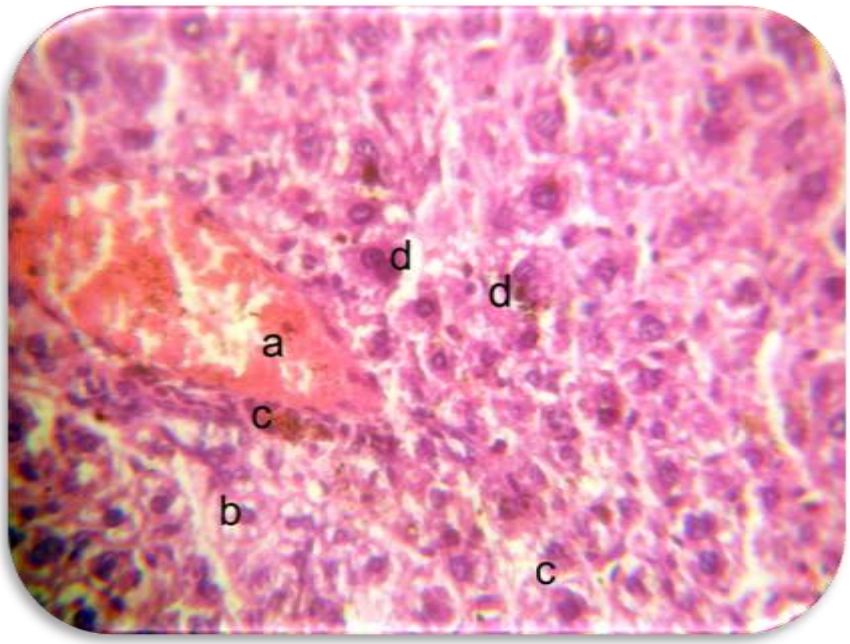

Fig.2: Histological section of mice liver treated with gentamycin for 8 days , showed congestion (a), sever vacuolar degeneration(b), hemosiderin (brown) pigment (c), in addition to apoptosis (d).H&E, X

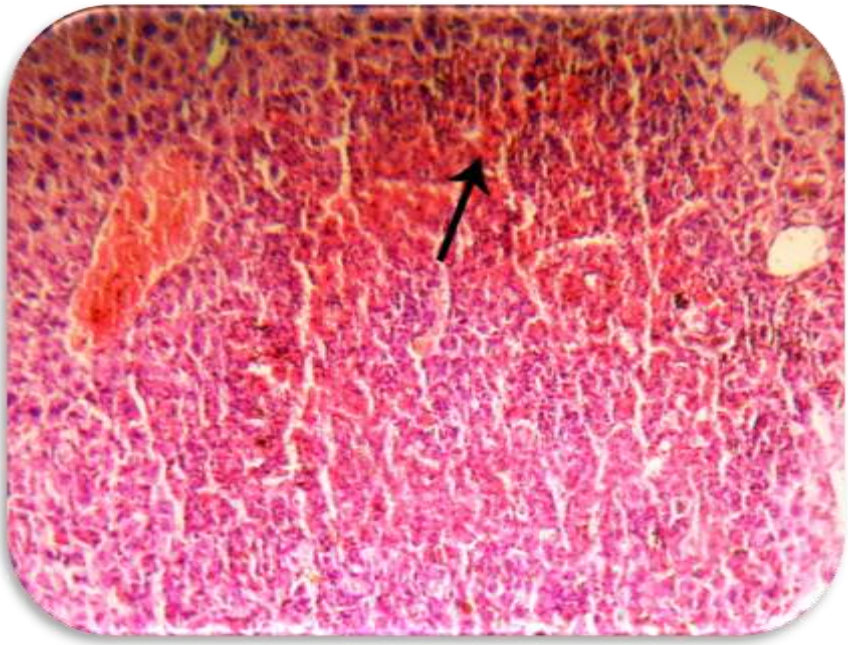

Fig. 3: Histological section of mice liver treated with gentamycin for 8 days , showed focal area of hemorrhagic necrosis (arrow).H&E, 145X

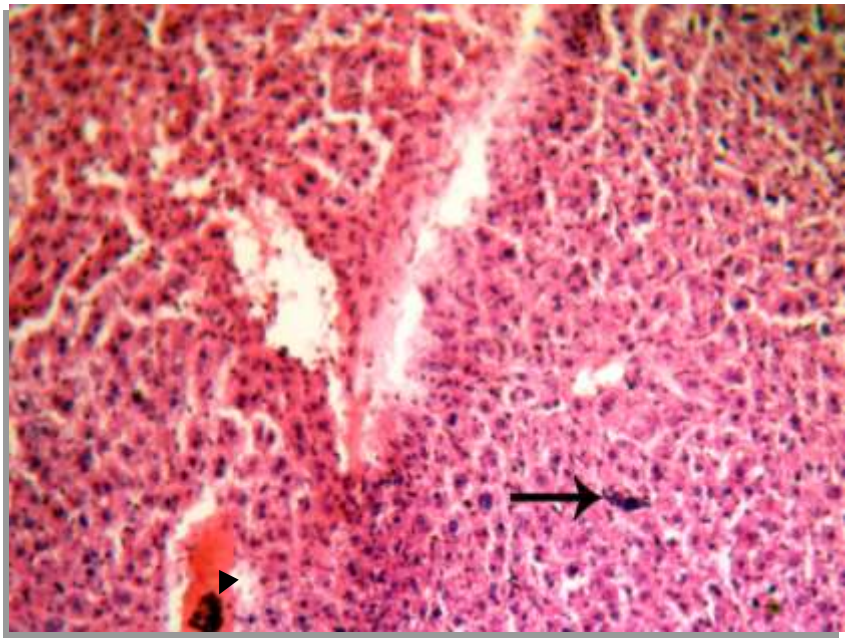

Fig.4: Histological section of mice liver treated with gentamycin for 8days, showed many apoptotic cells (arrow) and apoptotic bodies (head arrow). H&E. 115X

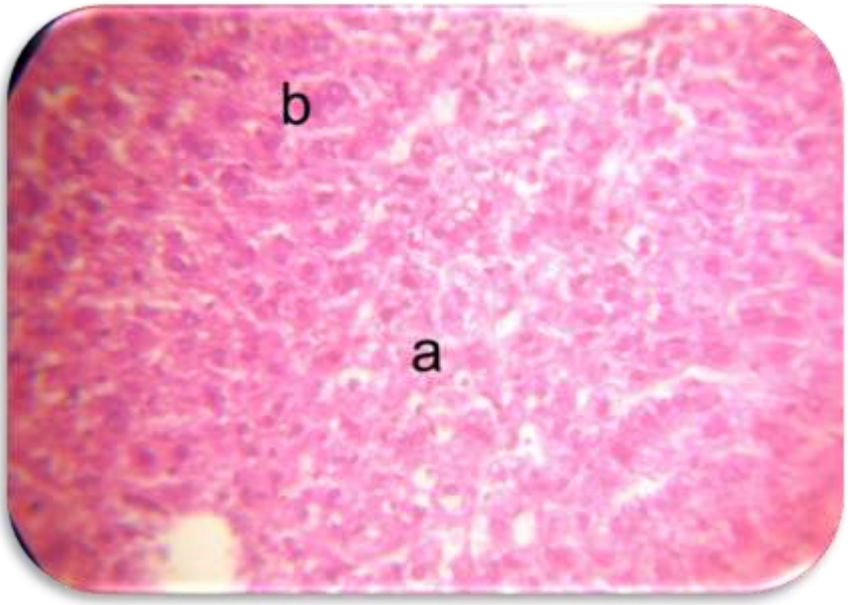

Fig.5: Histological section of mice hepatic tissue treated with gentamycin for 8day, revealed sever vacuolar degeneration (a), hepatomegacyte(b). H&E, 165X

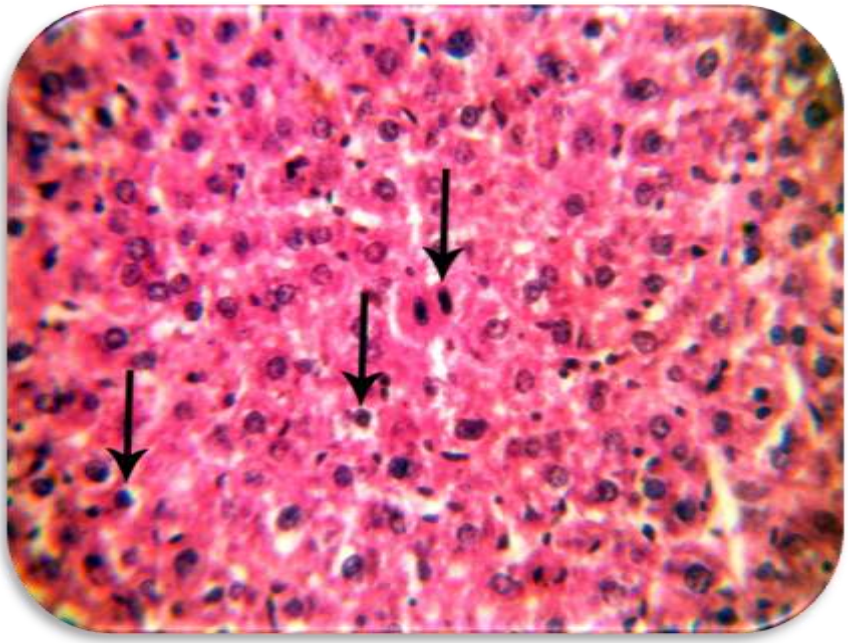

Fig. 6: Histological section of mice hepatic tissue treated with Ag for 8day, showed many apoptotic with cells apoptotic bodies (arrows). H&E, 165X

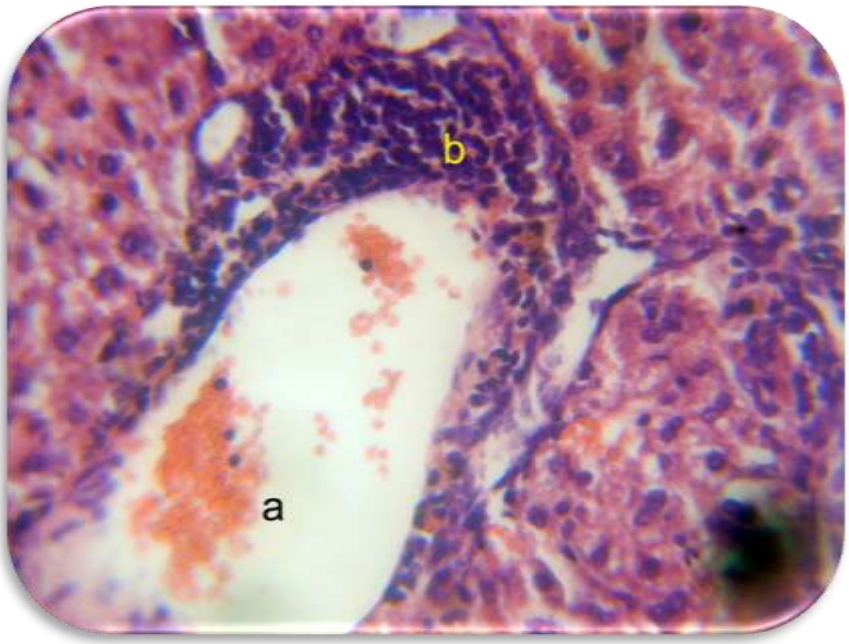

Fig.7: Histological section of mice liver treated with Ag for 8 days, showed congestion (a) infiltration of mononuclear inflammatory cells around blood vessels (b). H&E. 156X.

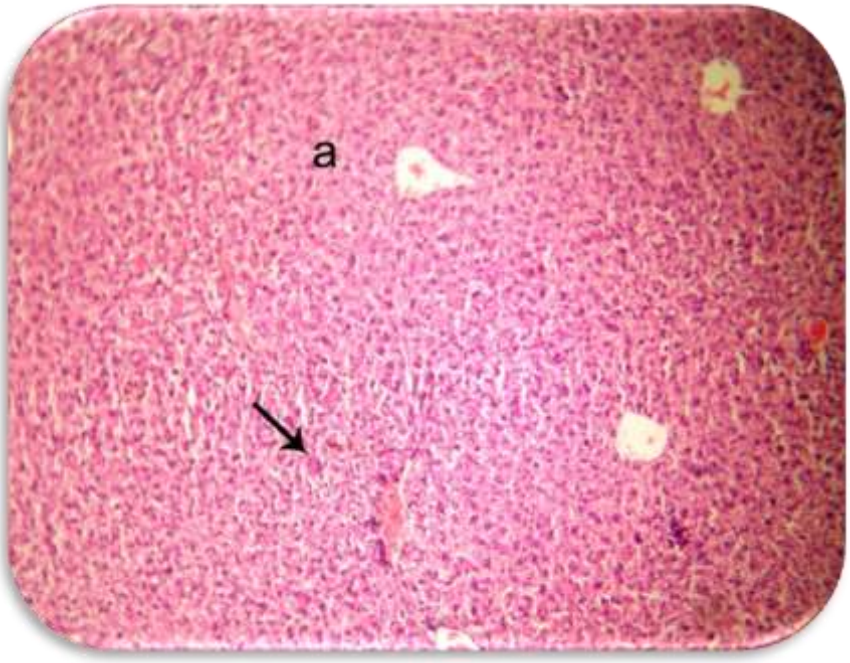

Fig. 8: Histological section of mice hepatic tissue treated with gentamycin +Ag for 8 days, showed disappeared of Vacuolar degeneration (a) and apoptosis (arrow). H&E, 100X

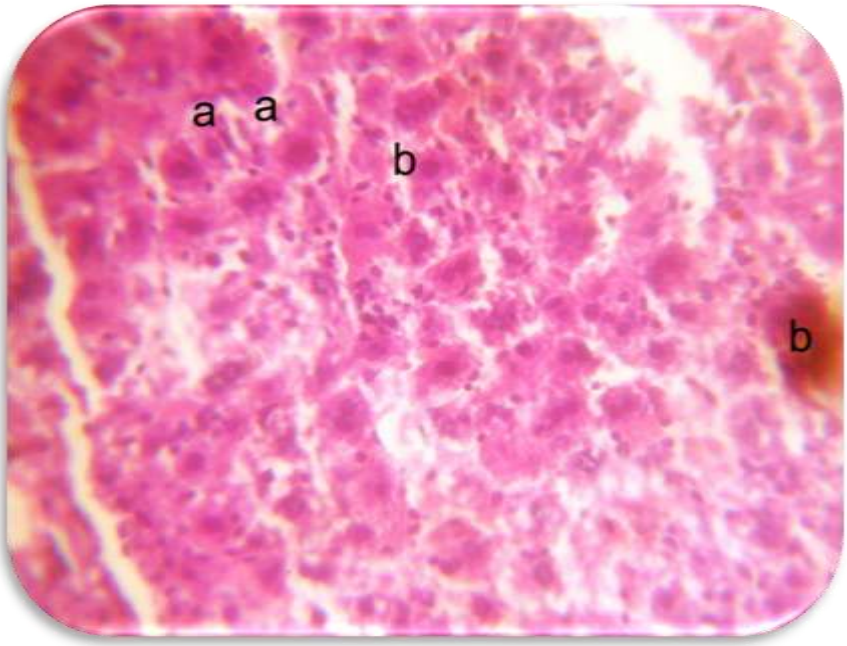

Fig.9: Histological section of mice liver treated with gentamycin and Ag for 8 days, showed megalohepatocyte (a), many apoptotic cells (b). H&E. 165X

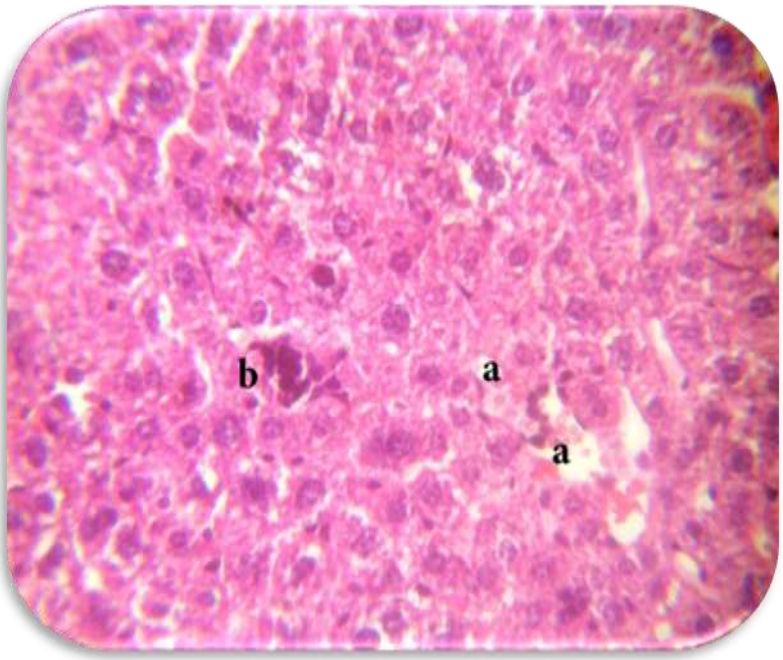

Fig. 10: Histological section of mice liver treated with gentamycin and Ag for 8days, showed mild Vacuolar degeneration (a) and apoptotic cells(b). H&E, 165X

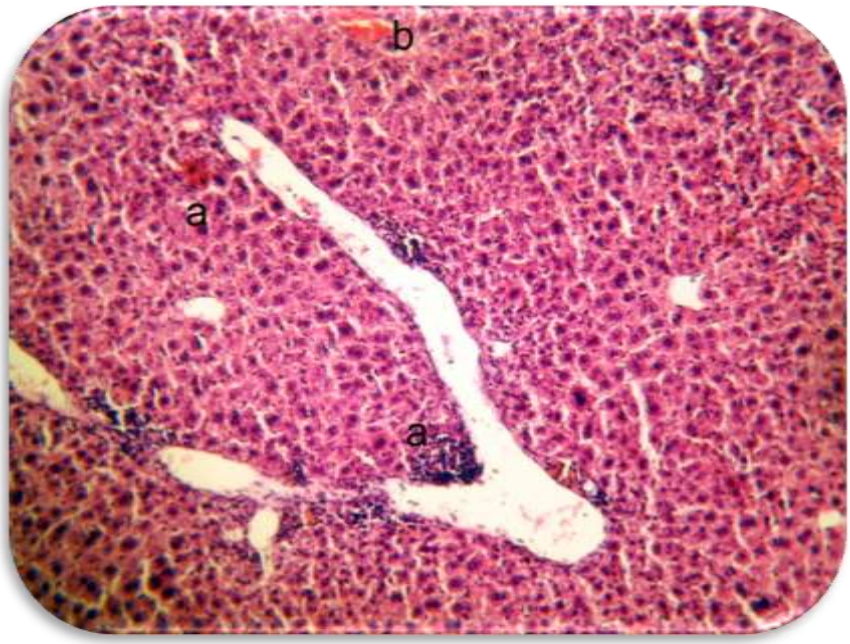

Fig.11: Histological section of mice liver treated with gentamycin and Ag for 8 days, showed focal area of apoptosis (a) and congestion (b). H&E. 115X.

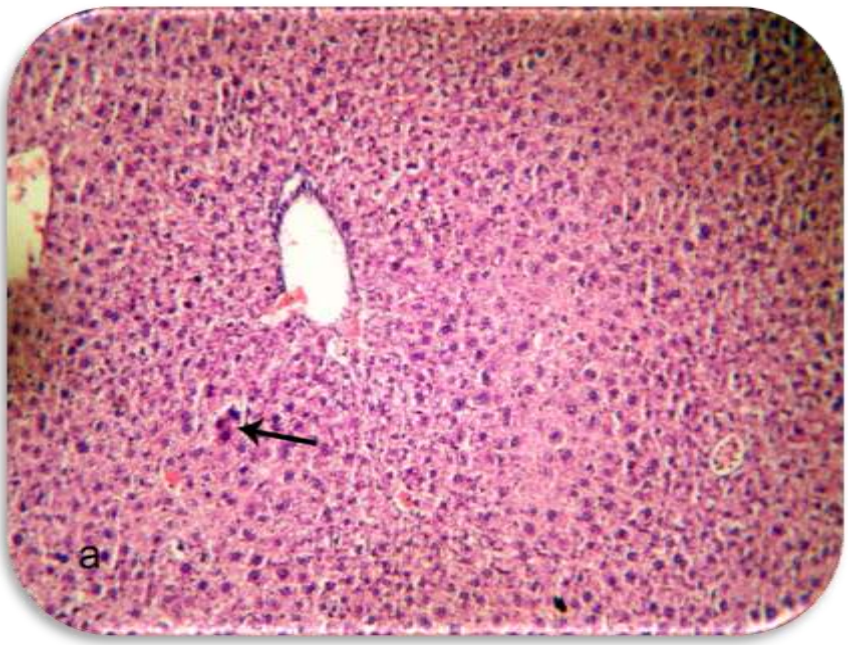

Fig.12: Histological section of mice liver treated with gentamycin and Ag for 8 days, showed sever apoptosis (arrow) and hypertrophy of kupffer cells associated with meliorating picture (a). H&E. 115X.

Discussion:

Biotransformation of drugs by the liver, which the major organ for xenobiotic metabolism has been well established. The information of reactive metabolites may indeed be underlying mechanism of variety of toxic drug actions aminolycosides (gentamycin) which damage kidney and the inner ear (2), also many evidence suggestive that theis drug can damage liver by formation of cytotoxin by a post mitochondrial "Sg" fraction and microsomal enzymes linked to cytochrome P450 (13).

This study showed that i.p. injection of 40mg/kg/ day of gentamycin caused significant liver injury in mice at 8 days after dosing as reflected by the progressive increase in serum enzyme specially ALT and AST although that gentamycin consistently produced nephrotoxicity but indirectly by hepato-renal syndrome gentamycin can produce hepatotoxicity. In the present study increase level of ALT and AST in the serum was highly significant differences between group treated with gentamycin G2 ,G3 and G4, this results are in agreement with (14) who noted that elevated in ALT and AST mean a marked for liver injury. Mice treated with Ag in G3 and G4 showed amelioration in enzymatic level (ALT +AST) but not reach to normal level as in control group, these results indicated that Ag have ability as a protective factor to decrease liver damage.

Our study, illustrated significant reduction in GSH in group treated with gentamycin (G2) and significantly increase in level of MDA, these results was in agreement with some studies suggest that aminoglycoside antibiotics can stimulate the formation of reactive oxygen species (ROS) which may directly involved in gentamycin induced in renal failure and membrane lipid peroxidation (15). While mice treated with Ag in G3 and G4 showed significant increase in level of GSH and significant reduction in MDA level in serum, these results indicated to that Ag have ability as antioxidant because have ameliorate hepatotoxicity by increase the level of GSH and reduction MDA although it haven't reach to normal value. its agreement with many research.

Who reported that Ag has free radical (nitric oxide) scavenging properties and macrophage inhibition functions (16). Oral administration of Ag protected mice from gentamycin induced hepatotoxicity, our results is in agreement with (17). Who suggest that Ag may find clinical application in a variety of condition where cellular damage is a consequence of oxidative stress. On other hand, this study showed the pathological change of liver that produce from treatment with 40 gm/kg/day gentamycin. Histopatologically, liver injury can be categorized by a number of systems, some systems

are based on the histological lesion produced, i-e, inflammation, necrosis, apoptosis and cholestasis. our study revealed that grouped mice treated with gentamycin revealed many lesions represented by hemorrhage, congestion, apoptosis of hepatocytes, pigmentation and necrosis, vacuolar degeneration as indicated of presence of liver injury, which considered as indirect drug induced liver injury, because the target organ of gentamycin are kidney and ear (2).So, in this study gentamycin responsible for hepatotoxicity may be through the depletion of GSH and initiates covalent binding cellular proteins, these events lead to mitochondrial dysfunction and oxidative stress. Apoptosis is another lesion produced by gentamycin and Ag , in course of apoptotic cell death intact cell organelles and cell membranes are fragmented into small membrane bound bodies. Cellular DNA is cleaved by endonuclease to 120-180 base pair fragments (18, 19). Classically apoptosis can be triggered via two basic mechanisms in hepatocytes, interactions between death lignads (Fas-ligand, TNF) and death receptors (Fas and TNFR-1) that trigger caspase 8 acting cytochrome activation or damage to mitochondrial inner membrane releasing cytochrome c (13, 20, 21).

In gentamycin treatment trigger apoptosis may be occurs due to activation of protein kines G and mitochondrial injury (increase ALT + AST) in addition to bioactivation by the cytochrome P450 system can produce reactive molecules that engender oxidative stress which can then be a stimulus to induce synthesis of Fas ligand and increase susceptibility of hepatocytes to apoptosis (20). In group treated with Ag and Gentamycin , the histopathological changes revealed more apoptosis of hepatocytes in addition to meliorating hemorrhage and removed hemosiderin pigment, these changes occurs due to that Ag may induce the immune system of liver (innate and adaptive) particularly tissue macrophage (KC) for clearance of particulates and soluble molecules and also induction of tolerance to food antigens derived from gastrointestinal tracts. For this tolerance, including apoptosis (22). KC play important role to removal of pathogens entering the liver via portal venous blood upon activation, KC produce various cytokines and other mediator including prostanoids, nitric oxide and ROS (23). The effect of Ag to reduce the damage of hepatic tissue take place due to have ability to scavenging nitric oxide in order to blocking Oxidative stress (17). Moreover, Ag was found to blocking function hepatic macrophage to prevent release nitric oxide (24). So Ag has strong antioxidant properties and major mechanism for the induction of these toxicities is the generation of free radicals (25, 26). In conclusion, the present study revealed that administration of Ag orally to mice at concentration

40gm/kg/for eight days, have a significantly meliorating the pictures of liver and reducing MDA in addition to ALT in serum of mice.

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