Journal of Functional Foods 74 (2020) 104200

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Garcinia mangostana peel extracts exhibit hepatoprotective activity against thioacetamide-induced liver cirrhosis in rats

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ARTICLE INFO

Keywords:
Traditional therapy
Garcinia mangostana
Thioacetamide
Liver cirrhosis
Hepatoprotective effect

ABSTRACT

The study *in vivo* evaluated the influence of ethanolic extract of *G. mangostana* peel on the histology, immunohistochemistry, and biochemistry of thioacetamide (TAA) induced liver cirrhosis in *Sprague Drawly* (SD) rats. The rats were distinctly administered with two doses of *G. mangostana* (250 mg/kg and 500 mg/kg) with TAA showed a significant reduction in liver index and hepatocyte proliferation with much lesser cell damage. These groups were significantly down-regulated the PCNA, α-SMA, and TGF-β1. The liver homogenate exhibited increased antioxidant enzymes (SOD and CAT) activities accompanied with decline in malondialdehyde (MDA) level. The serum level of bilirubin, total protein, albumin and liver enzymes (ALP, ALT, and AST) were restored to normal and were comparable to that normal control and silymarin with TAA treated groups. Thus normal microanatomy of hepatocytes, suppression of PCNA, α-SMA, TGF-β, improved antioxidant enzymes, reduced MDA with restoration of liver biomarkers demonstrates hepatoprotective influence of *G. mangostana*.

1. Introduction

Mangosteen (*Garcinia mangostana*) is a tropical edible fruit belonging to the Clusiaceae family. It is apparently originated from Southeast Asian countries and has been used as traditional medication (Pedraza-Chaverri, Cárdenas-Rodríguez, Orozco-Ibarra, & Pérez-Rojas, 2008). The mangosteen peel typically used to treat urinary tract infections (UTIs), diarrhea, eczema, ulcers, obesity and gastrointestinal disorders (Akao, Nakagawa, & Nozawa, 2008; Obolskiy, Pischel, Siriwatanametanon, & Heinrich, 2009; Abuzaid, Sukandar, Kurniati, & Adnyana, 2016; Karim, Jenduang, & Tangpong, 2018). The main constituents of *G. mangostana* pericarp are polyphenolic acids including xanthones and tannins. The biological activities namely antioxidant, anti-tumor, anti-inflammatory, anti-glycemic, antiallergy, antibacterial, anti-fungal, and anti-viral were reported from the pericarp of this fruit (Pedraza-Chaverri et al., 2008; Ibrahim et al., 2014) and might be beneficial for the treatment of liver fibrosis (Chin, Shin, Hwang, & Lee, 2011). Reduction of high fat-diet induced hepatic steatosis was observed in experimental rat upon administration of pericarp of *G. mangostana* (Tsai et al., 2016). The *in vivo* antioxidative potential of *G. mangostana* peel extract was earlier documented (Ibrahim et al., 2014; Samuagam, Sia, Akowuah, Okechukwu, & Yim, 2015) hence this functional food could be explore to cure oxidative stress-mediated liver cirrhosis in humans.

There have been numerous reports available regarding the oxidative stress-mediated hepatotoxic action of TAA leading to liver cirrhosis in experimental animals (Alshawsh, Abdulla, Ismail, & Amin, 2011; Kadir, Othman, Abdulla, Hussan, & Hassandarvish, 2011; Alkiyumi et al., 2012; Salama, Abdulla, AlRashdi, & Hadi, 2013; Bardi et al., 2014; Rouhollahi, Moghadamtousi, Abdulla, & Mohamed, 2015; Urrutia-

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https://doi.org/10.1016/j.jff.2020.104200
Received 15 July 2020; Received in revised form 2 September 2020; Accepted 5 September 2020
Available online 18 September 2020

Hernández et al., 2019; Yang et al., 2019). The TAA principally damage the liver cells wherever an extra quantity of cytochrome P450 oxidases simplify its alteration to poisonous intermediates, followed by a rise in reactive oxygen species, rise in MDA, and declaration of pro-inflammatory cytokines (Luster, Simeonova, Gallucci, Matheson, & Yucesoy, 2000). The elevated free radicals damages to proteins, adipocytes, and DNA of nuclei. TAA encourages hepatic cell damage following its interruption to TAA sulphene as well sulphone, via a thoughtful trail which comprises CYP4502E1-enabled bio *trans*-development (Ramaiah, Apte, & Mehendale, 2001). Chronic liver cirrhosis accompanying fibrosis, hepatocyte damage, and liver dysfunction cause elevated serum level of liver enzymes viz. alkaline phosphatase (ALP), Alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The proliferating cell nuclear antigen (PCNA) is a nuclear protein that is expressed in the mitosis phase. Earlier immune-staining has revealed increased nuclear PCNA staining in liver cirrhosis in rats (Bardi et al., 2014; Salama et al., 2018). The liver cirrhosis contains abundant extracellular matrix (ECM) collagen (Types 1 and 3), due to the effect of transforming growth factor beta 1 (TGFβ1) and alpha-smooth muscle actin (α-SMA). TGFβ1 is imperative cytokines in the commencement and development of cirrhosis in the liver by stimulation of hepatic stellate cells (HSC) increase synthesis of α-SMA in ECM protein (Kadir et al., 2014; Salama et al., 2018). TGF-β is pro-fibrotic cytokine since it excites ECM synthesis and prevents ECM deprivation (Kadir et al., 2014; Yang et al., 2019).

Silymarin is a flavonolignan that has been existing fairly recently as a hepatoprotective mediator. Silymarin is used for the dealing of plentiful liver ailments characterized by deteriorating necrosis and functional weakening (Abenavoli et al., 2018). Many studies by various co-researchers used silymarin as standard or reference drug for the hepatoprotection against TAA-induced liver injuries in rats have been previously reported elsewhere (Amin et al., 2012; Wong et al., 2012; Kadir, Kassim, Abdulla, & Yehye, 2013; Salama, Abdulla, Alrashdi, Ismail, et al., 2013; Alshawsh, Amin, Ismail, & Abdulla, 2015; Bagherniya, Nobili, Blesso, & Sahebkar, 2018; Mousa et al., 2019).

In scientific literature, there are several studies regarding the hepatoprotective activity of medicinal plants against TAA-induced liver injuries in rats that were previously reported by several academics (Alshawsh et al., 2011; Amin et al., 2012; Rouhollahi et al., 2015). The goal of the existing education was to measure the hepatoprotective influence of *G. mangostana* in TAA-induced oxidative stress-mediated liver cirrhosis in the rats. Here, we focused on the *in vivo* evaluation of gross morphology, liver histology, immunohistochemistry, and biochemistry of the rats upon TAA-induced liver cirrhosis in the presence of the ethanolic extract of *G. mangostana* peel.

2. Material and methods

2.1. Ethics declaration

This research approved by the Ethics Committee for Animal Experimentation, Faculty of Medicine, University of Malaya, Malaysia (Ethic No. PM/12/05/2019 MAA (R)). Altogether rats acknowledged humane attention giving to principles drawn in Guide for Care and Use of Research Laboratory Animals prearranged by the United States National Academy of Sciences and Issued by National Institutes of Healthiness (OECD, 2002).

2.2. Preparation of mangosteen pericarp ethanolic extracts

G. mangostana mature fruit was obtained from Malaysian farmers in Selangor and identified by herbarium staff with the voucher number SK 3283/18 placed in Herbarium of Institution of Bioscience, University Putra Malaysia. The pericarp was collected from *G. mangostana* fruit, formerly dehydrated in shade and milled into fine powder. Next, it was extracted using one liter of 95% alcohol for 3 days. The extract was sieved via filter paper and purified underneath condensed pressure in a rotary evaporator then freeze-dried and *G. mangostana* pericarp ethanolic extract in dried powder form was produced. The dried extract was then liquified in 10% Tween 20 and fed po to animals at a dosage of 250 and 500 mg/kg (Almagrami et al., 2014).

2.3. Acute toxicity test

Acute toxicity was required for checking the safety of *G. mangosteen's* biologic activity is extremely advised to prevent any undesirable adverse side effects. Acute toxicity test was conducted according to Organization for Economic Cooperation and Development (OECD) guidelines, 2002. Experimental rats acquired from Animal House Experimental Unit of University Malaya. Acute toxicity test was performed to fix toxic dose of *G. mangostana* extract. Rats were fed normal rat pellets ad libitum and tap water. Thirty-six rats (18 males and 18 females) assigned similarly each into 3 groups and administered vehicle (10% Tween 20); 2000 mg/kg and 5000 mg/kg of *G. mangostana,* respectively. These doses were selected based on the previous efficacy studies (Bunyong, Chaijaroenkul, Plengsuriyakarn, & Na-Bangchang, 2014). Experimental rats starved over-night (food) but allowed excess to water before to start treatment. Rats were watched for 30 min and 1, 2, 3, and 24 hrs for any toxic signs or death. Then rats fasted overnight on day14th and sacrifice on day 15th using general anesthesia, i.e. Ketamine (30 mg/kg, 100 mg/mL) and Xylazine (3 mg/kg, 100 mg/mL) (Farghadani et al., 2019). Blood was collected by intracardiac puncture for liver and kidney function tests. All the rats were subsequently sacrificed by cervical dislocation. Histopathology of liver and kidney stained by H & E stain and analyzed for any structural changes (Alsalahi et al., 2012).

2.4. Thioacetamide preparation

TAA was obtained from Sigma-Aldrich, Switzerland, then was melted in 10% Tween 20 and mixing well until complete dissolved. At that time, 200 mg/kg was injected i.p to rats three times per week for 2 months. TAA induced changes in both biological in addition to morphological structures similar to that of human liver fibrosis (Amin et al., 2012).

2.5. Silymarin preparation

Silymarin (International Laboratory, USA) is a reference medication was liquefied in 10% Tween 20 and fed animals po at a dose of 50 mg/kg daily for two months (Salama, Bilgen, Al Rashdi, & Abdulla, 2012).

2.6. Experimental animals for induction of liver cirrhosis

Adult male SD rats with average weights of 155–165 g obtained from the Experimental Animal Unit and reserved in polypropylene cages (6 in each cage). Animals had allowed free access to water and normal laboratory pellet. Rats adapted under typical workroom environments for duration of one week earlier initial experimentation (Kadir et al., 2011).

2.7. Experimental design

Thirty adults male rats haphazardly separated into five clusters with six rats each.

Group 1 (normal cluster) rats fed po 10% Tween 20 and inoculated i.p with distilled water thrice weekly for 8 weeks (Alshawsh et al., 2011).

Group 2 (TAA control group) rats fed po 10% Tween 20 and injected i.p with TAA (200 mg/kg) three times per week for 8 weeks.

Group 3 (reference group) rats administered po silymarin (50 mg/kg) every day and injected i.p TAA (200 mg/ kg) thrice weekly for

Table 1
Effect of ethanol extracts of *G. mangostana* on body weight, liver weight and liver index of TAA-induced liver cirrhosis rats after 8 weeks. Data expressed as mean ± S.E.M. (n = 6 rats/group). One-way ANOVA followed by Tukey's posthoc multiple comparisons test executed to measure the significance of differences between groups. ****P < 0.0001 compared with TAA control group, #P < 0.0001 compared with normal control group.

Group	Body weight (BW) (g)	Liver weight (LW) (g)	Liver index = (LW × 100 / BW)
Normal control	304.1 ± 3.71	8.53 ± 0.022	2.80 ± 0.062
TAA + 10% Tween 20	177.4 ± 1.49$^{\#}$	11.06 ± 0.047$^{\#}$	6.23 ± 0.024$^{\#}$
TAA + Silymarin	279.5 ± 1.86****	8.42 ± 0.074*	3.01 ± 0.031****
TAA + LD 250 mg/kg	260.0 ± 1.45****	8.36 ± 0.023*	3.20 ± 0.024****
TAA + HD 500 mg/kg	276.7 ± 1.35****	8.44 ± 0.014*	3.05 ± 0.022****

8 weeks.

Groups 4 and 5 (*G. mangostana* fed clusters) rats fed po *G. mangostana* extract Low dose (LD) 250 mg/kg and High dose (HD) 500 mg/kg daily, respectively and injected ip TAA (200 mg/kg) each thrice weekly for 8 weeks (Abdulaziz Bardi et al., 2013). At the end of 2 months, rats were fasted overnight and sacrificed under universal anesthesia (Farghadani et al., 2019).

2.8. Gross appearance of liver

Livers were removed and washed in cold phosphate buffer saline punctually, blotted on filter paper, weighed using electrical digital balance and carefully inspected for any gross pathological variations by taking images (Bardi et al., 2014).

2.9. Histopathology and immunohistochemistry of liver tissue

Hepatic tissue was placed in freshly prepared 10% phosphate buffered formalin (PBF) fixative, after 6 h tissues were trimmed to small pieces (2 cm thick) and put them in cassettes, re-fixed in PBF overnight for complete fixation and embedded in paraffin using Mechanical Tissue Processing Machin. Slices at 5 μm thickness stained with H & E stain (Farghadani et al., 2019), Masson trichrome stain (Abood et al., 2015) and immunohistochemistry for histopathological assessment (Bardi et al., 2014; Kadir et al., 2014).

2.10. Mitotic figure index (PCNA stain)

Liver sections were stained immunohistochemistry (PCNA stain). Calculation of proliferative hepatic cells index of liver sections was measured via total percentage of stained cells with PCNA cells per 1000 hepatocytes, and numeral of mitotic figure cells were stated as mitotic figure index as described previously by Kadir et al. (2014).

2.11. Liver tissue homogenate for endogenous enzymes activities and lipid peroxidation (MDA)

A lobe of liver specimens was rinsed promptly with cold PBS in cold pH 7.2 and homogenate by tephlon homogenizer. Then centrifuge at 3500 rpm for 15 min at −4 °C. Supernatants were used for assessment of superoxide dismutase (SOD) and catalase (CAT) activities, and MDA level (Cayman Chemical Company, USA), according to constructer protocol (Cayman Chemical Company, USA) (AlRashdi et al., 2012).

2.12. Liver function markers

Blood was collected via intracardiac puncture under universal anesthesia, and placed in gel-activated tubes, centrifuge and examined at Clinical Diagnosis Laboratory of University Malaya Hospital for liver biomarkers parameters (Farghadani et al., 2019; Alnajar, Abdulla, Ali, Alshawsh, & Hadi, 2012).

2.13. Statistical analysis

The results were presented as mean ± standard error of mean. The significant differences between group means were analyzed with one way analysis of variance (ANOVA) followed by the Tukey's posthoc multiple comparisons test. Results were considered as the statistical significant when p < 0.05. The statistical analysis and figures were generated using Graph Pad Prism 8.4.3.

3. Results

3.1. Acute toxicity study

Experimental rats fed with *G. mangostana* at a dose of 2000 and 5000 mg/kg were under observation for 14 days. Rats have persevered active with no noticeable signs or symptoms of toxicity. Besides, no behavioral changes, body weight aberrations, no gross finding, and no kind of morbidity or mortality seen to finish two weeks of observation. Histopathology analyses of liver and kidney and serum biochemical markers showed no considerable changes among various groups. According to our results, *G. mangostana* was not toxic even at high dosage (Data not showed).

3.2. Effect of ethanol extracts of G. mangostana on body weight, liver weight, and liver index

Body weight of all rats calculated weekly and former to sacrifice. Body weights and liver masses after eight weeks of *G. mangostana* or silymarin feeding are shown in Table 1. The one way ANOVA analysis of the data showed significant differences (****p < 0.0001) between all treated groups in the body weights and liver weight with F (4, 25) value 419.5 and 649.9. The Tukey's posthoc multiple comparisons test analysis demonstrated that oral administration of 200 mg/kg TAA reduces body weights significantly in comparison to normal control group (****p < 0.0001). The liver weights of rats belonging to this group was found to increase significantly as compared to normal control group (****p < 0.0001). However, the oral administrations of *G. mangostana* peel extracts i.e. LD 250 mg/kg and HD 500 mg/kg and silymarin increases body weights with reduction in liver masses. The significant deviation in body weights of rats were observed in both administered doses of *G. mangostana* peel extract (***P < 0.001) and were comparable to that of silymarin treated hepatoprotective group. Liver weights reduced to higher extents in both doses of *G. mangostana* in comparison to TAA treated group and were comparable to that of silymarin treated and normal control groups (^{ns}p > 0.05). The uppermost liver index (liver/body weight ratio) was detected in the hepatotoxic TAA-treated group (6.23 ± 0.024). Rats fed with *G. mangostana* (LD 250 mg/kg − 3.20 ± 0.024 and HD 500 mg/kg 3.05 ± 0.022) remarkably dropped liver index analogous to rats fed 50 mg/kg silymarin (3.01 ± 0.031).

3.3. Effect of G. mangostana on macroscopic appearance of liver

The livers of normal control group rats were reddish brown in color

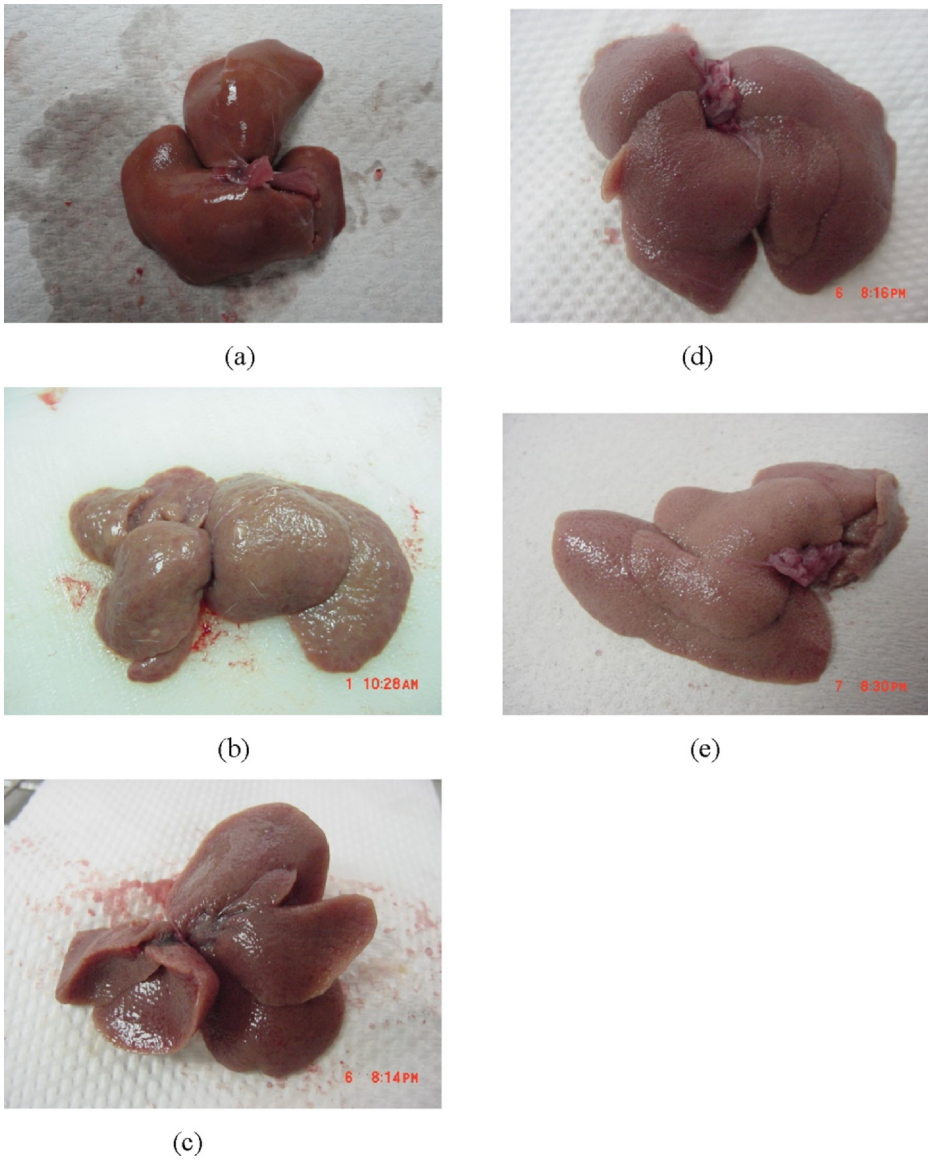

Fig. 1. The macroscopic liver morphology: Effect of *G. mangostana* peel extracts on TAA-induced liver cirrhosis in rats. (a) Normal control group, (b) TAA- treated group (Hepatotoxic control), (c) TAA + Silymarin group (Hepatoprotective), (d) TAA + 250 mg/kg *G. mangostana* group and (e) TAA + 500 mg/kg *G. mangostana* group (Original magnification × 40).

with smooth surfaces devoid of nodules (Fig. 1a). Hepatotoxic TAA treated control group rats produced liver cirrhosis. The liver appears rough and nodular, with uniform micro-nodules and macro-nodules surface (Fig. 1b). Rats fed with two different doses of *G. mangostana* extracts expressively improved TAA persuaded liver injury to close normal morphology (Fig. 1d and e). However, the livers of rats fed with HD 500 mg/kg *G. mangostana* peel extract have normal appearance and color, smooth surfaces with relatively minor micronodules. These effects were slightly superior as compared to rat treated with silymarin, a well-known hepatoprotectant (Fig. 1c).

3.4. Histopathological analysis by hematoxylin and eosin (H&E) staining

The normal control group showed normal liver with hepatic cords, sinusoidal spaces, and central vein (Fig. 2a). Hepatic cells were intact well-conserved cytoplasm and protuberant nuclei. In the hepatotoxic group, the liver showed structural cell damage, inflammation, vacuolation, fatty changes, sinusoidal dilatation, focal centrilobular necrosis, and hepatocyte proliferation (Fig. 2b). Collagen fiber deposition between lobules forming large fibrous septum associated with bile duct proliferation, mononuclear cell infiltration, and changed hepatic tissue construction was seen. Degeneration of hepatic parenchymal cells, loss of nuclei, and focal necrosis were observed. The livers tissues of rats fed with LD 250 mg/ml *G. mangostana* extracts showed minor and narrow fibrotic septum and reduced inflammation and necrosis of hepatic parenchyma (Fig. 2d). These outcomes showed hepatoprotective action of *G. mangostana* extracts comparable to that of silymarin treated group (Fig. 2c). Conversely, the livers of rats fed with HD 500 mg/kg *G. mangostana* extracts were normal in architecture and showed minor-micro-nodules with less fibrous septae with extension of normal hepatic parenchyma comparable to that of normal control group (Fig. 2e). These photographic evaluations confirmed that oral administration *G. mangostana* peel extracts efficiently protected the liver from further cirrhosis in a dose dependent manner.

3.5. Histopathological analysis by Masson's trichrome staining (MT staining)

MT staining determined the degree of fibrosis in liver sections. Liver slices of normal rats revealed normal and intact and no signs of collagen

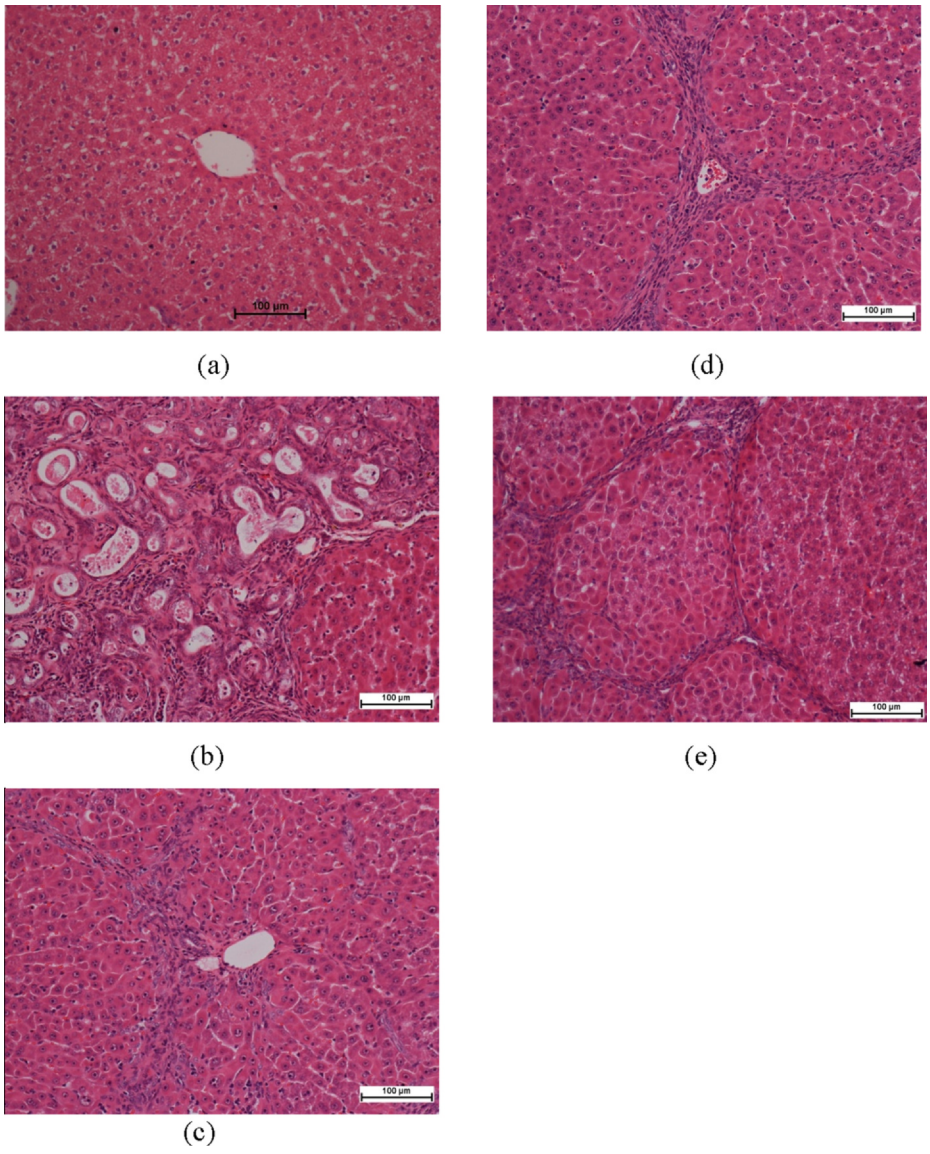

Fig. 2. Histopathology by H & E staining: Effect of *G. mangostana* on histopathology of liver in TAA-induced hepatotoxicity in rats. (a) Normal control group, displayed normal histology structure; (b) TAA- treated group (Hepatotoxic control), displayed severe structural injuries and progress of pseudo lobules with thick fibrotic septa with propagation of bile duct and centrilobular necrosis; (c) TAA + Silymarin group (Hepatoprotective), exhibited mild inflammation and fewer fibrotic septum; d) TAA + 250 mg/kg *G. mangostana* group, presented moderately preserved hepatocyte and slight area of necrosis and narrow fibrotic septa arisen in the liver; (e) TAA + 500 mg/kg *G. mangostana* group, showing relatively conserved hepatocyte and insignificant ranges of necrosis were noticed in the liver (H & E stain, magnification 20x).

expansion or hepatic tissue necrosis (Fig. 3a). In the hepatotoxic group, liver sections showed an increase of collagen fibers deposition with dense fibrous septa between lobules and around the congested central vein and bile duct proliferation in hepatic trade (Fig. 3b). Liver tissues from Silymarin-treated showed slight collagen fibers deposition with less dense fibrous septa deposition indicating minimal fibrosis (Fig. 3c). Alike liver tissues of rats fed with LD 250 mg/ml *G. mangostana* showed moderate collagen fiber deposition between lobes in liver parenchyma (Fig. 3d), while liver tissues of rats fed with HD 500 mg/ml *G. mangostana* displayed mild collagen deposition with mild congestion around the central vein (Fig. 3e). These microscopic evaluation of liver sections confirmed that *G. mangostana* extract competently resisted hepatocytes fibrosis analogous to that of silymarin.

3.6. Immunohistochemical stain of liver sections

TAA-induced liver damage and the consequence of *G. mangostana* - peel extract were examined by immunohistochemical staining of PCNA, TGF-β1 and α-SMA expression in the liver parenchyma using specific antibodies. Normal control group presented down-regulation of PCNA, α-SMA, and TGF-β1 staining, representing no cell renewal happening (Fig. 4A, B, and C). On the contrary, the TAA treated hepatotoxic control group had remarkable PCNA, TGF-β1 and αSMA expression suggesting upregulation of these proteins with higher level of hepatocyte fibrosis. This group elevated the mitotic figure index significantly (****$p < 0.0001$) with F (4, 25) value 99.46, signifying propagation to renewal extensive hepatic injuries produced by TAA (Table 2).

Rat fed 500 mg/kg *G. mangostana* had condensed hepatic cells renewal in comparing to the TAA treated hepatotoxic control group, as indicated by PCNA expression and a significant reduction of the mitotic index at ****$p < 0.0001$. These results were relatively comparable to that of silymarin treated hepatoprotective group ($^{ns}p > 0.05$). These two groups were analogous and resist hepatocyte fibrosis by down-regulating TGF-β1 and α-SMA expressions. Whereas LD 250 mg/kg *G. mangostana* extract displayed moderate expressions of PCNA, TGF-β1 and α-SMA within the hepatocytes with significant decrease in mitotic figure index but not comparable to silymarin treated hepatoprotective group (***$P < 0.001$). These results suggest *G. mangostana* peel extract had an admirable hepatoprotective influence by inhibiting fibrosis of hepatocytes and ameliorating proliferation.

3.7. Effect of G. mangostana on oxidative stress and lipid peroxidation

The one way ANOVA analysis of the data showed significant differences (****$p < 0.0001$) between all treated groups in the levels of endogenous antioxidative enzymes; superoxide dismutase (SOD) and

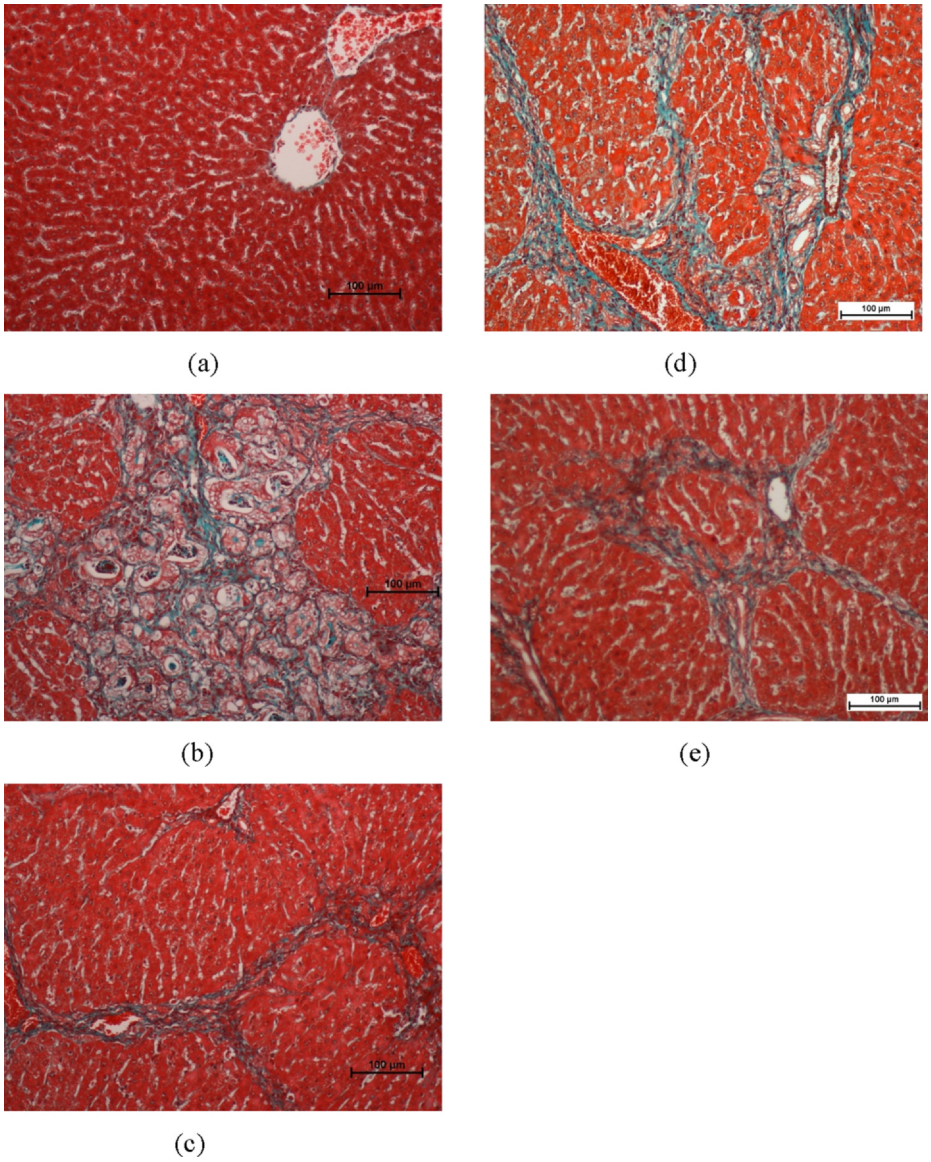

Fig. 3. Histopathology by Masson's trichrome staining: Effect of *G. mangostana* on extent of liver fibrosis in TAA-induced liver cirrhosis in rats. (a) Normal control group, normal liver structure with no signs of collagen deposition; (b) TAA- treated group (Hepatotoxic control), severe collagen expansion and widespread fibrosis were seen; (c) TAA + Silymarin group (Hepatoprotective), insignificant collagen statement; d) TAA + 250 mg/kg *G. mangostana* group, moderated collagen deposition; (e) TAA + 500 mg/kg *G. mangostana* group, slight collagen statement was observed (Masson's Trichrome stain, magnification 20x).

catalase (CAT) with F (4, 25) values 92.53 and 191.2 respectively (Fig. 5). The MDA levels has been altered with F (4, 25) values 858.7 at ****$p < 0.0001$. Tukey's posthoc multiple comparisons test analysis demonstrated that oral administration of 200 mg/kg TAA reduces SOD and CAT activities significantly with augmented MDA level (**** = $p < 0.0001$). However, the oral administration of *G. mangostana* peel extract i.e. LD 250 mg/kg and HD 500 mg/kg) induced a significant (**** = $p < 0.0001$) elevation of SOD and CAT in comparison to TAA treated group restoring levels of these antioxidative enzymes close to normal and comparable to silymarin treated group. Interestingly, SOD level greatly augmented in liver of rats fed with LD 250 mg/ml *G. mangostana* extract and CAT level in rats fed with HD 500 mg/ml *G. mangostana* extract were comparable to silymarin at $^{ns}P > 0.05$ and $^{*}P < 0.05$ respectively. Rats fed with both doses of *G. mangostana* extract significantly lowered the MDA level compared to TAA treated group (**** = $p < 0.0001$). However rats fed with HD 500 mg/ml *G. mangostana* extract was found to reduce MDA approximately analogous to that of silymarin (Fig. 5).

3.8. Effect of G. mangostana on serum parameters of liver damage

There were significant variations (**** = $p < 0.0001$) between all treated groups in the serum levels of ALP, ALT and AST with F (4, 25) values; 3152, 2320 and 3446 respectively. Tukey's posthoc multiple comparisons test analysis showed that administration of TAA induced a significant (**** = $p < 0.0001$) elevation in liver enzymes, ALP, ALT, and AST in rats (Fig. 6). However, the oral administration of *G. mangostana* peel extract (LD 250 mg/kg and HD 500 mg/kg) reduced TAA-induced increase in these liver damage markers significantly (**** = $p < 0.0001$), compared to the TAA-treated control group. Importantly, *G. mangostana* peel extract (HD 500 mg/ml) induced effects were similar (ns = $p > 0.05$) to as observed in the silymarin treated group. The serum level of other liver damage parameters viz. bilirubin, total protein and albumin altered significantly (**** = $p < 0.0001$) in all treated groups with F (4, 25) values 1382, 70.66 and 965.3 respectively. *G. mangostana* peel extracts was found to restore normal level of these parameters comparable to silymarin treated group at * = $p < 0.005$ and ns = $p > 0.05$ respectively

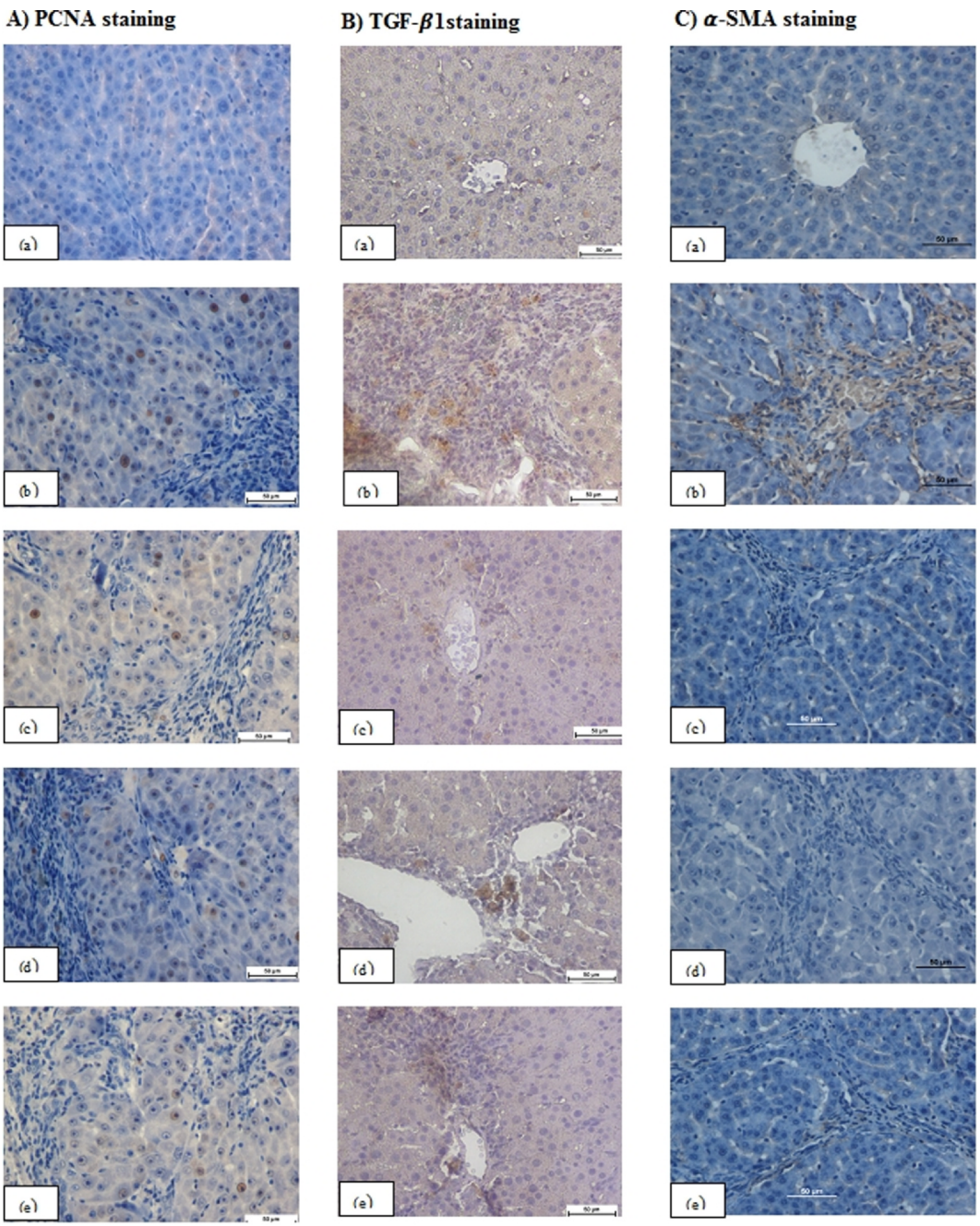

Fig. 4. Immunohistochemistry: A) Effect of ethanolic extracts of *G. mangostana* on **PCNA staining** of liver, (a) Normal control group, no PCNA staining (down-regulation); (b) TAA- treated group, PCNA expression hepatocyte nuclei (up-regulation); (c) TAA + Silymarin group, mild PCNA expression of hepatocytes nuclei (down-regulation); (d) TAA + 250 mg/kg *G. mangostana* group, mild to moderate expression PCNA positive hepatocyte nuclei (down-regulation); (e) TAA + 500 mg/kg *G. mangostana* group, mild PCNA expression hepatocyte nuclei (down-regulation) (PCNA stain, magnification 40x). **B) TGF-β1staining**, (a) Normal control group, no TGF-β1expression (down-regulation); (b) TAA- treated group, TGF-β1expression of hepatocytes were seen (up-regulation); (c) TAA + Silymarin group, mild TGF-β1expression (down-regulation), (d) TAA + 250 mg/kg *G. mangostana* group, mild TGF-β1expression(down-regulation); (e) TAA + 500 mg/kg *G. mangostana* group, mild TGF-β1expression(down-regulation) (TGF-β1 stain, magnification 40x). **C) α-SMA staining,** (a) Normal control group, no α-SMA expression (down-regulation); (b) TAA- treated group, α-SMA expression of hepatocytes (up-regulation); (c) TAA + Silymarin group, mild α-SMA expression (down-regulation); (d) TAA + 250 mg/kg *G. mangostana* group, mild α-SMA expression (down-regulation); (e) TAA + 500 mg/kg *G. mangostana* group, mild α-SMA expression (down-regulation) (α-SMA stain, magnification 40x).

(Fig. 7). The above outcomes advocate the hepatoprotective influence of *G. mangostana* peel extract against TAA-produced liver cirrhosis in rats.

4. Discussion

The current investigation was initiated with an oral acute toxicity test of *G. mangostana* on experimental rats, the outcome revealed promising safety of this plant extract with no morbidity and mortality during the entire experimental period even at higher concentrations i.e. 5000 mg/kg of *G. mangostana*. Consistently, many studies by various co-researchers using different medicinal plant extracts displayed safe, and no symbol of toxic effect was reported (Friedman, 2008; Salama et al., 2012; AL-Wajeeh et al., 2016; Farghadani et al., 2019; Saremi

Table 2
Effect of ethanol extracts of *G. mangostana* on PCNA staining and mitotic index. Data expressed as mean ± S.E.M. (n = 6 rats/group). ****P < 0.0001 compared with TAA control group, #P < 0.0001 compared with normal control group.

Groups	PCNA Staining	Mitotic Index
Normal control	0.00	0.00
TAA + 10% Tween 20	22.5 ± 1.6#	63.2 ± 4.1#
TAA + Silymarin	1.8 ± 0.5****	11.0 ± 1.1****
TAA + LD 250 mg/kg	7.3 ± 1.3****	29.7 ± 2.4****
TAA + HD 500 mg/kg	5.2 ± 0.6****	16.3 ± 2.4****

et al., 2019).

The TAA treated, hepatotoxic group displayed a substantial reduction in body weight go together with improved liver weight compared to normal healthy rats. Improved liver weight/body weight percentage in the hepatotoxic control group might be due to the accumulation of lipid and deterioration in hepatocytes, or due to the accumulation of ECM protein (collagen) (Amin, Alshawsh, Kassim, Ali, & Abdulla, 2013). Likewise, reduced in the body weight and rise liver heaviness in the hepatotoxic group was formerly stated by many investigators (Salama et al., 2012; Abdulaziz Bardi et al., 2013; Kadir et al., 2014; Azab & Albasha, 2018; Mousa et al., 2019). *G. mangostana* fed rats decreased liver weight to nearly normal ranges compared to the hepatotoxic group. The reduction of liver/body weight ratio observed in *G. mangostana* fed rats might be due to the reduction of hyper-lipidemic or might be due to decreased inflammation (Aoyama, Paik, & Seki, 2010; Amin et al., 2012). Similarly, numerous researchers used abundant medicinal plant extracts established reduction in liver weight/body weight ratio compared to hepatotoxic group (Wong et al., 2012; Amin et al., 2013; Kadir et al., 2014; Rouhollahi et al., 2015; Salama et al., 2018; El-Baz, Salama, & Salama, 2019).

TAA injection produced liver cirrhosis in rats. However, rats fed with *G. mangostana* could significantly accelerate the recovery of liver injuries meaningfully evades the influence of TAA harmfulness. The consequences of this learning are also in line with previous studies described by several academics using various herbal plants in the inconsistency of TAA produced liver damage in animals (Alshawsh et al., 2011; Rouhollahi et al., 2015; Salama et al., 2018; Said, Waheed, & Khalifa, 2019). Results of the current study displayed decreased collagen construction synthesis in *G. mangostana* fed rats as seen by Masson's trichrome stain of liver sections. The outcomes were similar to the earlier reports those displayed a decrease of collagen fibers against TAA-induced liver fibrosis using medicinal plant extracts (Amin et al., 2012; Salama, Abdulla, Alrashdi, Ismail, et al., 2013; Kadir et al., 2014; Kaur, Sharma, Singh, & Kaur, 2019). Oxidative stress and lipid peroxidation play an important role in the initiation of cirrhosis in the TAA control cluster. TAA metabolized in the body its toxic metabolites causes chronic liver injuries and accumulation of collagen in the ECM leading liver fibrosis (Bardi et al., 2014; Salama et al., 2018).

In our investigation, normal liver groups or silymarin fed groups exhibited down-regulation of PCNA, suggesting the non-appearance of cell regeneration. Up-regulation of PCNA appearance hepatocytes was observed in a hepatotoxic group, representing extensive proliferation, possible exertion to repair tissue injury. Otherwise, rats fed with silymarin or *G. mangostana* significantly reduced cell proliferation numbers because of a decrease in PCNA stain. Earlier down-regulation of PCNA was observed in TAA-induced liver cirrhosis experimental animals when fed with medicinal plants extracts (Salama, Abdulla, Alrashdi, & Hadi, 2013; Bardi et al., 2014; Kadir et al., 2014).

In the hepatotoxic group, TAA produced reactive-oxygen-species (ROS) causing activation of hepatic satellite cells (HSC) which is the major source of ECM production in chronic liver cirrhosis and up-regulation of TGF-β1 and α-SMA. Activation of HSC is accompanied by cell proliferation and improvement of ECM production, expression of α-SMA to myofibroblasts (Kadir et al., 2014). TGF-β1 was localized in the portal area and in dense fibrous septum where a proliferation of bile ducts parallel to the spreading of collagen fibers (Kadir et al., 2014; Yang et al., 2019). The marks of our research presented *G. mangostana* feeding down-regulate appearance of TGF-β1 and α-SMA compared to the hepatotoxic group which showed marked up-regulation of TGF-β1 and α-SMA. *G. mangostana* significantly avoids HSC activation by preventing the production of ROS. Numerous studies by diverse investigators established the downregulation of TGF-β1 in TAA-induced liver cirrhosis (Kadir et al., 2014; Yang et al., 2019; Ujiie et al., 2020).

In the liver tissues homogenate, the level of SOD and CAT enzymes significantly reduced in the hepatotoxic group compared to the normal

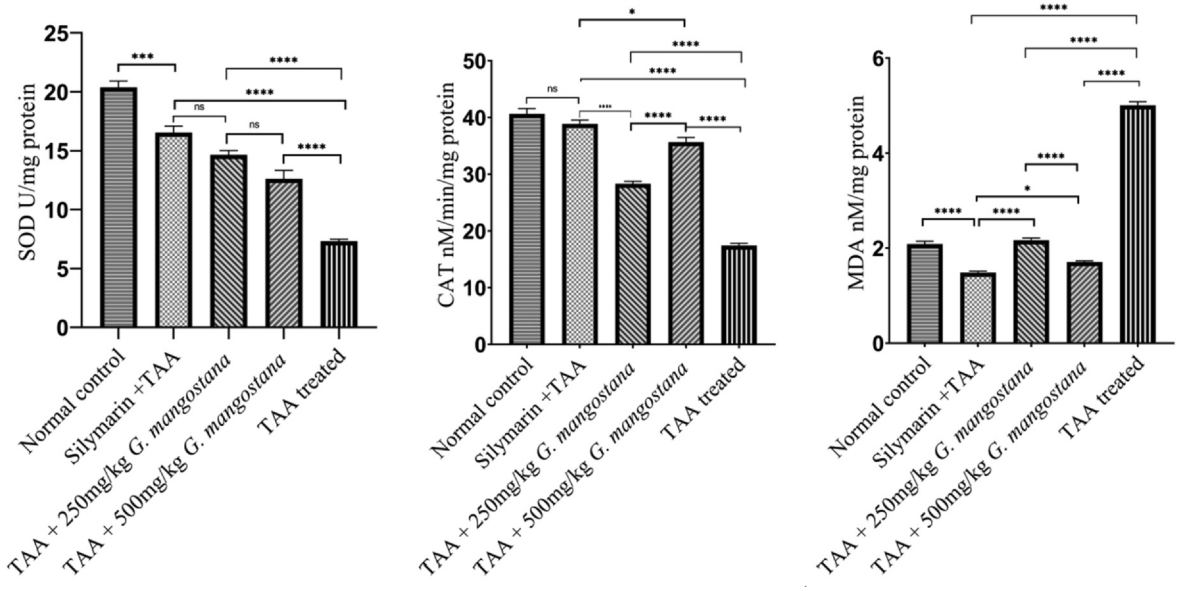

Fig. 5. Effect of ethanolic extracts of *G. mangostana* on the levels of SOD, CAT and MDA in liver tissue homogenate of TAA produced cirrhosis in rats at the end of 8 weeks study. Statistical significant differences were examined using one way ANOVA and Tukey's posthoc multiple comparisons test. ****P < 0.0001, ***P < 0.001, **P < 0.005, *P < 0.05 above columns specify significant differences from the normal control group and above lines for the differences between treated groups. nsP > 0.05 denotes to non-significant differences.

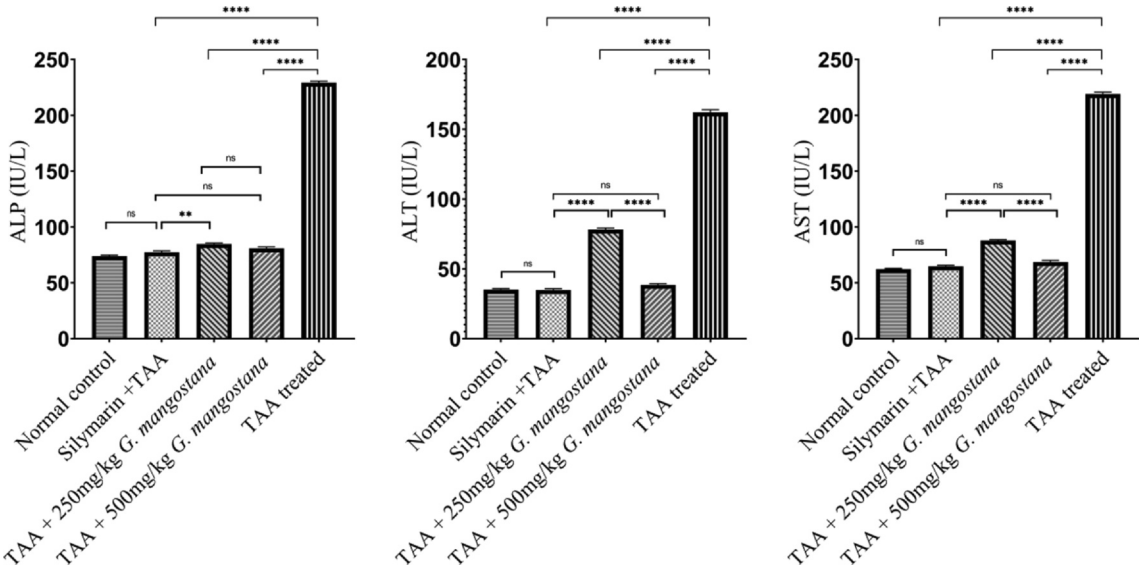

Fig. 6. Effect of ethanol extracts of *G. mangostana* and silymarin on serum hepatic markers in TAA-induced liver cirrhosis in rats. Five groups of experimental rats (n = 6 in each in group) were treated with or without ethanol extracts of *G. mangostana* or Silymarin. Levels of serum ALT, ALP and AST were measured as mentioned in the method section. Statistical significant differences were examined using one way ANOVA and Tukey's posthoc multiple comparisons test. ****P < 0.0001, ***P < 0.001, **P < 0.005, *P < 0.05 above columns specify significant differences from the normal control group and above lines for the differences between treated groups. ^{ns}P > 0.05 denotes to non-significant differences.

group. In the hepatotoxic group, both enzymes are fading by radical's resultant liver impairment. *G. mangostana* significantly raised the concentration of serum CAT and SOD, by defending the liver from the damaging properties of free radicals compared to the hepatotoxic group. Similar results have been reported previously by numerous investigators (Alkiyumi et al., 2012; Amin et al., 2012; Kondeva-Burdina et al., 2018). Lipid peroxidation is a normal injurious process. MDA levels elevated in hepatic tissue homogenate indicate lipid peroxidation. Rise MDA enhanced lipid peroxidation causing damage and adversity of anti-oxidant protection devices to prevent progress extra free radicals. Existing investigation showed TAA formed an increase in the MDA amount has been hopefully reduced by *G. mangostana* extract. Comparable results have been earlier reported by immeasurable co-researchers elsewhere (Alshawsh et al., 2011; Bardi et al., 2014). Decreased hepatic SOD and CAT action sin hepatotoxic group perhaps clarify raised MDA. Hepatoprotective effect of *G. mangostana* assumed to be related to its free radical scavenger effect and antioxidant activities, which declines oxidative stress produced by TAA-mediated ROS released (Salama et al., 2018).

The hepatotoxic group was accompanied by a noticeable increase in serum markers ALP, ALT, AST, total protein and bilirubin levels. The elevation in serum liver biomarkers reflects hepatocellular injury. These values were significantly reduced approximately to close normal values upon *G. mangostana* feeding groups. This indicates a stabilization of plasma membrane and repair of hepatic tissue damage induced by oral administration of TAA. Analogous explanations of serum liver markers

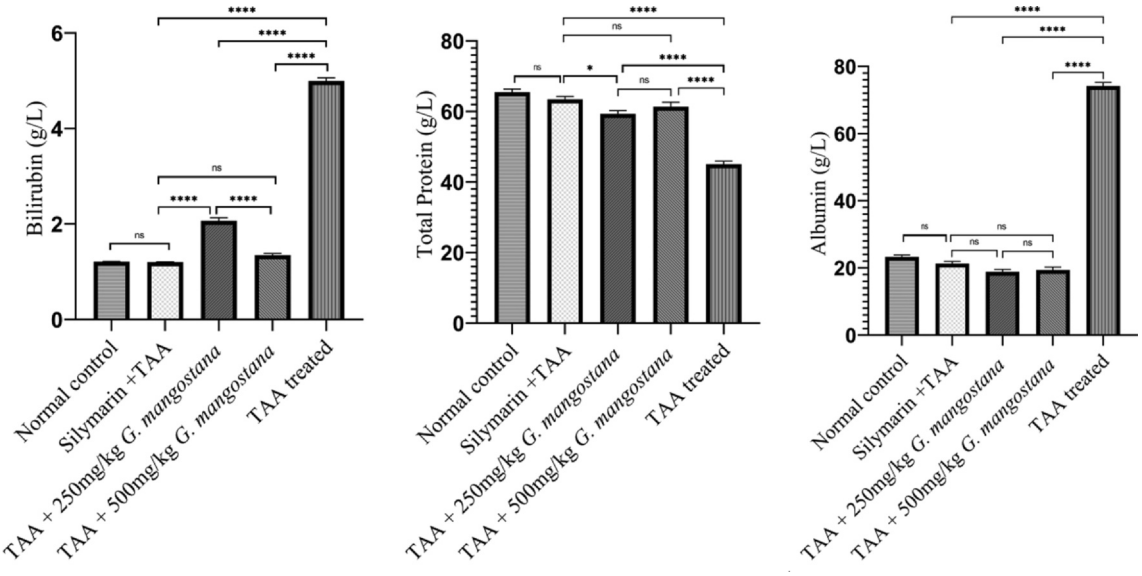

Fig. 7. Effect of ethanol extracts of *G. mangostana* and silymarin on the level of serum bilirubin, total protein and albumin in TAA-induced liver cirrhosis in rats. Statistical significant differences were examined using one way ANOVA and Tukey's posthoc multiple comparisons test. ****P < 0.0001, ***P < 0.001, **P < 0.005, *P < 0.05 above columns specify significant differences from the normal control group and above lines for the differences between treated groups. ^{ns}P > 0.05 denotes to non-significant differences.

improvement by using different medicinal plants were formerly informed earlier (Bardi et al., 2014; Salama et al., 2018; Azab & Albasha, 2018). The hepatoprotective activities maybe because of its effect against cell leak and injury of valuable honesty of hepatic cell sheath. TAA specified to delay with RNA drive from the nucleus to the cytoplasm, starting casing injury which outcomes in the rise statement of serum liver markers (Friedman, 2008; Alshawsh et al., 2011). In the present study, total albumin amounts in serum were reduced in the hepatotoxic group. Though, silymarin or *G. mangostana* feeding groups bring back albumin to an approximately normal level. Correspondingly, abundant investigators displayed that rats received silymarin or various plant extracts brought the albumin and protein to normal levels (Kadir et al., 2011; Alkiyumi et al., 2012; Amin et al., 2013; Bardi et al., 2014; Mousa et al., 2019).

5. Conclusion

According to the significances of the existing study, *G. mangostana* revealed significantly hepatoprotective results in inhibition of TAA-induced hepatic injury in rats as accepted by macroscopic appearance, histology, immunohistochemistry, and biochemical liver parameters. *G. mangostana* intensely increases the serum concentration of CAT & SOD, while significant reduction of hepatic MDA. *G. mangostana* extract effectively prevents TAA-induced liver cirrhosis by marked down-regulation of hepatic PCNA, TGF-β1, and α-SMA expression. Defensive result of *G. mangostana* in TAA-induced hepatotoxicity could be due to its capacity to prevent hepatocyte proliferation, decrease oxidative stress, and lipid peroxidation, and it's antioxidant and free radical scavenger possessions. The future prospective of this research includes identification of molecular contents of *G. mangostana* peel extract accountable to hepatoprotective property and analysis of pharmacological potential by mapping the molecular pathways.

6. Ethics statement

(1) This research approved by the Ethics Committee for Animal Experimentation, Faculty of Medicine, University of Malaya, Malaysia (Ethic No. PM/12/05/2019 MAA (R)). Altogether rats acknowledged humane attention giving to principles drawn in Guide for Care and Use of Research Laboratory Animals prearranged by the United States National Academy of Sciences and Issued by National Institutes of Healthiness (OECD, 2002).
(2) This manuscript has not been published in whole or in part elsewhere.
(3) The manuscript is not currently being considered for publication in another journal.
(4) All authors have read and approved the manuscript.

CRediT authorship contribution statement

WNA, MAA: Conceived and designed the experiments. WNA, SWB, NS, and MHM: Performed the experiments. WNA, FKS, MAA, RF: Data collection and analysis. FKS, MAA, SWB: Wrote and formatted manuscript for publication: TDK, NFSA, ASA: Intellectual input and feedback to manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank the University of Malaya, Malaysia for providing grant funding to conduct this study.

Funding statement: This study was financially supported by IPPP (PG263-2015B) and UMRG (RPO43A15HTM) grants from University of Malaya, Malaysia. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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