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Role of *Panax Ginseng* as an Antioxidant and Hepatoprotective after Liver Toxicity caused by Flutamide in Adult Male Rats

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ABSTRACT

Aim: This study was conducted to determine antioxidant, hepatoprotective and anti – inflammatory effects of *panax ginseng* extract
against hepatotoxic effect of flutamide, a drug widely used in the treat ment of metastatic prostate adenocarcinoma.

Materials and Methods: Thirty rats (weight 200-300 g) were used and had access to water and food in the animal house for two week.
The rats were divided randomly into six equal groups: group (A): received normal saline as control, group(B): received flutamide (25 mg/kg b.wt) orally for 7 days, group (C): injected *p. ginseng* extract (200 mg / kg b.wt) intraperitonialy daily for 30 days, group (D):injected *p. ginseng* extract (400mg/kg b.wt) intrapretonialy daily for 30 days, group (E):received flutamide (25mg/kg b.wt) orally for 7 days with *p. ginseng* extract (200 mg / kg b.wt) intrapretonialy daily for 30 days, group (F): received flutamide (25mg/kg b.wt) orally for 7days with *p. ginseng* extract (400 mg/kg b.wt) intrapretonialy daily for 30 days. In this study, we assessed the hepatoprotective effect of red ginseng in rats treated with flutamide. Serum concentrations of the hepatic marker enzymes alanine amino transferase (ALT), aspartate amino transferase (AST) and histopathological analysis have been performed for this purpose. The hepatic antioxidant status was assessed using measurement of GSH levels.

Results: Administration of flutamide to adult male rats causes severe hepatic injury. Hepatosomatic index, ALT and AST were significantly increased in comparison with control and ginseng treated groups. While a significant decrease in the contents of reduced hepatic glutathione (GSH) was observed
Histological examination of liver tissues showed that flutamide caused significant increase in the diameter of central vein, bloody congestion in the central vein with infiltration of Inflammatory cells, swelling of hepatocytes, narrowing of blood sinusoids and vacoules appear inside the hepatocytes.

Conclusion: The results of this current study indicate that the administration of ginseng (200,400 mg / kg b.w) to flutamide treated animals resulted in an improvement in the histological picture of the liver as well as biochemical parameters, mainly through down regulation of oxidative stress and inflammatory response.

Key words: *Panax ginseng* extract, flutamide, anti-inflammatory activity, antioxidant activity, aminotransferase, reduced glutathione, hepatotoxicity

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INTRODUCTION

Flutamide is an anti-androgenic and non-steroid drug commonly used to treat prostate cancer, this works as an antagonist by competing for the androgen receptor in the prostate gland with testosterone and active metabolite dihydrotestosterone, it can also prevent prostate cancer cells from growing and spreading(Elks, 2014) . This antiandrogen drug is also used for the treatment of hirsutism in combination with oral contraceptives, in addition, flutamide works as an inhibitor to release gonadotrophins from the pituitary gland by its action to close testicular receptors, since flutamide has no hormonal activity (Lundgren, 1988). Side effects of flutamide appear at the functional and histological level of the organs where it causes diarrhea, vomiting, dyspepsia, insomnia, muscle cramps, galactorrhea, gyneocomastia and breast tenderness (Chu *et al.,* 1998).

It has been observed in the tissues of people taking flutamide as a treatment for hepatic failure, the spread of acute necrosis in the hepatocytes and multiplication in the bile duct upon examination of the liver taken from one of the bodies treated with flutamide (Corkery *et al*,1991; Wysowski *et al*, 1993).

Indeed, there have been several studies documenting the rel ationship between flutamide use and hepatotoxicity inciden ce (García-Cortés *et al*., 2001; Andrade *et al*., 2005; Dikensoy *et al*., 2009; Bruni *et al*., 2012).

Metabolism of flutamide by the cytochrome P450 system or other microscopic enzymes caused formation of reactive metabolites that may lead to lipid peroxide and thus to liver injury (Dourakis *et al*., 1994).

Flutamide treatment stimulate abnormal changes in liver biomarkers, such as transaminase activity in the blood and in certain cases, causes severe liver toxicity correlated with emergency liver transplantation or even death (Gomez *et al*., 1992; Braham *et al.,* 2011).Information obtained through laboratory experiments revealed hepatotoxicity after flutamide treatment in rats with a marked increase in transaminase activity and serum TB concentrations (Mannaa *et al*., 2005; Hamieda *et al*., 2016).

Additionally, studies have shown in vivo and in vitro that the molecular cytotoxic mechanisms of hepatotoxicity caused by flutamide involving induction of oxidative stress and lipid peroxidation associated with reducing antioxidants (Fau*et al.,* 1994).

It was found that the medicinal value of natural products is important, Because of its antioxidant activity to combat oxidative stress and oxidative stress caused by free radicals (Benzie, 2003; Hassan *et al*., 2014).

Red ginseng (*Panax ginseng*) is a traditional herbal medicine used to maintain and restore human health in Asian and Korean countries (Park *et al*., 2012; Kim &Park, 2011).

Panex ginseng root is used in herbal medicine as a dietary supplement, ginseng has a wide range of beneficial biological properties including anti-diabetic (Yuan *et al*., 2012) antioxidant (kim *et al*., 2010), anti-cancer (panwar *et al*., 2005), anti-aging (kang *et al*., 2009), immunomodulation (spelman *et al*., 2006), anti-inflammatory and neuro-protective effects (park, 1996).

The pharmacological effects of ginseng are primarily due to phenolic acids, flavonoids and triterpenoid saponins (kim *et al*., 2010).

Ginseng saponins is a forceful antioxidant and effective in reducing tissue damage caused by free radicals(Chang *et al*., 1999 and Sohn *et al*., 1993).It was documented that ginseng have a protective effect against many toxicants in human and laboratory animals (Jeong *et al.,* 1997) and it also can increase the body's resistance against many harmful factors and protect the tissues from damage when the body is under stress (Liu *et al.,* 1995).

Our research interest in flutamide-related hepatic injury was explained by biochemical and histological analyses and evaluate the chemoprotective function of active antioxidant components in the red ginseng extract to relieve hepatotoxicity in male rats caused by flutamide and restore function of the liver beyond normal levels.

Materials and Methods

Chemicals

Flutamide (tablet 250mg) was obtained frommedochemic limited –MLT Ciprus. Flutamide dissolved in 10ml of pure corn oil and each 1ml contains 25 mg of flutamide (Sanchez-Craido *et al*., 1999).

Preparation of aqueous extract of *Panaxginseng*

Korean ginseng root (*Panax ginseng*) was bought from Al-kawther herb in Hilla city-babylon, to prepare 200 mg or 400 mg of aqueous extract, add 40 mg or 80 mg of herb to 100 ml of cold water and mix in an electric mixture for 20 minutes. The mixture was centrifuged, the clear supernatant was collected carefully and then placed in the refrigerator at 2-8 °C for subsequent experimental treatmentand the dose was measured based on the body weight of the animal.

Animals

The current study was carried out on adult male albino rats weighing 200-300 g. Animals were provided from animal house, Faculty of Science, Kufa University. In a well ventilated animal environment, the rats are kept in metalic cages and are supply with a suitable standard diet and water *ad libitum*. After 14 days of adaptation, the rats were divided randomly into six groups of five rats as follows:

1) Control group: Animals were received 1 ml of distilled water orally daily for 30 days.

2) Flutamide treated group: Animals were received a dose of flutamide (25 mg / kg b.w) for 7 days orally daily using a metallic stomach tube.

3) *Panax ginseng* group: Animals were injected intrapretonialy (200 mg / kg b.w) daily for 30 days.

4) *Panax ginseng* group: Animals were injected intrapretonialy (400 mg / kg b.w) daily for 30 days.

5) Flutamide + *Panax ginseng* group: Animals were given orally flutamide (25 mg / kg b.w) for 7 days and then injected intrapretonialy with *Panax ginseng* (200mg / kgb.wt) for 30 days.

6) Flutamide + *Panax ginseng* group: Animals were given orally flutamide (25mg/kg b.wt) for 7 days and then injected intrapretonialy with *Panax ginseng* (400mg / kg b.wt) 30 days.

Absolute and relative weight

Livers of control and experimental groups were removed quickly, blotted with a piece of filter paper and weighed for representing the absolute liver weights.to obtain an accurate measurement of changes in organ weight:

$$\text{Hepatosomatic Index} = \frac{\text{Obsolute Liver}}{\text{Total Body Weight}} \times 100$$

Z

Blood and tissue sampling

After the last treatment at least 24 hours from the end of the experimental period, all rats used were weighed and sacrificed under anesthesia by heart puncture. Blood samples were collected in clean and dry centrifuge tubes. Sera were separated by centrifugation at 3000 rpm for 10 minutes and then frozen quickly at 20 ° C for biochemical analysis.

Liver enzymes ALT (Alanineamino transferase) and AST (Aspartateamino transferase) were measured in serum (Reitman and Frankle, 1975).

Hepatic GSH was estimated using the commercial assay kit (CS1020; Sigma, St. Louis, Missouri, USA) according to the manufacturer's instructions.

Histopathological study

Liver samples from control and treated groups were fixed in 10% formal saline solution for 24 hours, The standard method for dehydration, xylene clearing and paraffin embedding was used, 5µ thickness sections were cut by rotary microtome and stain with hematoxylin and eosin stains (Luna ,1968).Sections were examined using a light microscope to detect pathological changes.

Data analysis

Statistical analysis was conducted using the SPSS statistic program version 17. All data was expressed as an mean statistical Mean ± SEM Performed with ANOVA followed by Post Hoc Tukey multiple tests .$P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The results of this study showed that there was a significant increase ($P < 0.05$) in the hepatosomatic index in the flutamide group compared to the control group and treated groups (Figure 1).

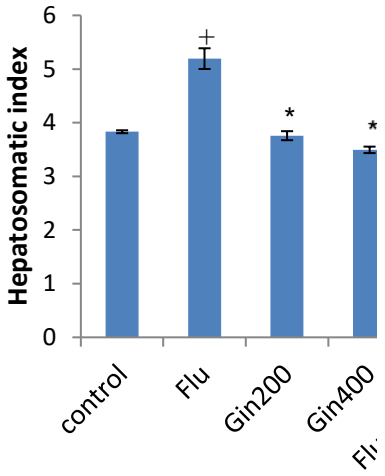


Figure 1: Hepatosomatic Index (HIS) in the control and treated groups, results are presented as Mean ± SEM, + P<0.05 compared to the control group, *P<0.05 compared to the flutamide group

These findings were supported by Dourakis *et al*, 1994 who founded that the administration of flutamide was related to development of hepatic encephalopathy which resulted in an increase in liver weight of patients with metastatic prostate cancer. This result was consistent with his findings Coe *et al*., 2006, who indicated that the increase in the hepatosomatic index was attributed to hepatocellular hypertrophy and increased activity of cytochrome P450 following administration of flutamide to rats. The increase in liver weight could be clarified by the extension of endoplasmic reticulum caused by induction of enzyme (Alkhamees., 2013).

The liver is the main source of biotransformation of foreign substances and is susceptible to chemicals, various enzymes are vulnerable to the effects of chemicals and their metabolites. Its main function is to maintain adequate levels of these metabolites in the plasma (Brahme*tal*,2011). In most cases, these enzymes leak into the blood serum from necrotic hepatocytes for abnormal amounts. Most of those soluble enzymes were known to be indicators of liver dysfunction and damage (Shaarawy*etal*.,2009) .

The obtained data showed that flutamide administration caused a significant increase (P <0.05) in the biochemical parameters of liver transaminase ALT and AST compared with control and treated groups Figure(2),(3)that have been commonly used as biomarkers of specific organ dysfunction in mammalian toxicity. In general, Increased transaminase activity is usually associated with damage of hepatocytes (Honda*etal*.,2010).These findings are in agreement with Matsuzaki et al's results (2006), who founded that the increase in transaminases and bilirubin levels was associated with the flutamide -induced hepatocyte toxicity via cytochrome P450-mediated metabolite formation .These increases in serum enzymes can be considered as a response to oxidative stress and may be also due to lesions in liver function after its cellular damage and consequently the release of its intracellular enzymes into the blood stream(Hassan *etal*,1994;Mannaa*et al*., 2005; Hemieda *et al.,* 2016).

Moreover, Gomez *et al*., 1992showed that the treatments with flutamide in prostate cancer resulted in various patterns of hepatic failure including: immune responses, necrosis, cholestasis and significant increases in the levels of transaminase and total bilirubin TB.

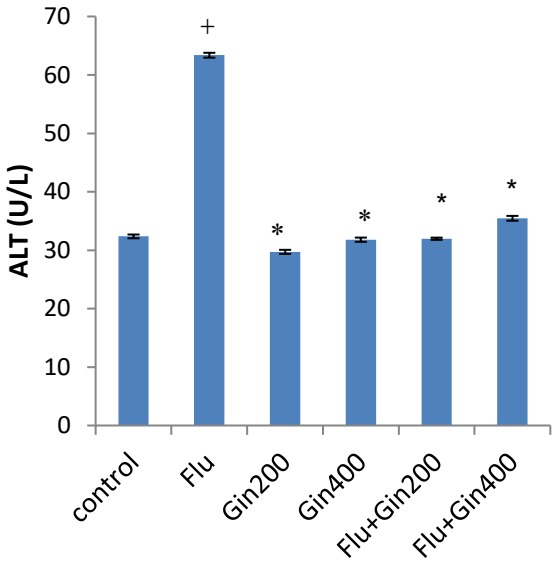


Figure (2): Serum levels of ALT ((U/L) in control and treated groups. Results are presented as Mean ± SEM, +P<0.05compared to the control, *p<0.05compared to the flutamide treated group.

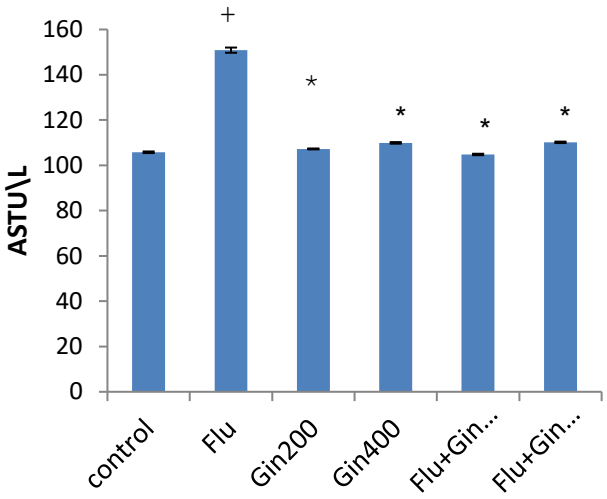


Figure (3): Serum levels of AST ((U/L) in control and treated groups. Results are presented as Mean ± SEM,+ P<0.05compared to the control ,*p<0.05compared to the flutamide treated group.

The endogenous defense system provides protection against oxidative damage under normal conditions, Including glutathione and antioxidant enzymes. The current study indicated a significant decrease (P <0.05) in GSH glutathione in flutamide group compared to control group and treatment groups (Figure 8).The primary endogenous antioxidant, has a multi-faceted role in antioxidant defence and it is a direct scavenger of free radicals (Winterbourn, 1995). Accordingly, the increased synthesis of GSH is an adaptive mechanism for cells exposed to oxidative stress. (Yi lmaz *et al*., 2006).The results obtained correspond to published data showing reduction in hepatic GSH in flutamide-treated mice (Ohbuchi *et al.,* 2009). In similar studies, flutamide has shown a marked decline in GSH in rat liver (Hemieda *et al*., 2016), rabbit serum (Ray *et al*., 2010) and isolated liver cells (Fau*et al*., 1994).

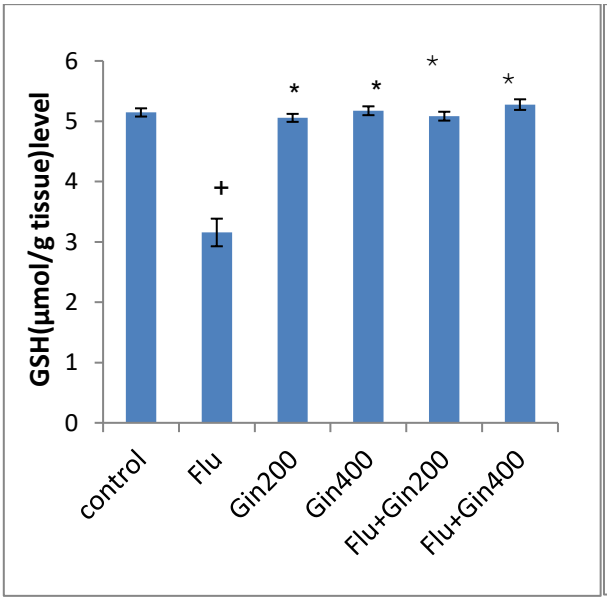


Figure 4: levels of GSH (µmol/g tissue) in control and treated groups. Results are presented asMean ± SEM , + P<0.05compared to the control , * p<0.05compared to the flutamide treated group.

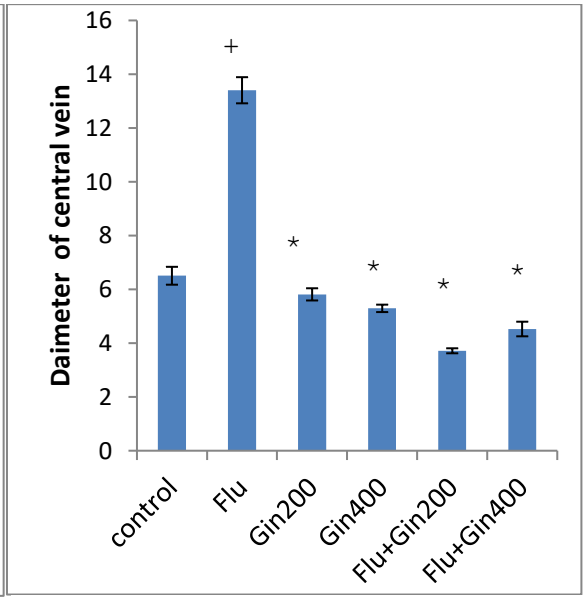


Figure 5: Diameter in central vein(µm) of liver tissue in control and treated groups. Results are presented asMean ± SEM ,+P<0.05compared to the control ,*p<0.05compared to the flutamide treated group.

The present investigation showed that ginseng decreased the harmful effect of flutamide on liver enzymes, as evidenced by significant inhibition of elevated levels of serum ALT, AST induced by flutamide Figure (2) (3).

On the other hand, Figure (1) showed that ginseng administration with flutamide group could minimize changes in liver weight relative to body weight. So far, various studies have demonstrated protective effects of ginseng in hepatic damage (Pradeep *et al*, 2007). Saller *et al*, 2007 revealed in his study that the ginseng extract minimized acute and chronic hepatitis treatment periods.

The results of this study are related with studies conducted by many other researchers in-vitro and in vivo (Hikino, 1985; Lin, 1995).

The observed increase in GSH level indicates that protection by ginseng can be mediated by modulating the levels of cellular antioxidants in Figure (4)

Ginseng therefore plays a significant role in maintaining this critical antioxidant in the liver and in enhancing hepatocyte antioxidant capacity. Evidence has shown that ginsenoside-Rg1 can recover GSH-cycle enzymes and protect cells from H2O2-induced cells death (opez, *et al*, 2007). Our results have shown that pretreatment with *Panax ginseng* can counteract oxidative stress caused by flutamide and protect the liver cells by stabilizing GSH levels.

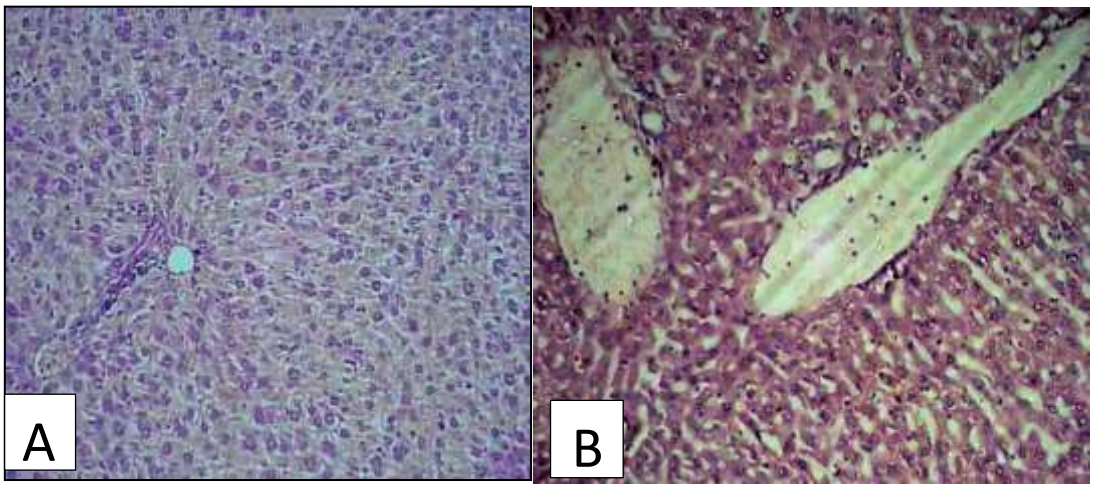
Ginseng enhanced the antioxidant defense mechanism and increased the activities of the self-antioxidant enzyme of superoxide dismutase (SOD), catalase (CAT), glutathione-peroxidase (GPx), reduced glutathione (GR), glutathione-S-tansferase (GSH) and hemooxygenase-1 in elderly rat liver and hepatotoxins- induced liver damage in rats (Shim *etal*, 2010).

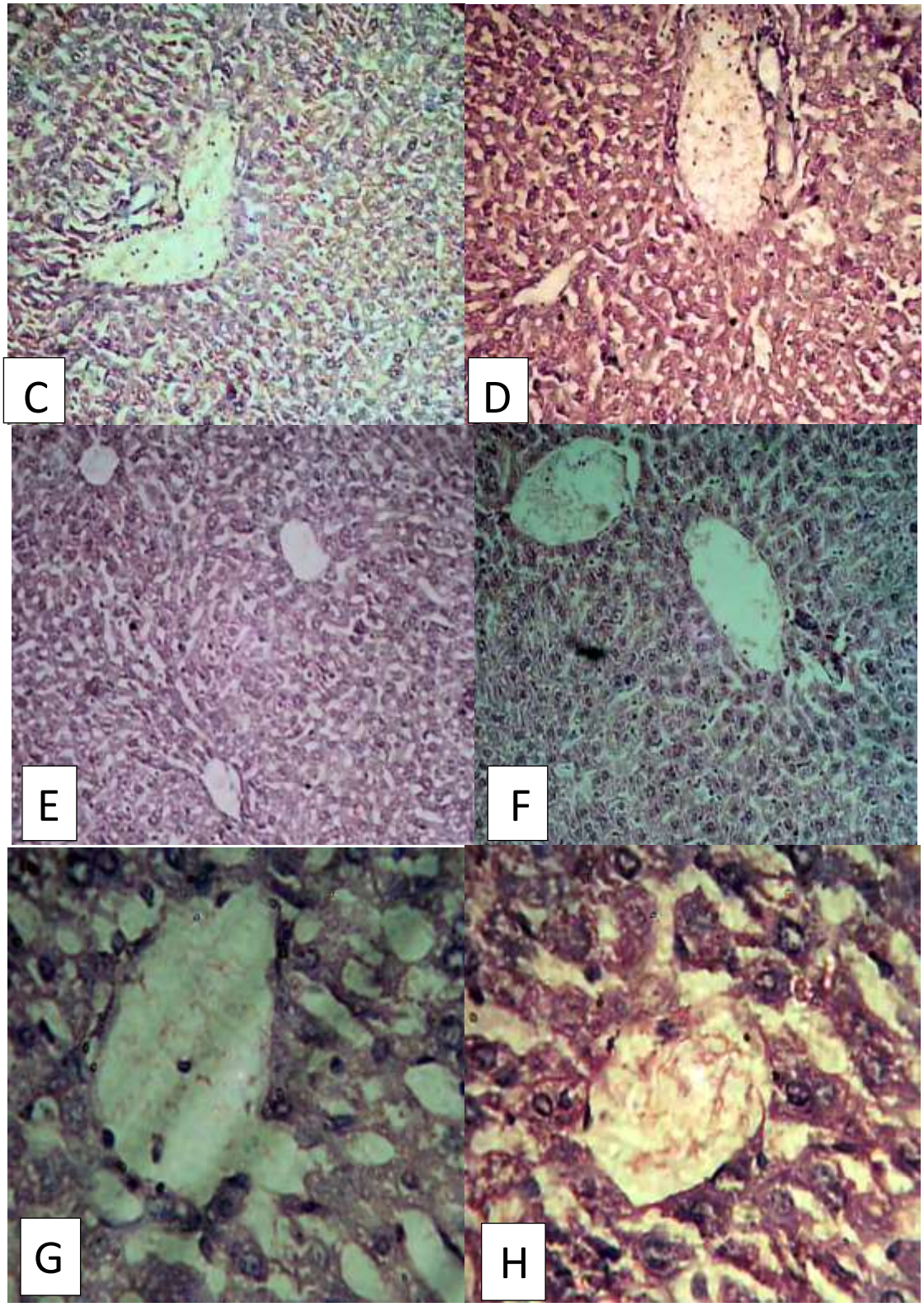
Treatment with ginseng suppresses oxidative damage, such as lipid peroxide, maldialdehyde, thiobarbituric, acid reactant, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and dehydrogenase lactate (LDH) (Kim *et al*, 2011; Ramesh *et al*, 2012; Yokozowa *et al*, 2007; Lee *et al*, 2012). The pharmacological properties of ginseng are primarily attributed to the major and bioactive constituents of ginseng saponins, widely known as ginsenoisdes (Ernst, 2010; Choi, 2008). It has antioxidant activity because it has beneficial protective effects against organ damage (Lobna *et al.*, 2014).

Recharding histological results, normal histological arrangement of the liver tissue of control group has been found with normal central vein and hepatic lobule in Figure 6(A). The liver tissue of the flutamide group showed significant increase in the diameter of central vein Figure 5, bloody congestion in the central vein with in filtaration of inflammantory cells, swelling of hepatocytes, necrosis of hepatocytes, narrowing of blood sinusoids and vacoules appear inside the hepatocytes in Fig 6 (B, C, D, E, F, G, H, I, J). No pathological changes could be noticed in the liver tissue of rats given *Panax ginseng* extract in Fig 6 (K, L). Liver sections in rats treated with flutamide + *Panax ginseng* showed regeneration of the normal liver structure in Fig 6 (M, N).

Histological results were consistent with measured activities for serum liver enzymes and provided supporting evidence for biochemical analysis, this can be explained by *Panax ginseng* components which are free radical scavengers, inhibit lipid peroxidation and protect cells and tissues from the oxidative stress caused by free radicals (Keum *et al*., 2000 and Lee *et al.,* 2002).According to Tran *et al*., 2002, the ginseng can inhibited apoptosis and suppressed hepatic necrosis.

In conclusion, the present results demonstrated that the histological and biochemical changes of liver induced by flutamide were significantly recovered by *Panax ginseng* roots.

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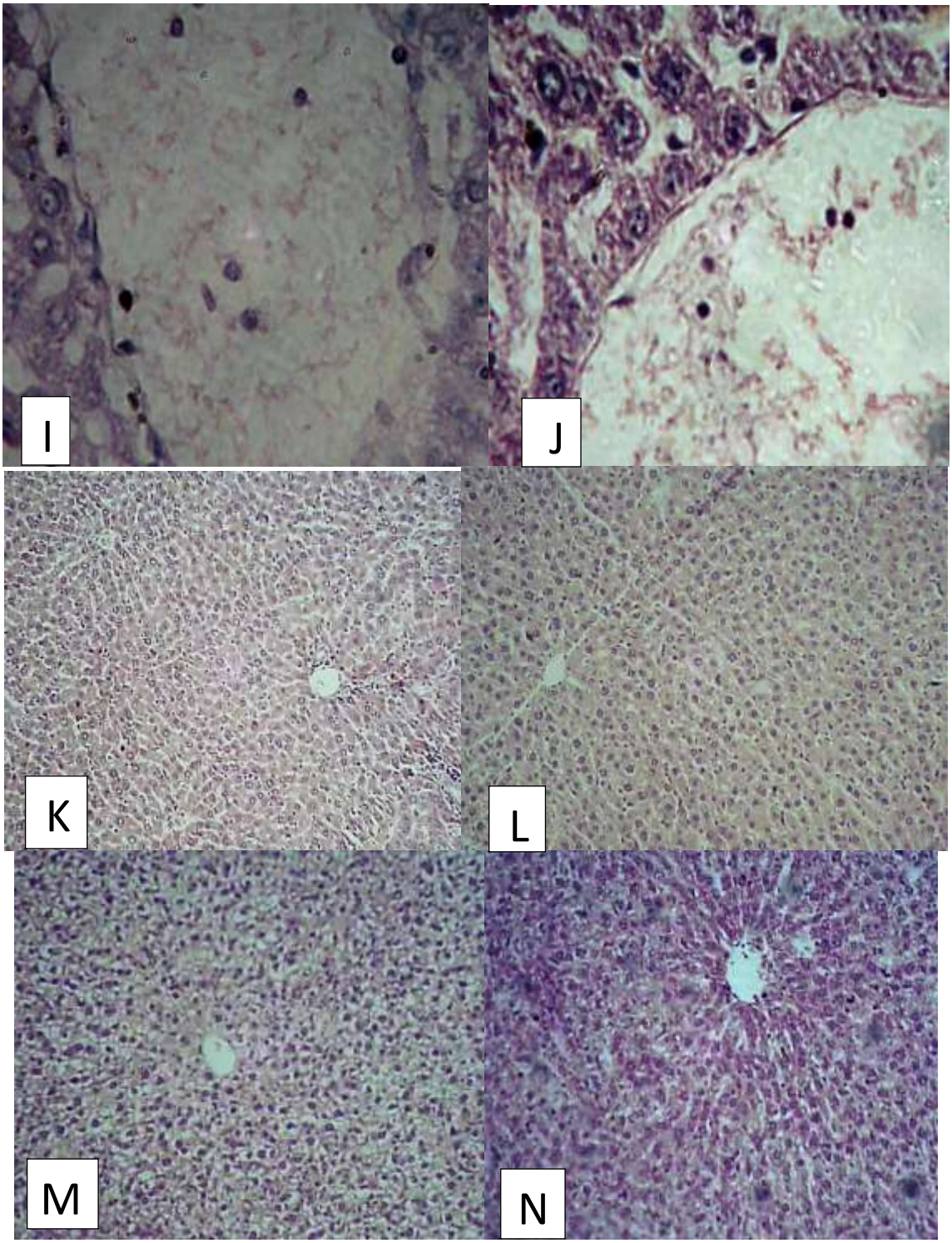

Figure 10: Photomicrographs showing sections of livers administered stained with H.E. (200X): (A) Normal hepatic tissue architecture, (B,C,D,E&F)are flutamide-treated rats show: bloody congestion in the central vein with infiltration of inflammatory cells ,increase in the diameter of the central vein, necrosis of hepatocytes,narrowing of the blood sinusoids .(G&H)are flutamide-treated rats stain with H.E. (400X) show : bloody congestion in the central vein with infiltaration of inflammantory cells, necrosis of hepatocytes and vacoules appear inside the hepatocytes.(I,J) are flutamide-treated rats stain with H.E. (400X) show : bloody congestion in the central vein with infiltaration of inflammantory cells , swelling of hepatocytes.(K,L) are ginseng –treated rats stain with H.E. (200X) show :no histopathological changes.(M,N) are recovery (flutamide + *Panax)* group stain with H.E. (200X) show: regeneration of the normal liver structure.

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