Experimental Comparative Study of the Possible Effect ofPanax Ginseng and Fish Code Oilagainst Acetaminophen Induced Hepatotoxicity

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Abstract: Background: Hepatic damage due to drugs, xenobiotics and environmental pollutants has been a serious concern worldwide. Acetaminophen is widely used as an analgesic and antipyretic drug, which can cause hepatic injury when given in high doses.*Panax ginseng*(Chinese medicinal herb) and Fishcode oil with antioxidant properties would attenuate the intensity of the oxidative stress often involved in thepathogenesis of various diseases andhave been studied in a variety of clinical conditions and found to protect against cellular damage .The objective of the present study was to study and compare the possible effect of ginseng and fish code oil against acetaminophen induced hepatotoxicity .Methods:36 rats were divided into six equal groups (n = 6):firstgroup (healthy control),second group (positive control group),they were given a single oral dose of acetaminophen (500 mg/kg),the third and fourth groups were given*Panaxginseng*(300 mg/kg) and fish oil (4 ml/kg) as protection for 7 consecutive days prior to single oral dose of acetaminophen (500 mg/Kg), the fifth and sixth groups were first taken single oral dose of acetaminophen (500 mg/Kg) and after 3 days the animals were treated with ginseng and fish code oil for 7 days (300mg/Kg and 4 ml/Kg, respectively).Blood was taken from the orbital sinus of the rats for biochemical testsserum transaminases (ALT ,AST) , Alkaline phosphatase (ALP), Total protein (T.P) and Albumin (Alb.) and at the same time liver was removed and kept in 10% formalin solution for histological analysis.All histological sections were subjected to morphometric study , pathological evaluation followed by statistical analysis.Results: The results showed that the acute elevation of serum ALT,AST, ALPand the acute reduction of T.P and Alb. due to acetaminophen induced hepatotoxicity were significantlycorrected in the groups receiving *Panax ginseng* and fish code oil. Necrosis of liver significantlydecreased according to histopathologic observation, and that fish code oil was significantly more effective against acetaminophen induced hepatotoxicity when compared with ginseng.Conclusion:It is concluded that both *Panax ginseng* and fishcode oil have protective and treating effect on hepatotoxicity induced by acetaminophen and that fish code oil has better effect than*Panaxginseng*.

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Key words:acetaminophen,ginseng , fish code oil,hepatotoxicity

1. Introduction

Liver, which is the key organ of metabolism and detoxification, is continually exposed to a variety of xenobiotics such as drugs, alcohol, tobacco products and environmental pollutants of industrial and agricultural origin **(Ali and Walford, 1985)**. Therefore, the associated disorders of liver are numerous and varied, often associated with centrilobular necrosis, periportal necrosis, fibrosis and initiation of hepatic cirrhosis (**Barker *et al.*, 1997)**. Acetaminophen (paracetamol) is the most commonly available analgesic antipyretic widely used worldwide. Though it is one of the most harmless drugs, its prolonged use or a single high dose causes hepatotoxicity, producing fulminate hepatic necrosis, which can be lethal in human and animal (**Kalantari*etal.*,2007)**. In the recent years, fish code oil has been evaluated clinically and experimentally for its use in some diseases like rheumatoid arthritis, coronary heart disease, etc. and its effect has been attributed to its high content of ω-3 polyunsaturated fatty acids of n-3 series, the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and vitamin A, D and E **(Goodfellow*et al.*, 2000;Kalra*et al.*, 2012).***Panax ginseng*** is a Chinese medicinal herb with antioxidant properties due to its ability to scavenge free radicals and to neutralize free ion-induced peroxidation would attenuate the intensity of the oxidative stress often involved in the pathogenesis of various diseases**(Karadeniz*et al.*, 2009).** Therefore, the purpose of this work was to study andcompare the possible effect of Ginseng and fish code oil against acetaminophen induced hepatotoxicity.

2.Material and methods:

Thirty six adult male albino rats weighing about 120-150 g were used.All rats included were fed on well balanced diet (rat chow) and allowed to adapt to the prevailing environment for one week prior to the beginning of the experiment in specific rat house at Faculty of Medicine (for Girls) AI-Azhar University.Animals were housed in plastic cages and received care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institute of Health **(National Health Institute, 1996).**

Rats were divided into six equal groups (n = 6): the first group (healthy controls),maintained on the commercial rat chow diet all over the experimental period, the second group positive control group (acetaminophen group),were given a single oral dose of acetaminophen (500 mg/kg body weight) according to **Kalra*et al.*, 2012**to induce acute hepatotoxicity. Both blood and tissue samples were collected 72 hours after acetaminophen administration, the third group received *Panax ginseng* (300 mg/kg orally, purchased from local herbal store, Cairo, Egypt) as protection for 7 consecutive days by the oral route using an orogastric tube and then the rats were given single dose of acetaminophen (500 mg/Kg). The dose and duration for *Panax ginseng* were chosen according to **Simseke*t al.*, 2007**. The fourth group was given fish code oil (manufactured by El Hawag Factory, Badr city, was purchased from local commercial source)(4ml/kgintrapretonially,ip) according to **Kalra*et al.*, 2012** as protection for 7 consecutive days prior to single oral dose of acetaminophen (500 mg/Kg), the fifth and sixth groupswere first given single oral dose of acetaminophen (500 mg/Kg) and after 3 days the animals were treated with ginseng and fish code oil for 7 days (300mg/Kg and 4 ml/Kg respectively).

Biochemical analysis:

Blood samples were obtained from the orbital sinus of therats under light ethyl ether anesthesia, with capillary tubesaccording to **Simmons and Brick, 1970**. The spurting blood was collected inclean centrifuge tubes and allowed to clot for an hour at room temperature, then centrifuged at a rate of 12,000 revolutions per minute (rpm) for 10 minutes.

The clear serumobtained was separated and labeled for the analyses of the serum levels of Aspartate aminotransferases (AST),Alanine aminotransferases (ALT) (obtained from Biorex Diagnostic Limited-United Kingdom) and Alkaline phosphatase (ALP) (obtained fromBio-diagnostic co.), Total protein (T.P) (obtained from Greiner Diagnostic GmbH-Germany) and Albumin (Alb.) (obtained from Unilab biotechnology).The analysis were carried out at thefaculty of pharmacy (for girls),Department of biochemistry, Al-AzharUniversity.

Histological analysis:

Allrats were sacrificed by decapitation after taking the blood samples.Small liver specimens were extracted,and fixed with 10% neutral formalin.Specimens were dehydrated andembedded in paraffin. Tissue sections of 5 µmwere stained with Haematoxylin-Eosin (Hx&E)andexamined under light microscope.All histological sections were subjected to morphometric study and pathological evaluation followed by statistical analysis.

Morphometric study: Hx&E stained sections were assessed by ordinary light microscope using an image analyzer computer system for histomorphometric measuring of the cellular (hepatocyte) area. The equipment consists of a digital camera attached to a light microscope and a computer system equipped with the software LeicaQuin500 (Leica Microsystems Inc., Switzerland) capable ofperforming high speed digital image processing. The imageanalyzer is calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. The area of hepatocytes was measured in 5 fields in each group, usingmagnification (x400) by light microscopy transferred to themonitor of the image analyzer. The outline of the cells wasmanually traced then the cellular area was automatically computed by the image analyzer (Fig.1). Mean values and standard error of mean (SEM) were calculated for each group.

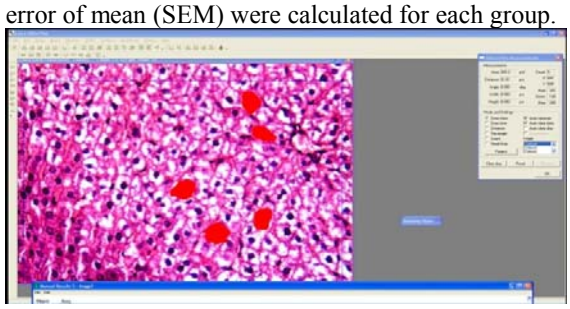

Fig. (1): Photograph showing histomorphometric measuring of the hepatocyte area.
(Leica Microsystems Inc., Switzerland)

Pathological evaluation: An experienced pathologist evaluated all histological sections in a blinded fashion. 5 fields were evaluated per tissue sample. The percent of necrosis was estimated by evaluating the number of microscopic fields with necrosis compared with theentire cross section, using magnification (x200).The criteria of necrosis included hyperchromatic, pyknotic, fragmented or lost nucleus and/or vacuolated cytoplasm and/or dilated congested veins as in (Fig.2). Necrosis was given scores as follows: (-) showing no necrosis, (+), (++) and (+++)

indicatingminimum, moderateand maximum necrosis respectively.

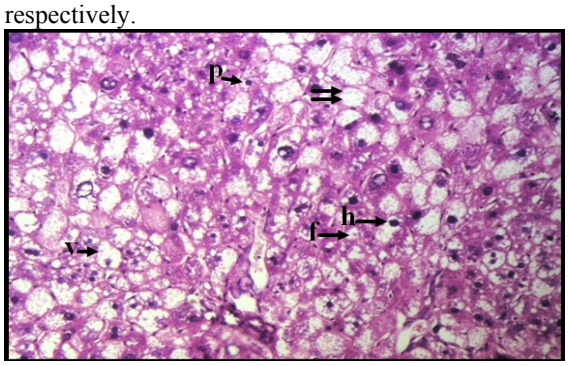

Fig. (2): Photomicrograph of a transverse section of liver of acetaminophen group showing the criteria of necrosis [hyperchromatic (h), pyknotic (p), fragmented (f) or lost nucleus (double arrow) and/or vacuolated cytoplasm (v)].(Hx&E X 200)

Statistical Analysis:

The data obtained were recorded and analyzed by unpaired student's't' test (GraphPad Instat version 6.0). The results were expressed as mean ± standard error of mean (SEM) and p value of less than 0.05 was considered significant.

3. Results:

This study was undertaken toevaluate and compare the possible effect of Ginseng and fish code oil against acetaminophen induced hepatotoxicity.

Biochemical results:

Biochemical analysis of blood showed a significant increase ($P< 0.05$) in the levels of ALT, AST, ALP. While T.P and Alb. levels showed a significant decrease ($P< 0.05$) in the acetaminophen treated rats when compared to the healthy control rats (Table 1). Administration of 300mg/Kg ginseng for 7 days before (as protective agent) and after (as a treating agent)acetaminophen showed a significant decrease in the serum levels of ALT, AST, ALP and significant increasein T.P and Alb. levels when compared to the acetaminophen treated rats(Tables 2, 3).While the administration of 4 ml/Kg of Fish code oilbefore andafteracetaminophen toxicity showed more significant decreases in the serum levels of ALT, AST, ALP, and more significant increase in T.P and Alb. when compared to the acetaminophen treated rats (Tables 4,5).Administration of fish code oil as a protective and treatment agent showed significant improvement in biochemical liver tests than administration of ginseng(Figs.3, 4).

Table (1): Biochemical liver test in the healthy control group as compared to the acetaminophengroup.

	ALT U/L	**AST U/L**	**ALP U/L**	**T.P g/dL**	**Alb. g/dL**
Healthycontrol group	7.66 ± 1.30	8.83 ± 0.70	46.33 ± 4.29	6.66 ± 0.24	3.50 ± 0.76
Acetaminophengroup	84.50 ± 3.22**** *p*<0.0001	71.0 ± 4.75**** *p*<0.0001	400.0 ± 8.87**** *p*<0.0001	3.46 ± 0.24**** *p*<0.0001	0.40 ± 0.057**** *p*<0.0001

Results are presented as mean ± SEM; *significance at $P<0.05$. **AST** = Aspartate Aminotransaminases, **ALT** = Alanine Aminotransaminases,**ALP**= Alkaline Phosphatase ,**T.P**= Total Protein and **Alb.**=Albumin.Acetaminophen group(single oral dose of acetaminophen 500 mg/kg)

Table (2): Biochemical liver test in Ginseng protected group compared to theacetaminophen group.(Before toxicity)

	ALT U/L	**AST U/L**	**ALP U/L**	**T.P g/dL**	**Alb. g/dL**
Acetaminophen group	84.50± 3.22	71.0 ± 4.75	400.0±28.87	3.46 ± 0.24	0.40± 0.05
Ginseng protected group	50.00 ± .67**** *p*<0.0001	43.17 ± 1.19*** *p*=0.0002	145.8 ± 4.36**** *p*<0.0001	5.13 ± 0.24*** *p*=0.0007	0.75 ± 0.04*** *p*=0.0007

Results are presented as mean ± SEM; *significance at $P<0.05$. **AST** = Aspartate Aminotransaminases, **ALT** = Alanine Aminotransaminases,**ALP**= Alkaline Phosphatase ,**T.P**= Total Protein and **Alb.**=Albumin.Acetaminophen group(single oral dose of acetaminophen 500 mg/kg).Ginseng protected group(300mg/Kgorallyfor 7 days before toxicity).

Table (3): Biochemical liver test in Ginseng treated group compared to the acetaminophengroup.(After toxicity)

	ALT U/L	**AST U/L**	**ALP U/L**	**T.P g/dL**	**Alb. g/dL**
Acetaminophen group	84.50± 3.22	71.0 ± 4.75	400.0±28.87	3.46 ± 0.24	0.40± 0.05
Ginseng treated group	52.33 ± 1.64**** *p*<0.0001	43.17 ± 1.86*** *p*=0.0003	151.7 ± 3.33**** *p*<0.0001	4.90 ± 0.31** *p*=00.51	0.76 ± 0.04*** *p*=0.0007

Results are presented as mean ± SEM; *significance at $P<0.05$. **AST** = Aspartate Aminotransaminases, **ALT** = Alanine Aminotransaminases,**ALP**= Alkaline Phosphatase ,**T.P**= Total Protein and **Alb.**=Albumin.Acetaminophen group(single oral dose of acetaminophen 500 mg/kg).Ginseng treated group(300mg/Kgorallyfor 7 days after toxicity).

Table (4): Biochemical liver test in Fish cod oil protected group compared to the acetaminophen group. (Before toxicity)

	ALT U/L	**AST U/L**	**ALP U/L**	**T.P g/dL**	**Alb. g/dL**
Acetaminophen group	84.50± 3.22	71.0 ± 4.75	400.0±28.87	3.46 ± 0.24	0.40± 0.05
Fish code oil protected group	34.83±1.53**** *p*<0.0001	22.17 ± 2.07**** *p*<0.0001	113.5 ± 2.43**** *p*<0.0001	6.133 ± 0.18**** *p*<0.0001	1.31 ± 0.16*** *p*=0.0004

Results are presented as mean ± SEM; *significance at *P*<0.05. **AST** = Aspartate Aminotransaminases, **ALT** = Alanine Aminotransaminases,**ALP**= Alkaline Phosphatase ,**T.P**= Total Protein and **Alb.=**Albumin. Acetaminophen group(single oral dose of acetaminophen 500 mg/kg). Fish code oil treated group (4ml/Kgipfor 7 days before toxicity).

Table (5): Biochemical liver test in Fish code oil treated group compared to the acetaminophen group. (After toxicity)

	ALT U/L	**AST U/L**	**ALP U/L**	**T.P g/dL**	**Alb. g/dL**
Acetaminophen group	84.50± 3.22	71.0 ± 4.75	400.0±28.87	3.46 ± 0.24	0.40± 0.05
Fish code oil treated group	34.83 ± 1.53**** *p*<0.0001	31.00 ±1.82**** *p*<0.0001	128.0 ± 2.63**** *p*<0.0001	6.03± 0.19**** *p*<0.0001	1.150 ±0.1147*** *p*=0.0002

Results are presented as mean ± SEM; *significance at *P*<0.05. **AST** = Aspartate Aminotransaminases, **ALT** = Alanine Aminotransaminases,**ALP**= Alkaline Phosphatase ,**T.P**= Total Protein and **Alb.=**Albumin. Acetaminophen group(single oral dose of acetaminophen 500 mg/kg). Fish code oil treated group (4ml/Kgipfor 7 days after toxicity).

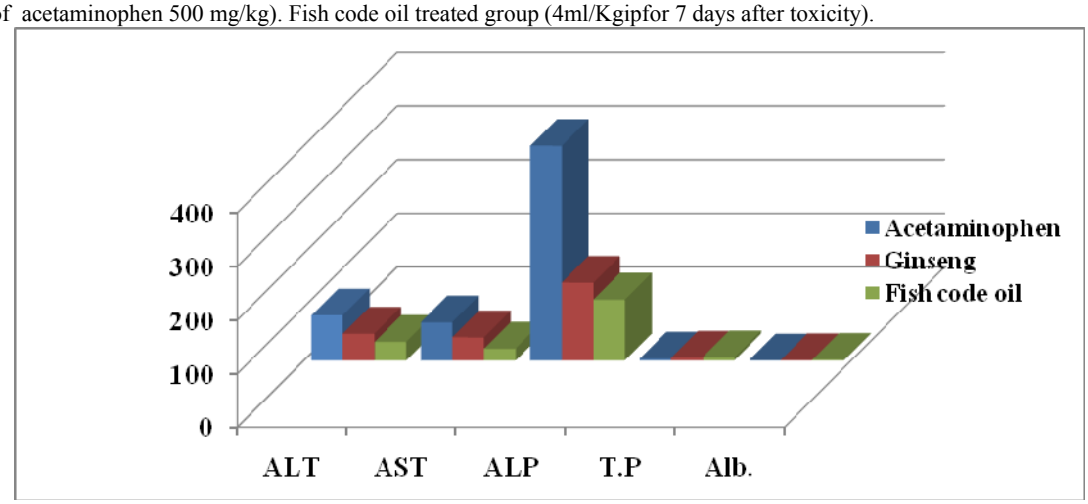

Fig. 3: Comparison between protected groups withfish code oil and ginseng according tobiochemical liver tests in relation to acetaminophen group.

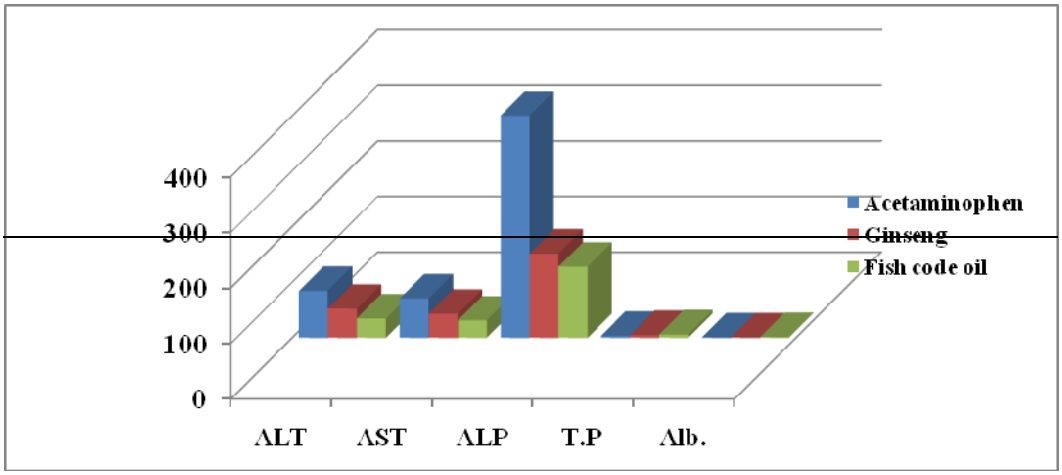

Fig. 4:Comparison between treatedgroups withfish code oil and ginseng according tobiochemical liver test in relation to acetaminophen group.

Histopathologicalresults:

The healthy control group: Examination of transverse sections of liver of the healthy control group revealed that the liver was formed of hepatocytes arranged in cords of 1-2 cells thick. The cords were radiating from central vein and separated by blood sinusoids. Areas of portal tracts appeared to be arranged at the periphery of the hepatic lobule (Fig.5a). The hepatocytes were polygonal in shape with eosinophilic cytoplasm and rounded basophilic nuclei containing prominent central or eccentric nucleoli. The blood sinusoids were lined with flat endothelial cells and Von Kupffer cells (Fig.6a). The portal areas contained a branch ofportal vein which was lined with flat endothelial cells and a branch of bile duct which was lined with cubical epithelial cells (Fig.7a).There was no necrosis (-).

Theacetaminophen group:Examination of transverse sections of the liver of acetaminophen treated group showed that the central vein became dilated and congested. There was extensive necrosis which was marked at the periportal areas and only in small areas near the central veins (Figs.5b & 6b). In the periportal area there was loss of hepaticarchitecture. The hepatocytes became disorganized, lost their characteristic polygonal shape and appeared to be enlarged in size. Thecytoplasm of the majority of the hepatocytes showed numerous variable size vacuoles that in most of the cells became large enough to replace all the cytoplasm. The nuclei assumed many patterns of degeneration. Most of the nuclei became hyper chromatic, irregular and eccentric with disappearance of the nucleoli. Some nuclei became pyknotic, fragmented and some completely disappeared. Blood sinusoids appeared very narrow or obliterated (Fig.7b).The area of necrosis was about (65% ± 2.09) indicating necrosis of score (+++).

Theginseng protected group:Examination of transverse sections of ginseng protected group showed moderate improvement as compared to acetaminophen group. One central vein appeared normal and the other one appeared congested but not dilated (Fig.5c). The hepatocytes around the central vein appeared organized like thecontrol (Fig.6c). The hepatocytes in theperiportal area appeared disorganized and few of them had vacuolated cytoplasm with irregular hyperchromatic peripheralnuclei (Fig.7c).The area of necrosis was significantly reduced ($p<0.05$) in comparison to acetaminophen group from (65% ± 2.09) to (32% ± 0.8) indicating necrosis of score (++).

TheGinseng treated group: Examination of transverse sections of ginseng treated group showed mild improvement as compared to acetaminophen group. The hepatic architecture was preserved around the central vein but there was still necrosis at the periportal area (Fig.5d). The central veinappeared dilated and congested (Fig.6d). The hepatocytes at the periportal area were disorganized and most of them showed vacuolated cytoplasm but the size of the vacuoles was less than that in acetaminophen group. Most of the nuclei were irregular and eccentric. Some nuclei are fragmented (Fig.7d).The area of necrosis was significantly reduced ($p<0.05$) in comparison to acetaminophen group from (65% ± 2.09) to(37.27% ± 0.9) indicating necrosis of score (++).The improvement with ginseng treated group was less than that with ginseng protected group.

TheFish cod oil protected group: Examination of transverse sections of fish cod oil protectedgroup showed marked improvement with nearly normal hepaticarchitecture (Fig.5e). The central vein appeared slightly congested and hepatocytes around it was normal (Fig.6e).Hepatocytes at periportal area appeared normal (Fig.7e).There was marked significant reduction ($p<0.025$) of necrosis in comparison to acetaminophen group from (65% ± 2.09) to(29.4% ± 0.09) indicatingnecrosis of score(+).

TheFish cod oil treated group:Examination of transverse sections of fish cod oil treated group showed marked improvement with nearly normal hepatic architecture (Fig.5f). The central vein appeared moderately dilated and congested. The hepatocytes around it were nearly normal (Fig.6f). Hepatocytes at periportal area appeared normal but the blood sinusoids appeared narrowed (Fig.7f).There was marked significant reduction ($p<0.025$) of necrosis in comparison to acetaminophen group from (65% ± 2.09) to (31.4% ± 1.05) indicating necrosis of score (+).The improvement with fish cod oil treated group was less than that with fish cod oil protected group.

Statistical results according to the hepatocyte area:The greatest mean value of hepatocyte area was observed inacetaminophen group, followed by ginseng protected and ginseng treated groups respectively and the smallest mean hepatocyte area was found in Fish oil treated group(Fig.8).. Unpaired Student's t test revealed that the difference between the acetaminophen group (367.64±66.7) and the control group (184.76±39.13) was extremely statistically significant ($p=0.0007$) (table 6).And by using unpaired Student's t test for pair-wise comparison revealed a statistically significant difference between acetaminophen group and all experimental groups, except ginseng protected group (Table 7).

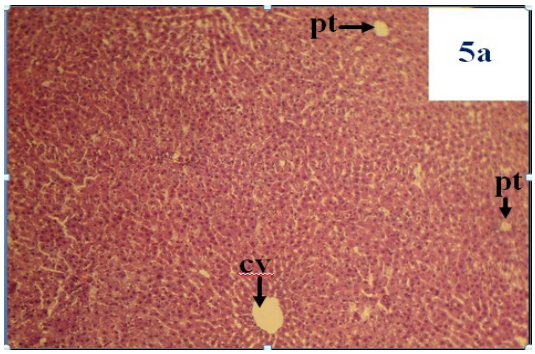

Fig. (5a): Photomicrograph of a transverse section of liver of control group shows the hepatic cords radiating from the central vein (cv). the portal tracts (pt) are arranged on the periphery of hepatic lobules.**(Hx&E X 100)**

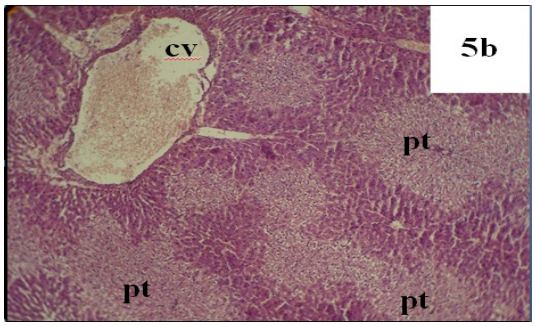

Fig. (5b): Photomicrograph of a transverse section of liver of acetaminophen group showing dilated and congested central vein (cv). Notice the necrosis around the portal tracts (pt).**(Hx&E X 100)**

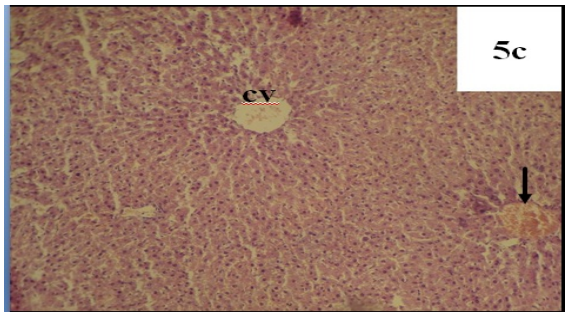

Fig. (5c): Photomicrograph of a transverse section of liver of ginseng protected group showing preserved hepatic architecture. Notice the presence of one normal central vein (cv) and the otherone is congested (arrow).**(Hx&E X 100)**

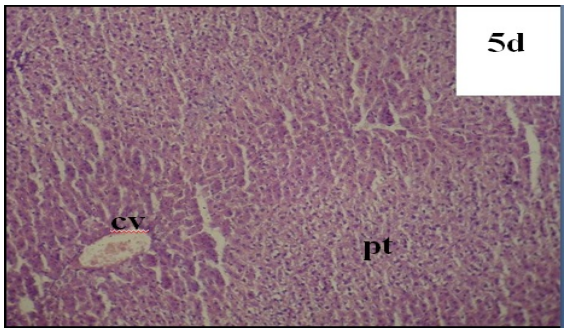

Fig. (5d): Photomicrograph of a transverse section of liver of ginseng treated group.the hepatic architecture is preserved around the central vein (cv) and disturbed at the periportal area (pt).**(Hx&E X 100)**

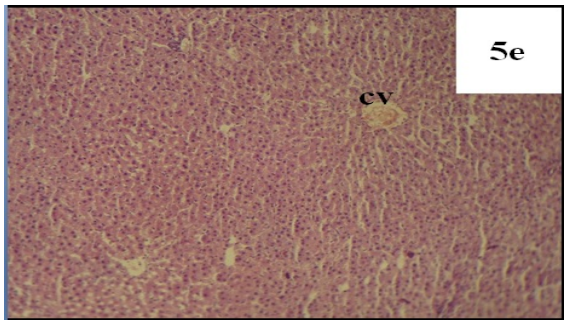

Fig. (5e): Photomicrograph of a transverse section of liver of fish cod oil protected group showing nearly normal hepatic architecture. Notice the slightly congested central vein (cv).**(Hx&E X 100)**

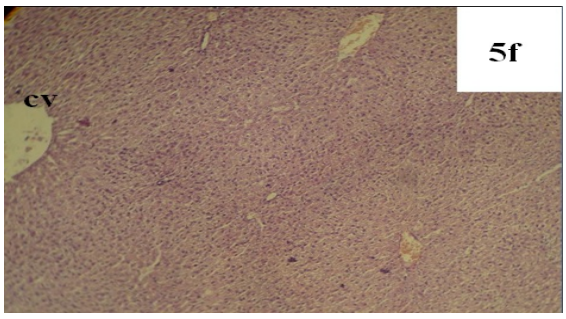

Fig. (5f): Photomicrograph of a transverse section of liver of fish cod oil treated group showing nearly normal hepatic architecture. Notice the moderately dilated central vein (cv). **(Hx&E X 100)**

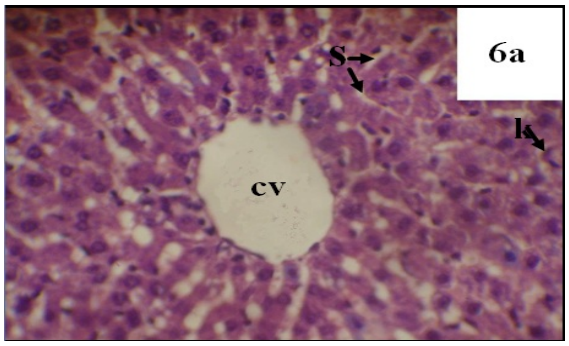

Fig. (6a): Photomicrograph of a transverse section of liver of control group shows the hepatocytes arrange in cords radiating from the central vein (cv). the blood sinusoids (s) are lined with flat endothelial cells and Von Kupffer cells (k)**(Hx&E X 400)**.

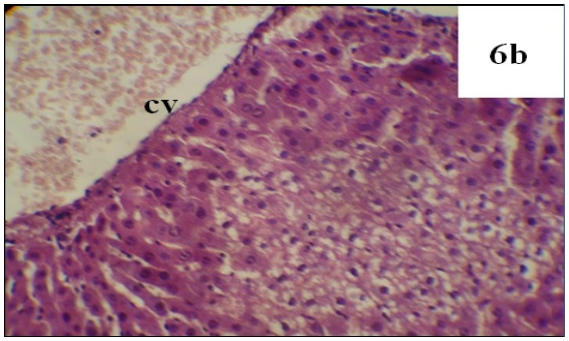

Fig. (6b): Photomicrograph of a transverse section of liver of acetaminophen group showing dilated and congested central vein (cv). Notice the presence of area of necrosis near the central vein.**(Hx&E X 400)**

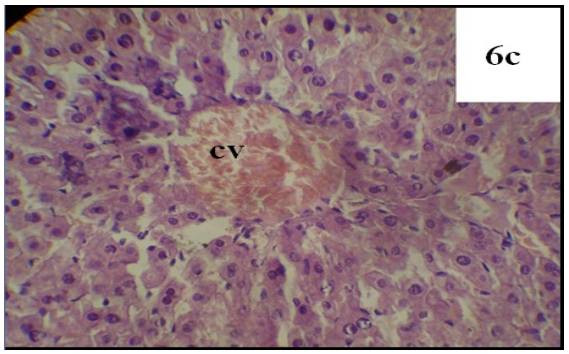

Fig. (6c): Photomicrograph of a transverse section of liver of ginseng protected group showing preserved hepatic architecture. Notice the presence of congested central vein (cv).**(Hx&E X 400)**

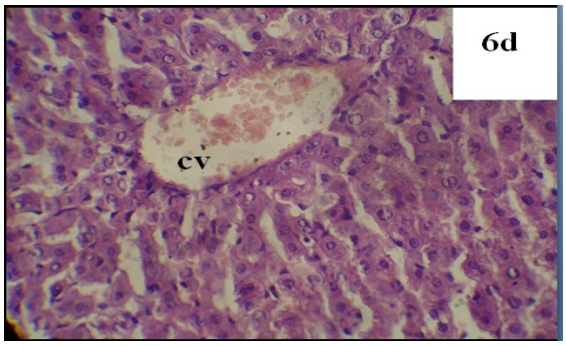

Fig. (6d): Photomicrograph of a transverse section of liver of ginseng treated group. Notice that the hepatic architecture is preserved around the central vein (cv). **(Hx&E X 400)**

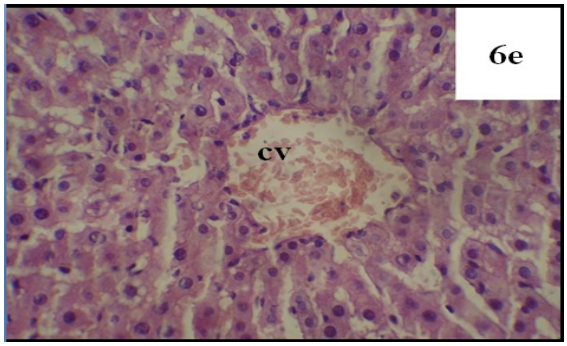

Fig. (6e): Photomicrograph of a transverse section of liver of fish cod oil protected group showing nearly normal hepatic architecturewith slightly congested central vein (cv).**(Hx&E X 400)**

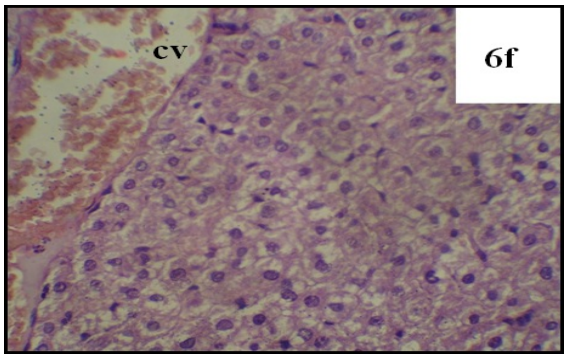

Fig. (6f): Photomicrograph of a transverse section of liver of fish cod oil treated group. Notice the moderately dilated and congested central vein (cv). Notice that the hepatocytes are slightly disorganized. **(Hx&E X 400)**

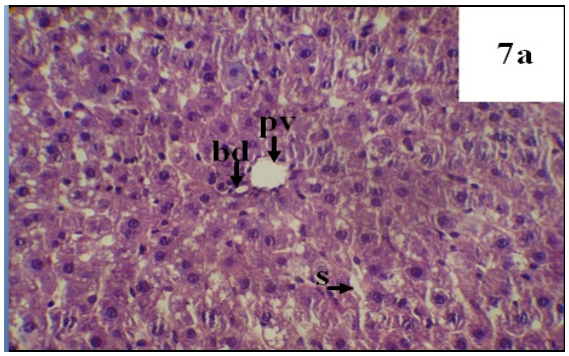

Fig. (7a): Photomicrograph of a transverse section of liver of control group to show the portal area containing a branch of portal vein (pv) and a branch of bile duct (bd).**(Hx&E X 400)**

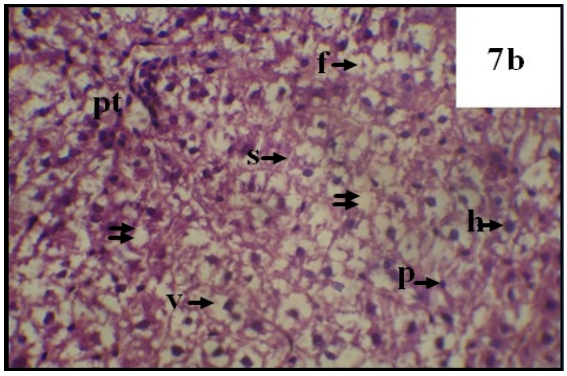

Fig. (7b): Photomicrograph of a transverse section of liver of acetaminophen group to show the necrosis around the portal tract (pt).The hepatocytes are disorganized, irregular and appear to be large in size, vacuolated cytoplasm (v), most of the nuclei are hyperchromatic (h). Some nuclei are pyknotic (p), fragmented (f) and some are completely disappeared (arrow). Blood sinusoids are narrow (s) or obliterated.**(Hx&E X 400)**

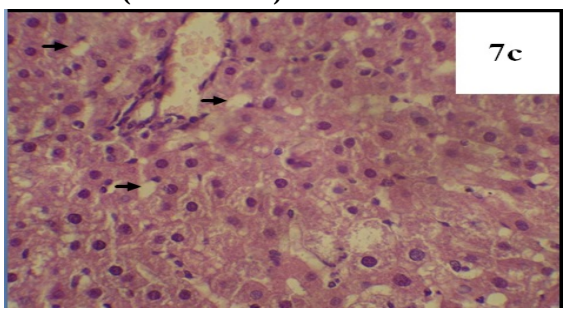

Fig. (7c): Photomicrograph of a transverse section of liver of ginseng protected group showing that the hepatocytes at the periportal area are disorganized and few of them have vacuolated cytoplasm with irregular hyperchromatic peripheral nuclei (arrow).**(Hx&E X 400)**

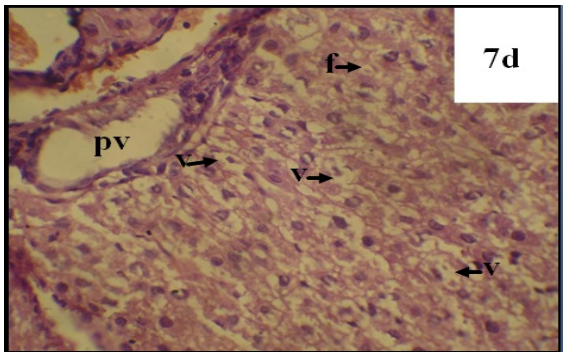

Fig. (7d): Photomicrograph of a transverse section of liver of ginseng treated group shows that the hepatocytes are disorganized and their cytoplasm is vacuolated (v). Notice that most of the nuclei are irregular and eccentric. Some nuclei are fragmented (f). Notice also the thickening of the wall and dilatation of the portal vein (pv). **(Hx&E X 400)**

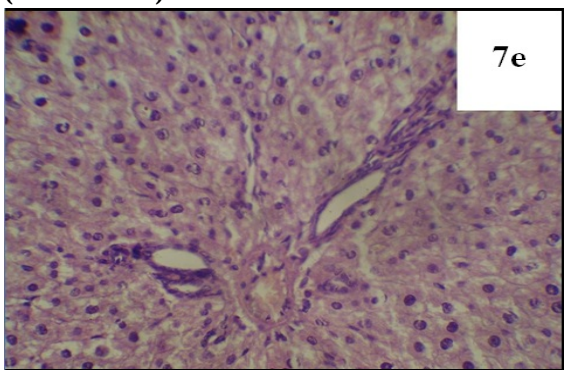

Fig. (7e): Photomicrograph of a transverse section of liver of fish cod oil protected group showing the normal hepatocytes at the peripotal area.**(Hx&E X 400)**

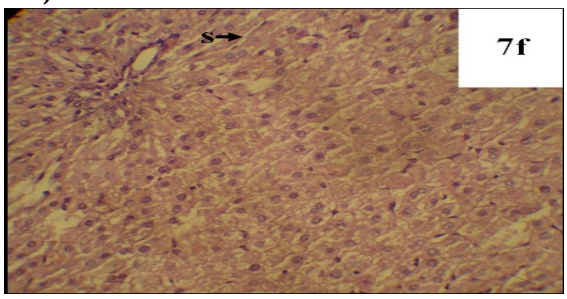

Fig. (7f): Photomicrograph of a transverse section of liver of fish cod oil treated group showing nearly normal hepatocytes at the periportal. Notice that the blood sinusoids are narrow (s). **(Hx&E X 400)**

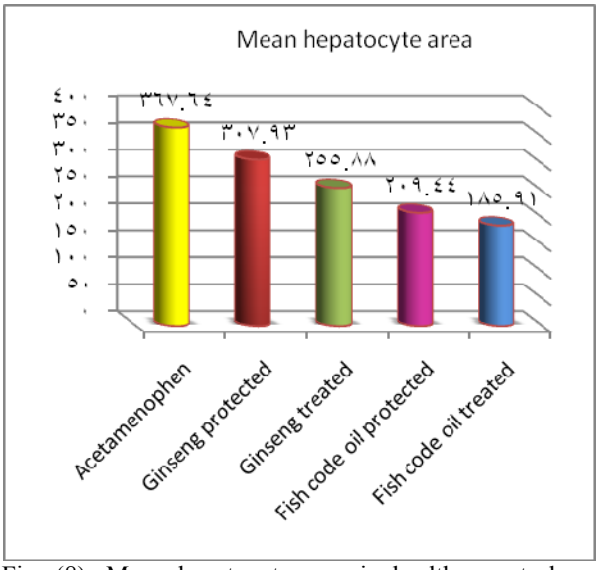

Fig. (8): Mean hepatocyte area in healthy control, acetaminophen and experimental groups.

Table (6) Mean hepatocyte area in healthy control and Acetaminophen. (Unpaired Student't test)

	Healthy Control	**Acetaminophen**
Mean ± SEM	184.76±17.5	367.64±29.83
Max	248.38	454.79
Min	142.26	289.26
t value	5.2881	
P value (t-test)	0.0007***	

*significance at $P<0.05$.

Table (7) Comparison between acetaminophen and experimentalgroups (Unpaired Student' t test)

Groups	t value	P value
Acetaminophen vs Ginseng protected	1.6980	0.1279
Acetaminophen vs Ginseng treated	2.3797	0.0446*
Acetaminophen vs Fish oil protected	4.3384	0.0025**
Acetaminophen vs Fish oil treated	5.9327	0.0003***

*significance at $P<0.05$.

4. Discussion

Acetaminophen is a widely used analgesic and antipyretic since its production in the mid- 1950 **(Garry and Kieran, 2005**). It isgenerally considered safe atthe therapeutic doses. However, it is known to cause severe hepatic necrosis at high doses both in man and experimental animals. It also causes liverdamage whentaken in therapeutic doses at certain circumstances (alcohol intake and fasting). In healthy individuals, there is apparently a considerable therapeutic range between harmless and harmful dose of acetaminophen (***Wills, 2005***).

Acetaminophen-induced hepatotoxicity has been the subject of extensive and expanding research efforts since the early 1970. This reflects its worldwide prominence in medicine and its general acceptance as a model for investigating mechanisms of hepatotoxicity **(Gary and Kieran, 2005**).Several studies in animals and human have demonstrated that acetaminophen overdose causes liver damage due to enhanced production and/or decreased glutathione conjugation of N-acetyl benzoquinoneimine (NABQI) which damage the cell membrane **(Kalra*et al.*, 2012).** The present study was undertaken to study and compare between the effect of both fish code oil (4 ml/kg, i.p. daily for 1 week) and *Panax Ginseng* (300 mg/kg oral daily for 1 week) against acetaminophen (500 mg/kg orally) induced hepatotoxicity. Administration of acetaminophen (500 mg/kg) to rats produced marked hepatic damage as reflected bythehistopathologicalfindings (extensive necrosis) and the results of liver function biochemical tests (the acute elevation of serum ALT, AST, ALPand the acute reduction of T.P and Alb.).these findings were consistent with the findings of (**Kamanaka*et al.*,2003, Helieh*et al.*,2004, Janbaz*et al.*,2004 and Omar *et al.*,2005)**who reported that, acetaminophen administration causes severe centrilobular necrosis resulted in markedreduction of albumin level and marked increase in serum ALTactivity.Also the marked increase in alkaline phosphatase (ALP) activity was supported by the findings of **Shivashangari*et al.*,2004**that the use of acetaminophen overdose is usually accompanied by the increase in ALP activityAccording to ginseng, the results of the present study showed significant improvement in liver function tests(significant decreases in the serum levels of ALT, AST, ALP, and significant increase in T.P and Alb.)and by the histopathologicalfindings(significant reduction of necrosis) in comparison to acetaminophen group,and this was in agreement with**Park *et al.*,2005** who mentioned that ginseng has been used as a valuable tonic and treatment of various diseases and coincided

with (**Shim *et al.*,2010 and Ramesh *et al.*,2012)** who reported that ginseng enhanced the antioxidant defense mechanism and increased self-antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GSH), and hemeoxygenase-1 in the aged-rat liver and hepatotoxins-induced liver damages in rats. Also **Kim*et al.*,2011**explained the hepatoprotective effect of ginsengby its antioxidation properties by inhibiting cytochrome- P450 associated monoxy-genase activities and throughout the supply of exogenous radical scavengers(phenolic acids, flavonoids and saponins). Also our results were in agreement with previous studies stated that ginseng treatment inhibited oxidative stress damage such as lipid peroxidation, **(Bak*et al.*,2012)** malondialdehyde**(Kim *et al.*,2011)**, thiobarbituric acid reactive substance **(Kang *et al.*,2007)**,decrease serum level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) **(Lee *et al.*,2012)**.

The results of the present study revealed that fish liver oil possesses marked hepatoprotective activity against acetaminophen -induced liver injury when used as a single daily dose for one week in both protected and treated group and resulted in significant improvement in liver functions test (highly significant decreases in the serum levels of ALT, AST, ALP, andhighly significant increase in T.P and Alb.)and by the histopathologicalfindings(highly significant reduction of necrosis) in comparison to acetaminophen group. These results were in agreement with those of **Kalra*et al.*, 2012.**and were supported by **Ko*et al.*,2000** who mentioned that fish liver code oil has been clinically and experimentally evaluated in previous studies for its beneficial effects in cardiovascular diseases, cancer, rheumatoid arthritis, bone disease, psychiatric and immune disorders **. Liu *et al.*,2000** attributed the protective effect of fish oil to its ingredients, particularly high contents of polyunsaturated fatty acids (PUFA) of n-3 and n-6 series namely eicosapentaenoicacid *(*EPA, C22: 5n-3) and docosahexaenoic acid (DHA, C22: 6n-3)**.** In the present study, according to the biochemical liver tests and the histopathological analysis it was noticed that fish code oil was significantly more effective than ginseng against acetaminophen induced hepatotoxicity.

Conclusion:

In conclusion it is clear from the above results and data obtained that acetaminophen induces marked oxidative stress in rat liver and that both Panaxginseng and fish oil have definite antioxidant properties which are responsible for their hepatoprotection effect and that fish code oil was significantly more effective against acetaminophen induced hepatotoxicity when compared with ginseng .However, these findings need to be further evaluated and more studies are needed for conclusive evidence.

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