ORIGINAL ARTICLE

Protective effect of dietary inclusion of *Aegle marmelos* fruit on gentamicin-induced hepatotoxicity in rats

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Abstract

Background: The present study was undertaken to investigate the modulatory efficacy of dietary inclusion of *Aegle marmelos* fruit against gentamicin-induced liver injury in Wistar albino rats. **Methods**: The animals were divided into five groups and each containing six animals. Group I and Group II received basal diets and basal diets with 4% w/w *A. marmelos*, respectively. Group III, Group IV, and Group V fed basal diets, basal diets containing 2% w/w, and 4% w/w *A. marmelos*, respectively for 27 days before gentamicin administration. The intraperitoneal administration of gentamicin (100 mg/kg body weight) for 3 days leads to disturbed the hepato-specific serum markers. Liver functions were measured by the valuation of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), serum acid phosphatase (ACP), and total bilirubin. The oxidative stress parameter and antioxidant markers were also evaluated. Moreover, histopathological evaluation was performed to assess liver case regarding inflammatory infiltration or necrosis. **Results:** Gentamicin produced significant changes in liver marker enzymes (increase in SGOT, SGPT, ALP, ACP, and bilirubin level) and histological (damage to hepatocytes). Gentamicin also leads to significant changes in the level of lipid peroxidation, superoxide dismutase, glutathione, and catalase in liver homogenate. The administration of basal diet supplemented with 2% and 4% *A. marmelos* to rats significantly reversed the above changes compared to the control group as observed in the gentamicin-treated rats. **Conclusions**: The results propose that dietary inclusion of *A. marmelos* fruits possesses promising hepatoprotective effects and could protect the liver against gentamicin-induced hepatic injury.

Key words: *Aegle marmelos*, dietary inclusion, gentamicin, hepatoprotective

INTRODUCTION

Gentamicin is the most commonly used aminoglycoside antibiotic and is indicated for moderate-to-severe bacterial infections caused by sensitive agents, primarily Gram-negative bacteria. Gentamicin is known for its hepatotoxicity, and one of the possible mechanisms suggested is damage due to the generation of free radicals. Mostly gentamicin induces a dose-dependent hepatotoxicity during therapeutic courses.[1] There are reports which suggest the role of reactive oxygen species/nitrogen species, in association with increased lipid peroxide formation and decreased activity of antioxidant enzymes in gentamicin-induced hepatotoxicity. The association of gentamicin with negatively-charged phospholipids and their accumulation in the lysosomes of tubular cells leads to phospholipidosis by inhibition of lysosomal phospholipases, which may trigger necrosis. It has been reported that, at the cellular level, aminoglycosides interfere with protein synthesis, especially by inhibition of translocation. Approximately 8-26% of patients who receive aminoglycosides for more than 7-10 days develop mild necrosis in the liver.[2,3]

Aegle marmelos belonging to the family *Rutaceae* has been widely used in indigenous systems of Indian medicine due

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to its various medicinal properties [Figure 1]. *A. marmelos* is a commonly used food, and their medical properties have been well recognized since time immemorial. *A. marmelos* fruit possesses anti-dyspepsia, antidiarrhea, and antidysentery. The fruit is used as a dietary supplement to cure intermittent fever, mental disease, hypoglycemic effect, antifungal effect, antimicrobial, analgesic, anti-inflammatory, antipyretic, anti-dyslipidemic activity, immunomodulatory activity, antiproliferative activity, wound-healing activity, antifertility, insecticidal activity, and many more.[4-6] Flavonoids, in particular, are polyphenolic compounds, widely distributed in the *A. marmelos* fruit, and exhibited various pharmacological activities including hepatoprotective activity.

Researchers all over the world are working on the development of hepatoprotective herbal drugs. However, through literature survey, it has been found that most of the work has been carried out on the plant extracts, and very little work done on the valuation of the medicinal properties of dietary supplement. The administration of food containing antioxidant components can definitely protect, it definitely protects the organs of those patients who are administrating synthetic drugs. The present works to also ensure to keep human beings healthy after completion of a course of synthetic drugs. The medicated dietary supplement is best alternative herbal preparation to replace the crude extracts which are currently in use. It is more convenient for administration by patients compared to crude extract. In view of its diverse spectrum of pharmacological properties, it was worthwhile to investigate and establish the hepatoprotective potential of dietary containing *A. marmelos* against gentamicin-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Collection and Authentication of Plant

Based on the information collected from the tribal people of Chhattisgarh, the fruit part of the plant for the proposed study was selected. The plant was initially identified by their vernacular names through consultations with the local people. Fruit parts of *A. marmelos* [Figure 1] was collected from the tribal belt of Bilaspur, Chhattisgarh, India, during October 2015. The collected plant was identified and authenticated by Dr. Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum, Delhi, Council of Scientific and Industrial Research-National Institute of Science Communication and Information Resources, New Delhi, India, and the voucher specimen (AML-1) was deposited in the herbarium in the Columbia Institute of Pharmacy, Tekari, Raipur, Chhattisgarh, India.

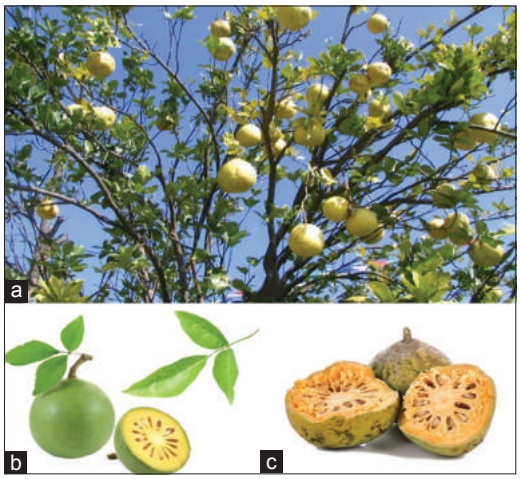

Figure 1: *Aegle marmelos* (Beal) with different parts, (a) tree; (b) fruit and leaf; (c) ripe fruit with pulp

The fruit was shade dried, reduced to a coarse powder, and stored in an airtight container till further use.

Experimental Animals

The adult Wistar albino rats of either sex weighing 150-180 g were used for the study. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed in a temperature of 24 ± 2°C and relative humidity of 30-70%. A 12/12 h light and dark cycle was followed. All animals were fed on a standard balanced diet and provided with water *ad libitum*. The experimental procedures and protocols used in the study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) registered under Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, with the registration number 1321/PO/ReBi/S/10/CPCSEA; dated 22/10/2014 Columbia Institute of Pharmacy, Tekari, Raipur, Chhattisgarh, India, and in accordance with the guidelines of the CPCSEA. The registration number is CIP/IAEC/2016-17/080.

Preparation of Dietary Inclusion

The basal diet (50% skimmed milk, 36% corn starch, 10% groundnut oil, and 4% mineral and vitamin premix) was prepared and fed to normal and control group animals. The basal diet supplemented with 2% w/w and 4% w/w powdered fruit pulp of *A. marmelos*, respectively, and fed to normal- and hepatotoxicity-induced animals.[7]

Gentamicin-induced Liver Injury

All the animals were divided into the five groups; each group consisted of 6 animals, and they received the treatment as follows:

1. Group I: Normal and received basal diet
2. Group II: Received basal diet + 4% w/w *A. marmelos* fruit
3. Group III: Control group received basal diet + gentamicin (100 mg/kg i.p.) for 3 days
4. Group IV: 2% w/w *A. marmelos* for 27 days + gentamicin (100 mg/kg i.p.) for 3 days

5. Group V: 4% w/w *A. marmelos* for 27 days + gentamicin (100 mg/kg i.p.) for 3 days.

The experiments were terminated after 30 days. The rats were subjected to an overnight fast, after which they were decapitated by cervical dislocation. The blood was rapidly collected in separate ethylenediaminetetraacetic acid bottles by direct heart puncture, centrifuged at 3000 r/min for 10 min to separate the plasma, and used for the estimation of various biochemical parameters. Similarly, the livers were isolated, rinsed in cold saline (0.9% NaCl), and homogenized in phosphate buffer (pH 6.9). The homogenates were centrifuged at 7500 r/min for 10 min to obtain the clear supernatant. The clear supernatant obtained was used for determination of *in vivo* antioxidant activity.[8]

Biochemical Studies

Serum separated by centrifugation was used to determine serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), serum acid phosphatase (ACP), and total bilirubin.[9,10]

Analysis of Antioxidant Enzymes of Liver Tissue

The antioxidant activities in the rat liver homogenate were assayed for superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and activity lipid peroxidation (LPO).[11-13]

Histopathological Studies

The liver was dissected out, and the liver samples were excised from the experimental animals of each group and washed with the normal saline. Initially, the materials were fixed at 10% buffered neutral formalin and then with a bovine solution. They were processed for paraffin embedding following the microtome technique. The sections were taken at 50 µ thicknesses processed in alcohol-xylene series and were stained with alum-hematoxylin and eosin. The sections were examined microscopically for the evaluation of histopathological changes in the liver.[14,15]

Statistical Analysis

The experimental results were expressed as the mean ± standard error of the mean for six animals in each group. The biochemical parameters were analyzed statistically using one-way analysis of variance, followed by Dunnett's multiple comparison test. The $P < 0.05$ was considered as statistically significant.

RESULTS

Table 1 showed the effect of various treatments on serum SGOT, SGPT, ALP, ACP, and bilirubin. The animals treated with gentamicin, developed significant liver damage, were observed from the alteration in the activities of serum enzyme (SGOT, SGPT, ALP, and ACP) and bilirubin in serum [Table 1]. The level of the SGOT, SGPT, ALP, ACP, and bilirubin values was significantly increased in the gentamicin-treated rats compared to normal animals fed with basal diet. The animals treated with diets containing 2% w/w and 4% w/w *A. marmelos* significantly reduced the serum level. The serum level of animals received diets containing only 4% w/w *A. marmelos* (Group II) was normal, and it indicates that liver functions were not affected.

Table 2 exhibited animals treated with gentamicin at a dose of 100 mg/kg body weight leads to significant reduction

Table 1: Effect of diets supplemented with *A. marmelos* on the liver function test for different parameters in animals treated with gentamicin

Group	Treatment	Serum level				Bilirubin (mg/100 ml of blood)	
		SGOT (U/L)	SGPT (U/L)	ALP (U/L)	ACP (U/L)	Direct (mg/dl)	Total (mg/dl)
I	Normal rats (basal diets)	68.14±5.42	87.36±7.14	142.83±4.76	125.61±5.85	0.29±0.07	0.64±0.06
II	*A. marmelos* (4% w/w) + Normal rats	69.84±5.74	89.26±8.11	154.23±5.85	131.61±6.15	0.32±0.14	0.69±0.26
III	Control rats gentamicin (100 mg/kg) + Basal diets	169.14*±5.12	175.42*±3.72	231.64*±4.27	231.24*±2.41	1.04*±0.07	1.83*±0.32
IV	*A. marmelos* (2% w/w) + Gentamicin (100 mg/kg)	98.53[a]±4.76	121.72[a]±3.18	173.21[a]±5.61	163.42[a]±4.53	0.62±0.43	1.05±0.27
V	*A. marmelos* (4% w/w) + Gentamicin (100 mg/kg)	65.39[a]±7.36	84.72[a]±2.82	138.54[a]±4.92	131.27[a]±3.71	0.31[a]±0.63	0.61[a]±0.72

Values are expressed as mean±SEM, n=6 in each group. *P<0.05 when compared with normal Group I and *A. marmelos* (4% w/w) Group II, [a]P<0.05 when compared with gentamicin (100 mg/kg)-treated Group III considered as statistically significant. SEM: Standard error of the mean, *A. marmelos: Aegle marmelos,* SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, ALP: Serum alkaline phosphatase, ACP: Serum acid phosphatase

Table 2: Effect of diets supplemented with fruits of *A. marmelos* on oxidative stress induced by gentamicin in the liver of experimental animals

Group	Treatment	Enzymes involved in oxidative stress in the liver			
		LPO (mole/g)	SOD (U/g)	GSH (µmole/g)	CAT (U/mg)
I	Normal rats (basal diets)	58.14±3.52	42.73±4.18	2.36±0.57	5.53±0.82
II	*A. marmelos* (4%) + Normal rats	57.26±3.19	40.71±2.34	2.46±0.48	5.71±0.54
III	Control rats (Gentamicin 100 mg/kg)	123.38±5.24*	11.18±3.59*	0.19±0.24*	0.83±0.61*
IV	*A. marmelos* (2%) + Gentamicin (100 mg/kg)	85.51±4.82[a]	31.29±5.24[a]	1.91±0.68[a]	3.42±0.47[a]
V	*A. marmelos* (4%) + Gentamicin (100 mg/kg)	65.42±3.29[a]	48.57±6.17[a]	2.42±3.52[a]	5.72±1.36[a]

Values are expressed as mean±SEM, n=6 in each group. *P<0.05 when compared with normal Group I and *A. marmelos* (4% w/w) Group II, [a]P<0.05 when compared with gentamicin (100 mg/kg)-treated Group III considered as statistically significant. SEM: Standard error of the mean, LPO: Lipid peroxidation, SOD: Superoxide dismutase, GSH: Glutathione, CAT: Catalase

in the activities of the antioxidant enzymes, namely, SOD, GSH, and CAT compared to the normal group, while increasing in LPO level. Conversely, feeding the gentamicin-treated rats with diets containing either 2% w/w and 4% w/w *A. marmelos* fruit inclusions (Groups IV and V, respectively) caused a marked reversal in the depleted antioxidant enzyme level.

Histopathological examination of liver sections of a normal Group I and Group II 4% w/w *A. marmelos* showed a normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and the central vein as shown in Figures 2 and 3. Disarrangement of normal hepatocytes with centrilobular necrosis, vacuolization of the cytoplasm (VC), and fatty changes (FC) were observed in Group III received gentamicin (100 mg/kg body weight i.p.)-intoxicated rat livers [Figure 4]. The liver sections of the rats in Group IV and V treated with 2% w/w and 4% w/w of *A. marmelos*, respectively, showed a sign of protection against gentamicin intoxication as evident by the presence of normal hepatic cords and absence of necrosis with minimal inflammatory conditions around the central vein [Figures 5 and 6].

DISCUSSION

The liver imparts chief role in metabolism, detoxification, and protein synthesis. Drug-induced hepatotoxicity is one of the major causes of human mortality worldwide. The researchers are working for the protection of the liver against gentamicin-induced toxicity.

The clinical uses of gentamicin have been narrow due to its associated side effects. It has been observed that most patients taking gentamicin encountered liver inefficiency problem. Consequently, taking these medications face limitations due to the fact that one of the major side effects of gentamicin is creating hepatotoxicity. The gentamicin induces the production of free radicals, which can be seen after the use of gentamicin in cells, is effective in inducing toxic impacts of this drug on the structure and function of tissues.[16-18]

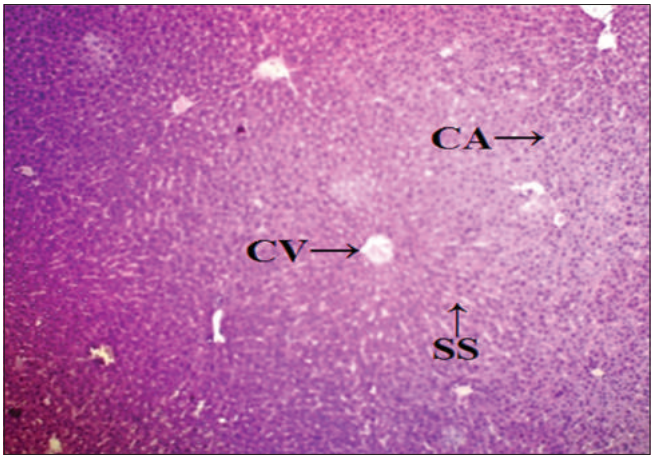

Figure 2: Microscopic photograph of the liver section of normal rats (Group I); showing histology H and E staining (×100) CA: Cellular architecture with distinct hepatic cells, SS: Sinusoidal spaces, CV: Central vein

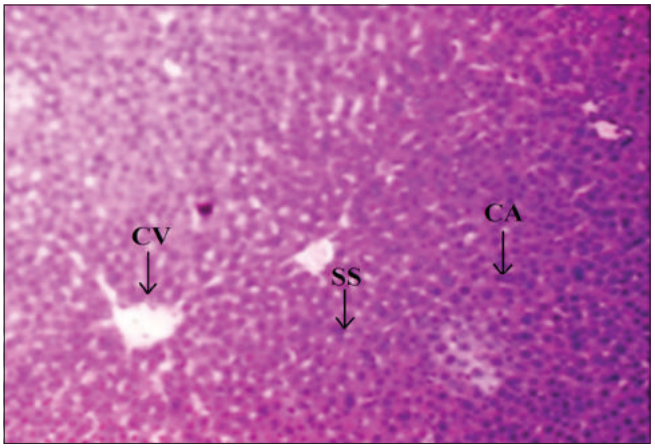

Figure 3: Microscopic photograph of a liver section of control rats with *Aegle marmelos* (4% w/w) (Group II); showing histology H and E staining (×100). CA: Cellular architecture with distinct hepatic cells, SS: Sinusoidal spaces, CV: Central vein

To evaluate the liver injury, biochemical markers (SGOT, SGPT, ALP, and ACP activity and serum bilirubin) levels were measured. The findings demonstrated the hepatotoxicity

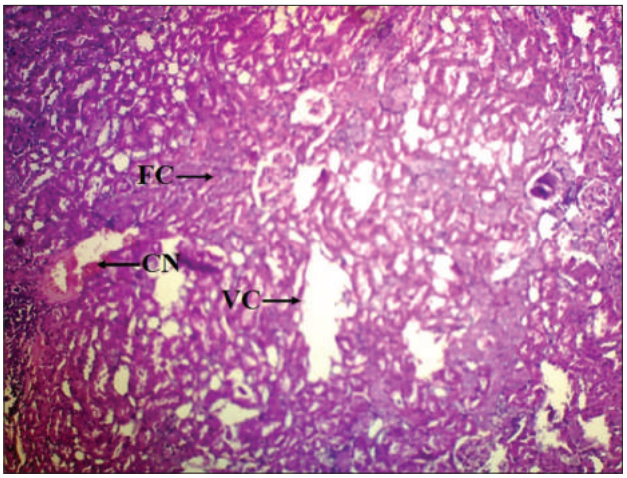

Figure 4: Microscopic photograph of liver section of gentamicin-treated rats (Group III); showing necrosis of the hepatic cells H and E staining (×100) CN: Centrilobular necrosis, VC: Vacuolization of cytoplasm, FC: Fatty changes

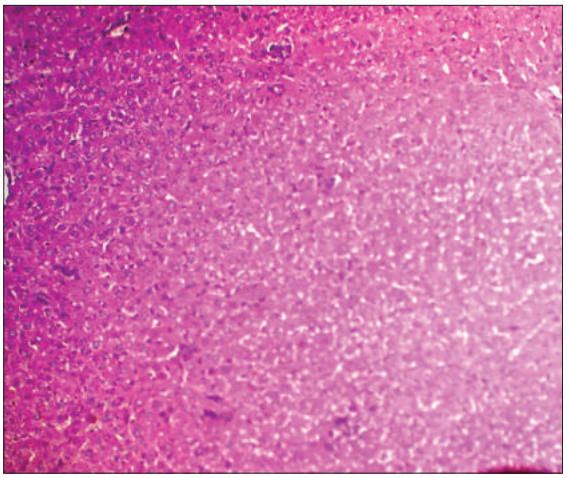

Figure 6: Microscopically photograph of liver section of Gentamicin (100 mg/kg) + *Aegle marmelos* (4% w/w)-treated rats (Group V) showing necrosis of the hepatic cells is almost prevented H and E staining (×100)

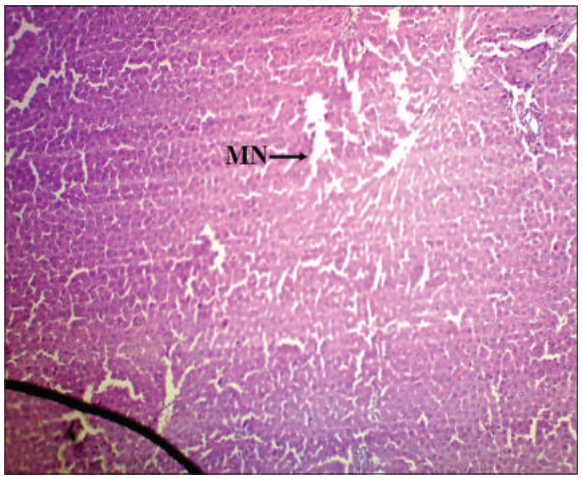

Figure 5: Microscopically photograph of liver section of Gentamicin (100 mg/kg) + *Aegle marmelos* (2% w/w)-treated rats (Group IV); showing necrosis of the hepatic cells is mildly prevented H and E staining (×100). MN: Mild necrosis

due to gentamicin was confirmed by elevated levels of biochemical parameters such as SGOT, SGPT, ALP, ACP, and total serum bilirubin. The impairment of hepatic cell or membrane leads to discharge enzyme into circulation, and it has been observed in the studies. A higher level of SGOT indicates the liver damage, due to oxidative stress produced from gentamicin during metabolism by hepatic microsomes which in turn cause peroxidation of lipid of cellular membranes. SGPT catalyzes the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, SGPT is more specific to the liver and is thus a better parameter for detecting liver injury. The increased level of SGOT and SGPT in gentamicin-induced liver injury is an indicator of cellular leakage and loss of membrane integrity of liver cells.[19] The animal fed with diets containing *A. marmelos* fruit reversed the increased levels of SGOT and SGPT as a result of the stabilization of plasma membrane and the repair of hepatic cell damage induced by gentamicin.

The serum level of ALP, ACP and bilirubin is directly related to the functions of hepatic cells. Increase in serum level of ALP and ACP is due to increased synthesis, in the presence of increasing biliary pressure. Hyperbilirubinemia was due to the excessive heme destruction and block of bile duct within the liver. Accordingly, there is a mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged hepatocytes.[20-22] The findings exhibited that the animals treated with diets containing *A. marmelos* fruit significantly inhibit the generation of ALP, ACP, and bilirubin. Effective control of bilirubin level and alkaline phosphatase activity point toward on early improvement in the secretory mechanism of the hepatic cell.

The LPO level in the liver tissue was markedly increased in response to gentamicin intoxication, indicating oxidative damage of the liver. Gentamicin administration also reduced the levels of SOD, GSH, and CAT in the liver tissue compared to the normal rats. The elevated LPO in the liver indicates failure of antioxidant defense mechanisms. The oxidative stress plays an important role for unbalance between the production of reactive oxygen species and antioxidant defenses, It leads to the damage of liver cells. The marked declined in the leakage of liver enzymes into the serum also confirmed the inhibitory effect of a diet containing *A. marmelos* fruit against LPO.[22-24] In contrast, there is a significant increase in SOD, GSH, and CAT levels compared to control group. A variation of these antioxidant defenses clearly contributed to the antioxidant and hepatoprotective activity of diet containing *A. marmelos* fruit.

Further, the biochemical findings established by histopathological results. Histopathological studies showed that gentamicin caused centrilobular necrosis, VC, and

FC changes of the liver tissue. This may be a result of the formation of free radicals and oxidative stress induced by gentamicin. The animal fed with diet containing *A. marmelos* fruit exhibited improved and protection of hepatic cells, this confirmed the results of biochemical studies. Hence, the adverse effects caused by gentamicin in rats receiving *A. marmelos* fruit supplemented diets can be attributed to the antioxidant property of *A. marmelos*.

CONCLUSION

Based on the results of this study, the hepatoprotective effect of a diet containing *A. marmelos* fruit is attributed to its ability to reduce the rate of LPO, to enhance the antioxidant defense status, and to guard against the pathological changes of the liver induced by gentamicin intoxication. The significant *in vivo* antioxidant activity also proposed that a dietary supplement of *A. marmelos* fruit may produce a beneficial effect against oxidative stress and protect the liver from intoxication. Further, dietary inclusion of *A. marmelos* fruit may be a cheap management strategy for the liver against acute hepatotoxicity or gentamicin-induced liver damage and helpful for the researcher, physicians, and medical scientific societies to overcome the problem arises with gentamicin treatment to the patients.

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