



RESEARCH ARTICLE

Assessment of Ginger Extract and Ginger Nanoparticles Protective Activity against Acetaminophen-Induced Hepatotoxicity and Nephrotoxicity in Rats

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ABSTRACT

Acetaminophen is widely used analgesics all over the world. However, hepatotoxicity and nephrotoxicity are the most features of acetaminophen overdose. Ginger is a medicinal plant that has anti-tumorigenic, anti-apoptotic, anti-oxidant activities. Recently, ginger nanoparticles have been investigated in liver protection against alcohol-induced liver damage and in treatment of bowel colitis. Our experiment was aimed to judge the protective activity of ginger nanoparticles relative to ginger extract at dose of 120 mg/kg against acetaminophen toxicity at dose of 375 mg/kg (1/10 LD₅₀) daily for three months in male rats from the histological and biochemical aspects. Serum Alanine aminotransferase and aspartate aminotransferase were determined. In addition, urea and uric acid levels were evaluated in serum. Moreover, oxidative stress was evaluated by determining malondialdehyde content and catalase enzyme activity. Meanwhile, histopathological changes in liver and kidney tissues were observed. The present study indicates that liver and kidney biochemical markers, oxidative stress and histopathological structure are improved in rat pretreated with ginger extract. However, rats treated with GNPs were more protective relative to ginger extract pre-treated rat.

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INTRODUCTION

Acetaminophen (APAP) is a commonly used as pain relieving drug. It is harmless at regular doses, but at overdoses it causes hepatotoxicity and nephrotoxicity (Kandemir *et al.*, 2017). The main reason of acetaminophen toxicity is persistent production of N-acetyl-p-benzoquinoneimine (NAPBQI) which is one of APAP metabolites. NAPBQI is directly conjugated with glutathione and consequently excreted as cysteine and mercapturic conjugates. Saturation of conjugation pathway occurred at high dose of acetaminophen consumption, thus APAP are metabolized by oxidation, consequently hepatic and renal damage occur due to depletion of intracellular glutathione (Mazer and Perrone, 2008).

Plant extracts which have natural antioxidants against chemically induced toxicities have got more interest. One of the most commonly used plant around the world is ginger which has active phenolic compounds such as

shogaols, zingerone, and gingerols (Poorrostami *et al.*, 2014). Also ginger has pharmacological activities, such as anti-inflammatory, cardiovascular activities, anti-cancer activities, and antioxidant activity which attributed to gingerols and shogaols existing in ginger (Zhang *et al.*, 2017).

Lately, natural nanoparticles had been fabricated from plants, characterized by easy preparation for large scale relative to synthetic nanoparticles (Zhang *et al.*, 2016). Natural nanoparticles from edible plants can distribute from intestine to the liver, thus they may have an important role in treatment regimen and delivery of drug to the target tissues and improvement of therapeutic results. Moreover, nanoparticles of natural compounds provide a safe carrier for improving drug bioavailability within the cells (Khalil *et al.*, 2016).

Recent studies demonstrated that ginger nanoparticles had anti-apoptotic activity (Abdu *et al.*, 2017), hepatoprotective effect against alcohol-induced liver damage (Zhuang *et al.*, 2015) and treatment of intestinal

disorders and colon cancer (Zhang *et al.*, 2017). Our study aimed to determine and compare the effectiveness of GNPs and ginger extract to protect adult male rat against paracetamol induced toxicity.

MATERIALS AND METHODS

Chemicals: Acetaminophen was obtained from Sigma Chemical Co. (USA).

Preparation of ginger extract and nanoparticles: Ginger roots were compressed to powder. 125g of the powder were macerated in one liter of distilled water for 12h at room temperature and were then filtered to obtain the extract. The concentration of the extract is 120mg/kg (Sakr *et al.*, 2011). For preparation of nanoparticles ginger roots were washed with PBS for three times. A total of 200 g of roots were crushed in a blender at the maximum speed for 10min. Then total ginger extract sample was exposed to serial centrifugations start with 1,000g for 10min, then 3,000g for 20min and 10,000g for 40min. To obtain ginger extract in nanoparticles size, the pellet after last centrifugation was resuspended in PBS for further centrifugation at 150,000g for 90min. The diameter of the nanoparticles ranged from 102.3 to 998.3 nm (Zhuang *et al.*, 2015).

Animals: Adult male Sprague Dawley rats (120-150g) were obtained from Vacsera Co, Egypt. The animals were adapted for 7 days before the beginning of study at $25\pm 2^{\circ}\text{C}$, and 12h of a light- dark cycle. The experiment protocol was accepted by the Institutional Animal Care and Use Committee (IACUC) as described in the Cairo University guidelines.

Experimental design: Ninety male rats were divided into six groups representing as G1-G6. G1: received distilled water daily by gavage for 3 months and served as control, while other groups received ginger extract (GE), ginger nanoparticles (GNPs) and APAP as follows: G2: GE (120 mg/kg) orally, G3: GNPs (120 mg/kg), G4: APAP (375 mg/kg) daily by gavage for 3 months, G5: APAP and GE. G6: APAP and GNPs. GE and GNPs were administered 2 weeks before APAP administration then continued for 3 months. Samples were collected at 6th and 12th weeks of the experiment.

Determination of biochemical markers: ALT, AST, urea and uric acid were measured in all groups using reagent test kits obtained from SPECTRUM co. according to (Ali *et al.*, 2016). While Lipid peroxidation (MDA) and Catalase (CAT) activity were evaluated by the method of (Netto *et al.*, 2002) using spectrophotometer.

Histopathological studies: Liver and kidneys tissues were fixed in 10% neutral buffered formalin for 48 h, samples were dehydrated through passing in ascending concentration of ethyl alcohol, then cleared in xylene and embedded in paraffin. Sections (5-6 μm thick) were fixed and stained with H&E stain for microscopic examination (Mahmoud and Mohammed, 2018).

Immunohistochemical staining (IHC): IHC was performed on liver sections of all groups at 12th week to

evaluate the expression of iNOS (inducible nitric oxide synthase) (Tache *et al.*, 2014).

Statistical analysis: The data expressed as Mean \pm SD. Data were statistically analyzed by ANOVA using Tukey comparisons post-test to determine the statistical difference between groups. Significance level was set at $P<0.05$ using GraphPad Prism software 2017.

RESULTS

Biochemical results: The serum AST and ALT activities of the GE and GNPs treated groups (G2&3) were non-significantly different compared with group1. While, they were significantly increased ($P\leq 0.001$) in group 4 that was treated with APAP in comparison to the control negative group. The parallel administration of GE with APAP (G5) had significant ameliorative effect ($P\leq 0.01$) on their elevation. GNPs co-administration with APAP exerted the higher significant effect ($P\leq 0.05$) on maintaining liver function markers more than GE protected group (Fig. 1).

GNPs protected group (G6) exhibit non- significant difference in AST and ALT levels compared with groups (1, 2 & 3). While GE protected group (G5) showed significant difference ($P\leq 0.05$) relative to control groups (Fig. 1).

APAP treated group showed the most significant drop in CAT as well as significant increase in MDA level at 6th and 12th week when compared to other groups at the same time points. Administration of GE and GNPs lead to improve the oxidative status of livers which was evident by significant reduction in MDA levels and increase in catalase activities ($P\leq 0.05$) in liver homogenates compared with groups (1, 2 & 3). The administration of GNPs achieved the best improvement of the hepatic oxidative status when compared with GE (Fig. 2).

The serum urea and uric acid levels of groups 2 & 3 were non-significantly different relative to group1. However, the administration of APAP caused significant elevation of urea and uric acid levels ($P\leq 0.001$) in comparison to the control group. Administration of GE and GNPs resulted in significant decrease of those parameters. GNPs co-administration with APAP exerted the higher protective effect ($P\leq 0.05$) relative to GE protected group ($P\leq 0.01$) (Fig.3).

Gross pathology: Apparently liver and kidneys of all groups were normal.

Histopathological results: Microscopic examination of liver from groups (1-3) revealed normal structure. While, livers of APAP treated rats revealed marked tissue alterations. At six weeks period, the hepatocytes suffered from vacuolar degeneration (Fig. 4a) and necrosis (Fig. 4b). Portal area showed edema with infiltration of fibrous connective tissue and inflammatory cells (Fig. 4c). Twelve weeks post APAP administration, cytoplasm showed fatty degeneration (Fig. 4d). Hepatic necrosis was increased with macrophages infiltration associated with sinusoidal dilatation (Fig. 4e). Portal area showed congestion and edema with inflammatory cells and appearance of newly formed bile ducts (Fig. 4f).

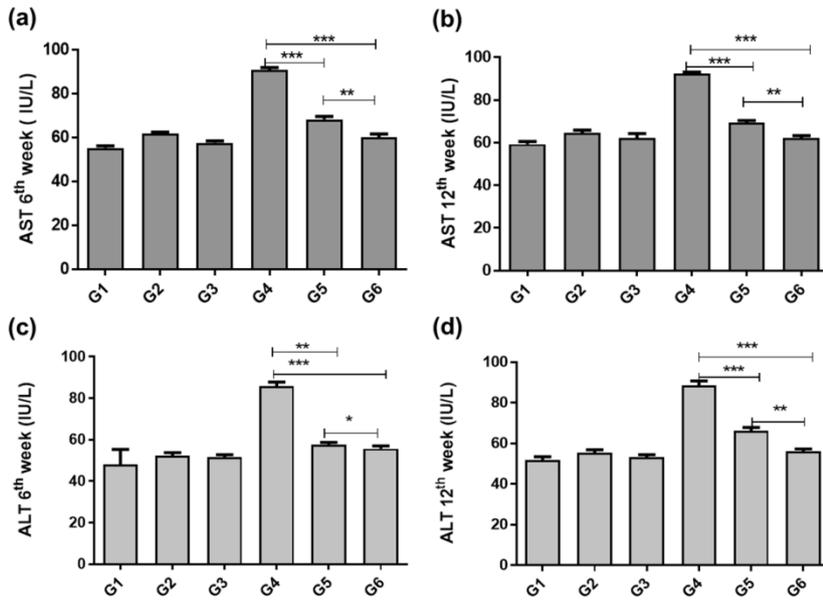


Fig. 1: Showing the mean values of serum AST (a&b) and ALT (c&d) in all groups; (*P<0.05, **P<0.01, ***P<0.001).

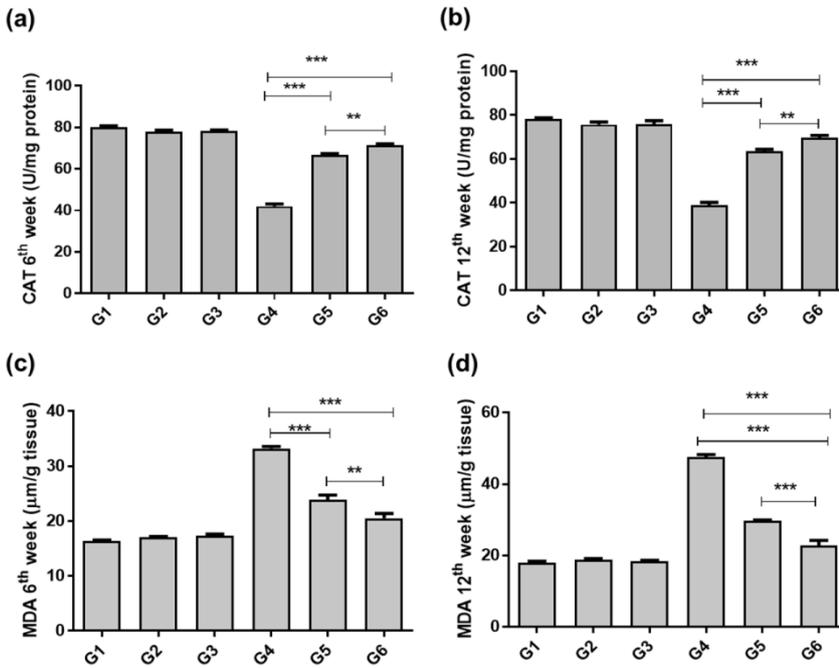


Fig. 2: Showing the mean values of liver tissue CAT (a&b) and MDA (c&d) in all groups; (*P<0.05, **P<0.01, ***P<0.001).

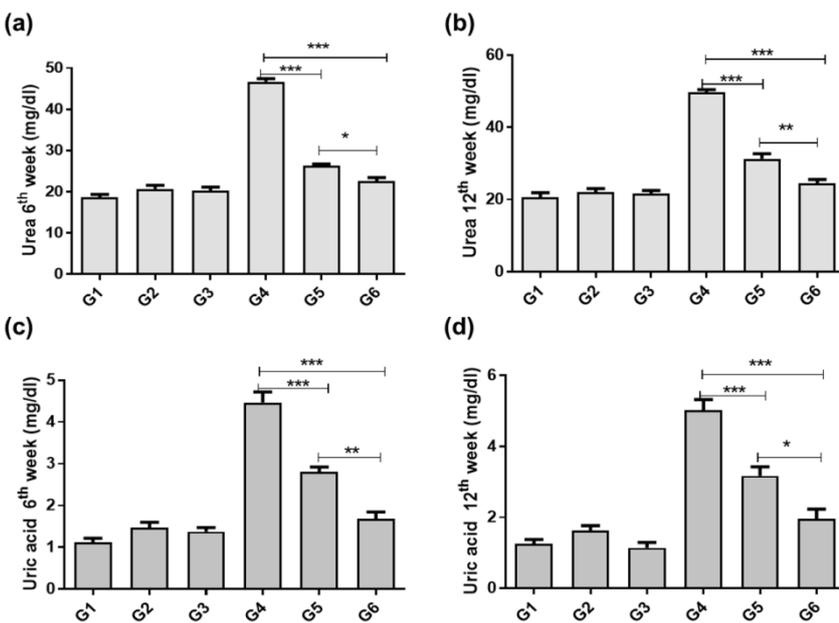


Fig. 3: Showing the mean values of urea (a&b) and uric acid (c&d) in all groups; (*P<0.05, **P<0.01, ***P<0.001).

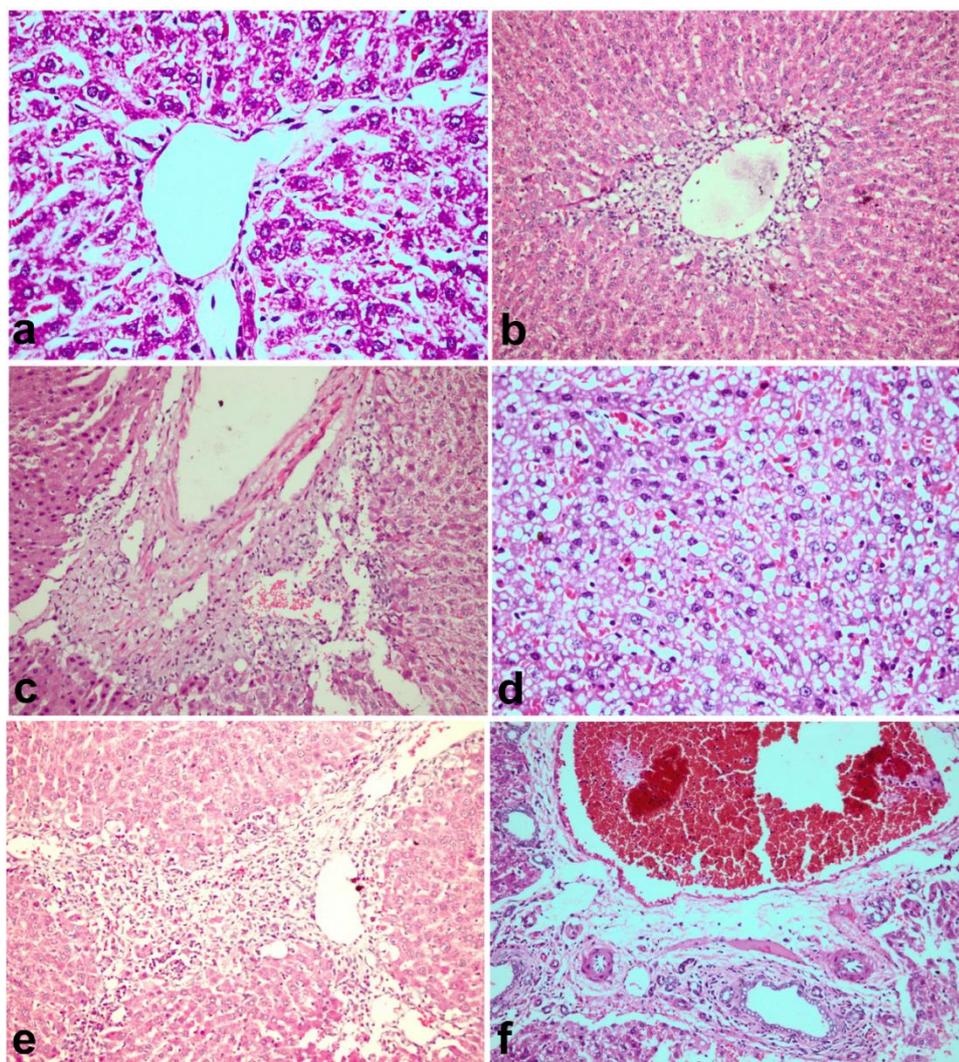


Fig. 4: (a, b and c) Liver of G4 rat (after 6 weeks) showing (a) vacuolar degeneration of hepatocytes with dilatation of sinusoid (X400). (b) centrilobular necrosis with infiltration of inflammatory cells (X 200). (c) periportal edema with infiltration of mononuclear cells and formation of fibrous connective tissue (X 200). d, e and f (after 12 weeks) showing (d) fatty change (X 400). (e) sever necrosis with macrophages infiltration associated with sinusoidal dilatation (X 200). (f) congestion in portal area with edema and infiltration of inflammatory cells and appearance of newly formed bile ducts (X 400). All pictures were stained with H&E.

Six weeks post concurrent administration of GE and APAP, mild vacuolar degenerative and necrotic changes were detected (Fig. 5a). Portal areas appeared slightly congested (Fig. 5b). While after 12 weeks, the hepatocyte showed scattered necrotic area replaced by inflammatory cells (Fig. 5c). Portal area appeared congested with periportal edema and formation of fine fibrous connective tissue (Fig. 5d). Liver sections of rats treated with GNPs and APAP revealed that GNPs were more protective than GE. Minor pathological alterations were noticed during the 6th week, including cytoplasmic granules within hepatocytes and inflammatory cells infiltration in portal area (Fig. 5e). At 12 weeks, vacuolar degeneration and apoptosis were observed (Fig. 5f). Portal area showed congestion.

Kidney sections of groups (1-3) revealed normal structure. Six weeks post administration of APAP many animals showed congestion in blood vessels and renal tubules suffering from vacuolation (Fig. 6a). Interstitial nephritis was detected (Fig. 6b).

At the end of experimental period, necrobiotic changes and renal cast were observed (Fig. 6c). Extensive interstitial nephritis was detected (Fig. 6d). Glomeruli showed widening of the Bowman's space (Fig. 6e) while some other glomeruli showed atrophy (Fig. 6f). Chronic interstitial nephritis which was characterized by the presence of collagen fibers surrounding the between collapsed renal tubules was detected (Fig. 6g).

Kidney sections of rats from G5 showed congested blood vessel with perivascular edema associated with infiltration of inflammatory cells at 6th week (Fig. 7a), in addition to vacuolar degeneration (Fig. 7b). At twelve weeks, renal tubules suffered from degeneration, necrobiotic changes and renal cast (Fig. 7c). Interstitial tissue showed infiltration of mononuclear inflammatory cells (Fig. 7d). Kidneys of rats from G6 revealed minor changes at six weeks of the experiment. Renal blood vessels suffered from congestion (Fig. 7e) Necrobiotic changes and dilatation in Bowman's spaces was detected at the end of experimental period (Fig. 7f).

Immunohistochemistry: Nitric oxide synthases (NOS) are the enzymes responsible for production of nitric oxide (NO). Nitric oxide is reactive oxygen species which play an important role in induction of oxidative stress. Immunostaining for inducible nitric oxide synthases (iNOS) revealed normal negative expression in the hepatocytes of rats from groups 1, 2 and 3 (Fig. 8a, b&c). However, livers of G4 rats showed strong positive expression of iNOS in the hepatocytes (Fig. 8d). However, moderate expression of iNOS was noticed in livers of G5 rats (Fig. 8e). Livers of rats of G6 showed weak scattered positive expression of iNOS in the hepatocytes (Fig. 8f).

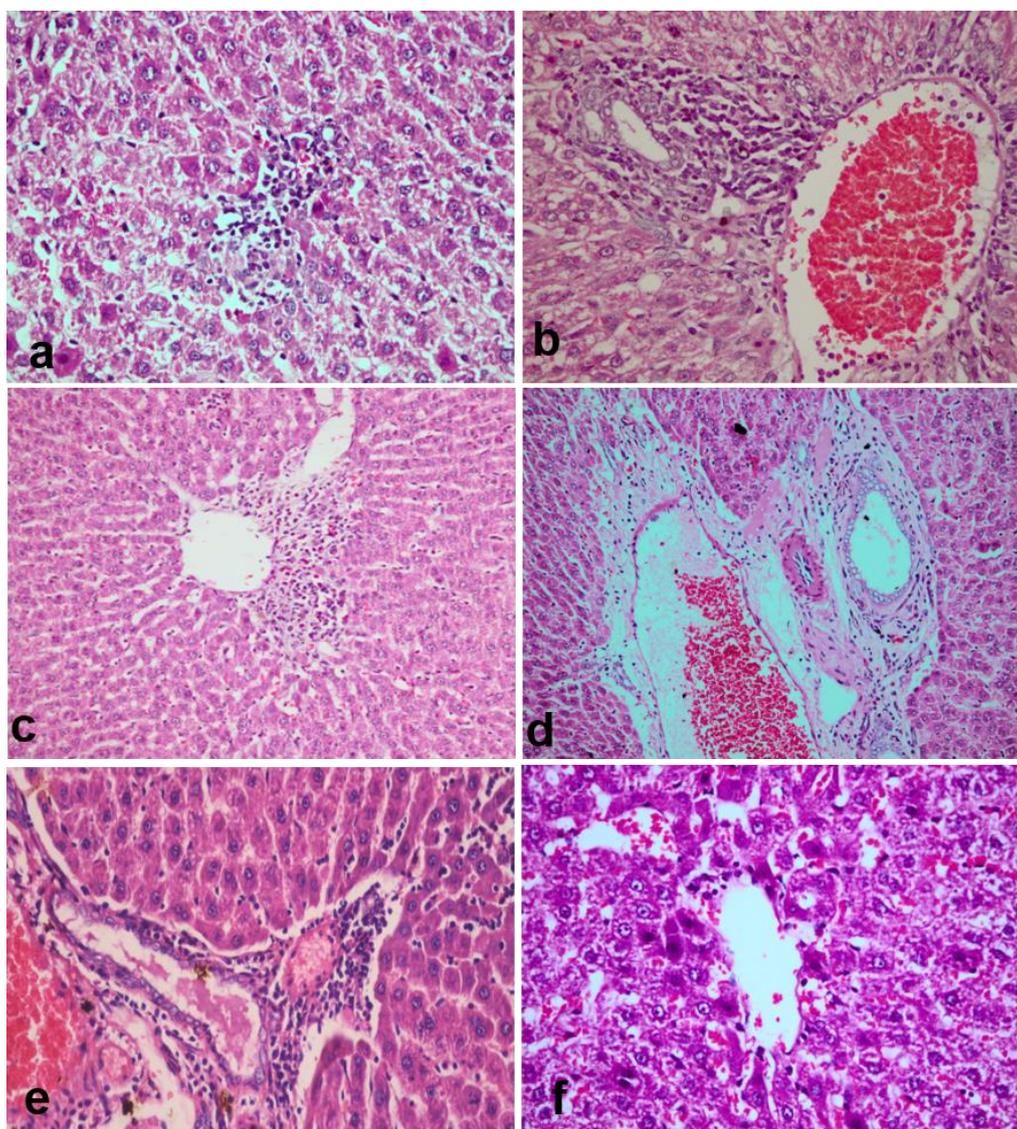


Fig. 5: (a and b) Liver of G5 rat (after 6 weeks) showing (a) showing focal area of necrosis associated with infiltration of inflammatory cells and kupffer cells activation, hepatocyte suffering from mild vacuolation and apoptosis (X 400). (b) mild congestion in portal area with infiltration of inflammatory cells (X 400). c and d liver of G5 (after 12 weeks) showing (c) necrosis of hepatocyte with macrophages infiltration associated with sinusoidal dilatation (X 200). (d) congestion of portal blood vessels with edema and formation of fine fibrous connective tissue (X 200). (e) Liver of G6 rat (after 6 weeks) showing infiltration of inflammatory cell in portal area and between hepatic cords (X 400). (f) Liver of G6 rat (after 12 weeks) showing vacuolar degeneration and apoptosis of sporadic hepatocytes (X 400). All pictures were stained with H & E.

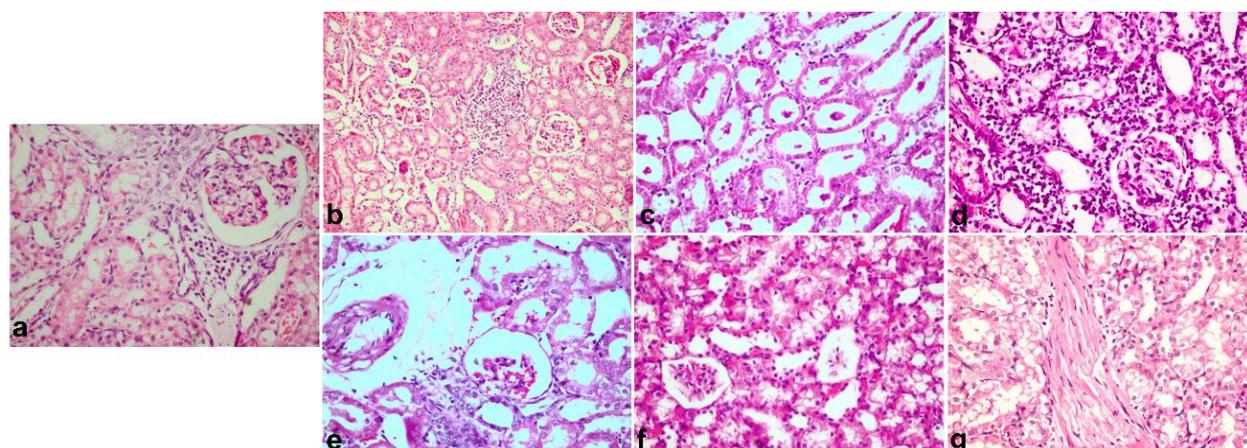


Fig. 6: (a and b) Kidney of G4 rat (after 6 weeks) showing (a) vacuolar degeneration of renal tubules with infiltration of inflammatory cells (X 400). (b) focal interstitial nephritis and degeneration of renal tubules. c, d, e and f (after 12 weeks) showing (X 200). (c) degeneration and necrosis of renal tubules. Notice the eosinophilic substance inside the lumen of tubules (X 400). (d) interstitial nephritis and degeneration in renal tubules (X 400). (e) perivascular edema and thickening in basement membrane of renal glomeruli with dilatation of bowman's space (X 400). (f) atrophy of renal glomeruli (X 400). (g) focal area of fibrous connective tissue proliferation with vacuolar degeneration (X 400). All pictures were stained with H & E.

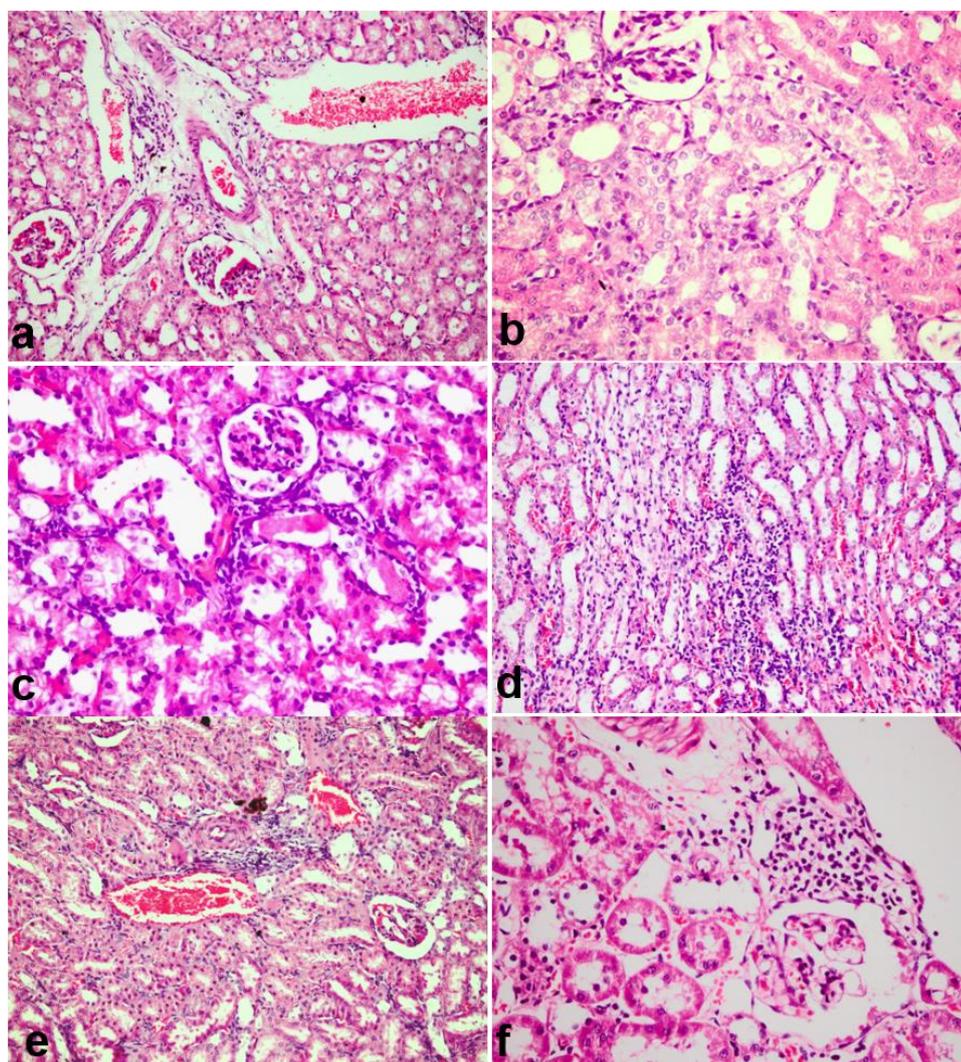


Fig. 7: (a and b) Kidney of G5 rat (after 6 weeks) showing (a) congested blood vessel with perivascular edema associated with inflammatory cells (X 200). (b) vacuolar degeneration. c and d (after 12 weeks) showing (X 400). (c) necrobiotic changes in renal tubules. Note the renal cast in the lumen of tubules (X 400). (d) interstitial nephritis and degeneration of renal tubules (X 100). (e) Kidney of G6 rat (after 6 weeks) showing congestion of renal blood vessels with infiltration of inflammatory cells (X 200). (f) Kidney of G6 rat (after 12 weeks) showing perivascular edema associated with infiltration of inflammatory cells, degeneration and necrobiotic changes in renal tubules and dilated Bowman's spaces (X 400). All pictures were stained with H & E.

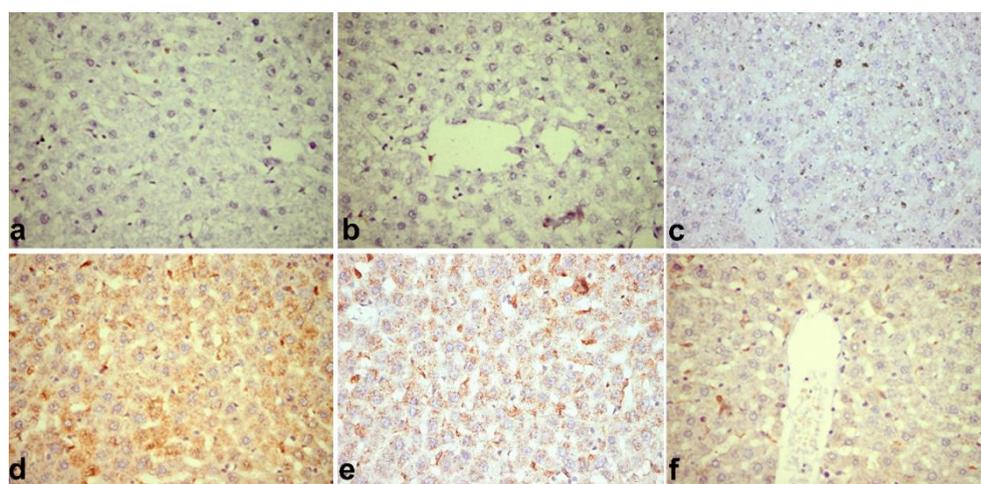


Fig. 8: Immunostaining for iNOS. (a, b and c): Liver of rats G1, G2 and G3 showing negative expression of iNOS (X 400). (d): Liver of rat from G4 showing marked strong positive expression of iNOS (brown color) in hepatocytes (X 400). (e) Liver of rat from G5 showing moderate expression of iNOS (X 400). (f) Liver of rat from G6 showing weak expression of iNOS (X 400).

DISCUSSION

Excessive using of synthetic drugs such as acetaminophen (APAP) could induce several side effects when used in repeated supra-therapeutic doses or in acute high dose (Ramachandran and Jaeschke, 2017). One of the major side effects of APAP is liver toxicity which confirmed in our study by elevation levels of AST, ALT and oxidative stress markers in addition to expression of inducible nitric oxide synthases (iNOS) which play an important role in induction of oxidative stress.

Acetaminophen absorbed rapidly after oral administration and metabolized by liver (Woolbright and Jaeschke, 2017). The liver toxicity is caused by production of N-acetyl-pbenzoquinoneimine (NAPQI) during APAP metabolism. At toxic doses of APAP, NAPQI bind with cellular protein and reduction of glutathione level within hepatocytes occurred. Glutathione depletion will lead to cellular damage by generation of ROS such as nitric oxide, activation of stress proteins, mitochondrial oxidative stress and injury of cell membrane (Hodgman and Garrard, 2012).

Pretreatment with ginger extract (9GE) markedly improved the histological structure of the liver cell membrane and decreased the release of aminotransferases from hepatocytes, in addition to decline the oxidative stress induced by APAP. These results may be attributed to the antioxidant effect of ginger and its capability to maintain the cell membrane integrity (El-Ghonaïmy, 2015). The antioxidant effect of ginger is associated with its active ingredients such as gingerols and shogaols which consider the most important components present in ginger root (Mekuriya and Mekibib, 2018).

Renal markers levels were significantly increased in acetaminophen treated rats, compared to the control group. These changes may indicate a reduction in the glomerular filtration rate because of renal dysfunction due to cumulative doses of the drug induced renal injury in rats (Adam *et al.*, 2016; Naseem *et al.*, 2018). Renal damage in acetaminophen overdose has been attributed to cytochrome P-450 mixed function oxidase isoenzymes, prostaglandin synthetase and N-deacetylase enzymes existing in the kidney, in addition to glutathione which involved in the formation of nephrotoxic compounds (Mazer and Perrone, 2008).

In this study, administration of GE to rats showed significant progress in renal function, these data were attributed to the critical anti-oxidative effect of ginger (Gabr *et al.*, 2019).

Acetaminophen induced damage in liver and kidney tissues. Histopathological results are in the same line with our biochemical and oxidative stress results. High doses of acetaminophen caused histological changes in numerous organs such as necrosis by activation of caspase-3 levels and inflammation through stimulation of caspase-1 and interleukin-1 β by inflammasome at the late stage of acetaminophen toxicity (Benjamin *et al.*, 2017). Also, these changes in cellular structure were due to the oxidative activity with consequent generation of lipid peroxidation which lead to injury of cell membranes with cellular edema (Sakr and Shalaby, 2012). Ginger extract improved the histological architecture of tissue, this may be attributed to anti-oxidant effect of ginger. Ginger was reported to has anti-inflammatory effects through decrease the production of proinflammatory cytokines such as tumor necrosis factor α (TNF- α), interleukin (IL)-1 β , and IL-12 in addition to inhibition of cyclooxygenase-2 (COX-2) expression, which play an important role in induction of inflammation by increase the level of prostaglandins (Aryaeian and Tavakkoli, 2015).

Ginger nanoparticles showed more hepatoprotective, reno protective and antioxidant effect against acetaminophen than ginger extract. Ginger nanoparticles are highly resistant to digestion in stomach and intestine. So, significant amounts of nanoparticles could reach to liver after oral administration (Zhuang *et al.*, 2015) that provide a safe carrier for delivery and improved drug bioavailability within the hepatocytes.

Ginger nanoparticles serve as stronger antioxidant against free radical compared to ginger extract. This may be due to presence of shogaols which carried by GNPs in bind form in contrast the shogaols carried by GE which present in free form. So, less amount of shogaol carried by GNPs required for obtaining the equal effect on the hepatocytes compared with GE (Zhuang *et al.*, 2015).

Shogaols regulate the genetic expression of many antioxidant and detoxication enzymes. In addition to play important role in reduction of inflammation by stimulation of nuclear factor-erythroid 2-related factor 2 (Nrf2) which has anti-inflammatory activity (Johnson *et al.*, 2010).

Our results showed improvement in histopathological structures of livers and kidneys in rats pretreated with GNPs relative to GE pretreated rats. Our results may be attributed to anti-oxidant and anti-apoptotic activities of GNPs. The cascades of apoptosis and necrosis are strongly regulated by a several factors; from these factors, Bcl2 as anti-apoptotic and Bax protein as apoptotic promotor, in addition to p53 protein (apoptotic inhibitor). The ratio of these proteins has been considered as a main indicator of the apoptotic process regulation, because Bax/Bcl-2 proteins ratio increases throughout the apoptosis (Karmakar *et al.*, 2016). GNPs control the ratio of these proteins and the expression of Bcl-2, Bax and p53 gene and decrease necrotic/apoptotic rate (Abdu *et al.*, 2017). GNPs were reported to reduce chronic inflammation after oral administration in rats through reducing the expression levels of pro-inflammatory cytokines (TNF- α , IL-6 and IL-1 β) and increasing the expression levels of anti-inflammatory cytokines (IL-10 and IL-22) (Soliman and Elfeky, 2016).

Conclusions: In our study we concluded that GNPs are more hepatoprotective and reno-protective against acetaminophen induced toxicity relative to GE. In addition, oral administration of GNPs alone didn't show hepatotoxicity or nephrotoxicity. Therefore, it is considered a novel study in developing new method in treatment and protection of liver and kidneys with minimal side effects.

Authors contributions: AFB run all experiments, statistical analysis and drafted the manuscript. SSA contributed in study design, interpreted histological results and drafted the manuscript. OSET contributed in study design, elucidated the toxicological results and drafted the manuscript. AMB contributed in study design, interpreted histological results and drafted the manuscript. All authors read and approved the final manuscript.

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