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Antioxidant effect of Flaxseed against liver Cirrhosis induced in Thioacetamide intoxicated rats

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Abstract:

Background/Aim:There has been a current upsurge in the medical implications of free radicals and related species during the past several decades. These chemical species are integral components produced during normal biochemical and physiological processes but leads to oxidative stress when produce in excess and causes potential damage to cells. A wide range of non-enzymatic and enzymatic antioxidant defenses exists to counteract the damaging effects of free radicals. There exist epidemiological evidences correlating higher intake of antioxidant rich foodstuffs with greater free radical neutralizing potential to lower incidence of several human morbidities or mortalities.

Material andMethods: liver cirrhosis model was induced by TAA,hepatobichemical and antioxidant parameters were assayed.

Results: Flaxseed induced enhancement in liver function test, antioxidant status and lipid profile.

Conclusion: Flaxseed can be used as hepatoprotective against liver cirrhosis induced with TAA as compared to reference drug Silymaringroup**.**

Keyword: liver Cirrhosis, Thioacetamide , Flax seed , Silymarin, Antioxidant.

Introduction

The liver plays a crucial role in the metabolic elimination of most drugs and other foreign compounds, thus making it an important target for toxicity. Hepatotoxic agents can react with the basic cellular components and consequently induce almost all types of liver lesions [1]. Toxins and drugs are among the basic etiopathogenet- agents of acute liver failure in Western countries [2].

Thioacetamide (TAA) was originally used as a fungicide to preserve agricultural citrus products, but later it was determined that it was a potent hepatotoxin and carcinogen because its thono-sulfur-containing compound endowed with liver-damaging and carcinogenic activities [3]. On a prolonged exposure, TAA leads to the formation of hyperplastic nodules, cell adenomas, hepatocarcinomas, andcirrhosis. TAA-induced cirrhosis in rats has been shown to be a suitable experimental model of this disease with etiology and pathology comparable to the one seen in humans [4].

In recent years, flaxseed is gaining importance in diet supplements, as they possessnutraceutical properties. Flaxseed is one of the oldest domesticated crops and it is being increasingly used in the human diet because of its potential health benefits, particularly for cardiovascular protection [5].

Flaxseed is the richest natural source of plant lignans, with secoisolariciresinoldiglucoside (SDG) being the principal lignan compound, chemically 2, 3-bis [4- hydroxy-3-methoxyphenyl)methyl]-1, 4-butane diglucoside. The concentrations of SDG in flaxseed vary with different cultivars. SDG concentrations in twenty-seven flaxseed speciesranged from 1.19 to 2.59% for SDG and 0.22 to 0.5% (w/w) for its diastereoisomer [6] . Flaxseed commonly contains 34–45.6% total fat, and a-

linolenic acid alonerepresents 45–60% of the total fatty acidcontent in flax oil. Although α-linolenic acid is an ω-3 fatty acid, dietary α-linolenic acid did not show cholesterol-lowering effects in several clinical trials [7].

Silymarin is a purified extract obtained fromthe seeds of the plant Silybummarinumand used widely as a supportive therapy for liver disorders such as cirrhosis, hepatitis, and fatty acid infiltration due to alcohol and toxic chemicals [8].

In this study, the hepatoprotective function of the hull flaxseed was further evaluated and its efficacy as a therapeutic agent was experimentally tested on a rat model of livercirrhosis induced chemically by TAA administration.

Material and methods

Animals

A total of 70 Sprague Dawley adult male albino rats weighing between 180-230 gmwere randomly divided into 7 equal groups. They were individually housed in metallic cages under constant healthy environmental conditions. Water and diet were provided ad- libitum.

Preparation of Flaxseed

Flaxseed **(L. usitatissimum**) was purchased from Agricultural Research Center. As A hull fraction of flaxseedwas grinded and mixed with basal diet treatment.

Preparation of Thioacetamide

TAA (Sigma-Aldrich) was prepared freshly by dissolving in sterile distilled water and stirred well until all crystals were dissolved. Then, 200 mg/kg body weight was administered intraperitoneally (ip) to the rats twice weekly for 4 consecutive weeks. The injection protocol above was according to the recommendation of Bruck [9]. Constant exposure of rats with this amount of TAA induces changes in its liver pathology from both biochemical and morphological aspects comparable to that of human liver cirrhosis [10] and therefore, used very often as a preferred model in experimental Studies of this disease.

Preparation of Silymarin

Silymarin (70% silibin, 16.5% silydianin, and silychristin.) was purchased from Sedico Pharmaceutical Company (Egypt). Silymarin which is a reference drug and (5mL/kg body weight) was dissolved in saline for oral administration to rats ina dose of 20mg/kg body weight according to the recommendation ofRastogi [11].

Animal diets

The stock basal diet was prepared according to the recommended proportions given by reeves [12], as shown in table (1).

Experimental design

Seventy male rats were randomly divided into seven groups, each of which with ten rats: Group1 (control group) rats were received basal diet for five weeks andintraperitoneally (ip) injected with saline solution. Group 2 and group 3 (Flaxseed control groups) rats were served as a flaxseed control group and they were received basal diet supplemented with flaxseed (5% and 10%) respectively for five weeks. Group 4 (cirrhotic group) was received the basal diet for five weeksafter the first week rats were intraperitoneally injected with thioacetamide at a dose of 200 mg/kg body weight twice weekly for 4 consecutive weeks. Group 5 (reference drug group) was received the basal diet for five weeks with oral administration of silymarin at a dose of 20 mg/kg body weight,after the first week rats were received the same course of thioacetamide as the cirrhotic group. Group 6 (5% Flaxseed group) was received flaxseed supplemented diet (5%) for five weeks, after the first week they were received the same course of thioacetamide as the cirrhotic group. Group 7 (10% Flaxseed group) was received flaxseed supplemented diet (10%) for five weeks, after the first week they were received the same course of TAA. Body weights of rats were recorded at three days interval to monitor body weight change and to determine the doses for induction of fibrosis and silymarin dose. At the end of five weeks(experimental period), the animals were fasted 12 hrs, then they were anaesthetized with diethyl ether and blood was collected.

Food intake and body weight variation.

Food intake was controlled, being the difference between the offered and leftover food. Body weight of each animal was evaluated 2 times a week, throughout the experiment, which lasted 35 days table.

Antioxidant Activity

Hepatic tissues from all livers were sampled from the same site, but away from the portal system. One gram of the sampled tissue was placed in 10mL (10% w/v) of PBS (phosphate buffer solution with pH 7.4), then homogenized and centrifuged at 4000 rpm for 10min at −4◦C.The supernatant was kept in a −80◦C freezer and it was used to determine the Glutahione-S-transferase (GST) , Reduced glutathione (GSH) , Malondialdehyde (MDA) and superoxide dismutases (SOD).

Hepatic Biochemical Parameters

Blood of each rat was collected and serum was separated for analysis and to determine the liver function enzymes such as alanine aminotransferase (ALAT), aspartate amino- transferase (ASAT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and arginae activity.

Serum Lipid profile

Collected serum was used to determine levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triacylglycerols (TAG) and very low-density lipoprotein cholesterol (VLDL-C) were determined.

Statistical Analysis

Statistical analysis was evaluated by using one-way analysis of variance (ANOVA) using QI macros 2010. Data were expressed as the mean ± SD.; a probability value less than 0.05 was consideredsignificant.

Results

Body weight change, hepatosomatic, Spleenosomatic,nephrosomatic , cardiosomatic , gonadosomaticindices and food intake

The hepatotoxic weight changeand food intake were extremely significantly decreased, but their indicesof hepatosomatic, Spleenosomatic ,nephrosomaticcardiosomatic and gonadosomaticweighted significantly more compared with the control rats. Rats subjected to the treatment with silymarinexhibited extremely significantly loss in the body weightchangebut the amounts were not as much as those in the cirrhotic group.Rats subjected to the treatment with 5% flaxseed exhibited extremely a significant elevation in the indices of hepatosomatic, Spleenosomatic ,nephrosomatic , cardiosomatic and gonadosomatic(figure1, figure2 and figure3).

Hepatic Biochemical Parameters

There were an extremely significant increased in the serum ALP, ALAT, ASAT and a significantincrease in LDH and arginasewere detected in the hepatotoxic rats compared to those in the control group.Silymarin therapy was effectively restored the activities of ALAT, LDH and arginase but showed extremely significant increase in ALP activityhowever the amounts were not as much as those in the cirrhotic group and showed extremely significant decrease in ASAT activity.Cirrhotic groups treated with 5% and 10 %flaxseed produced activities similar to their respective 5% and 10% flaxseed control groupsexcept in case of 10% flaxseed group which showed an extremely significant increase in ASAT and LDH activity as compared to cirrhotic 10% flaxseed group (figure4).

Antioxidant activity

Figure 5 lists the activities of enzymes involved in the hepatic antioxidant defense system. It showed that MDA andSOD enzyme levels were extremely increased in cirrhoticgroup when compared with the control groupwhile GSH and GST level were extremely decreasedwhen compared with the control group. GST and GSH enzymes were restored their level insilymaringroup while showed a significant increase in MDA and SOD levels as compared to control group

activity but the amounts were not as much as those in the cirrhotic group .the cirrhotic rats treated with flaxseed showed increasein antioxidant level as compared to control group.

Lipid profile

As shown in figure (6).Serum total cholesterol level was significantly decreased in 5%flaxseed control group and 10% flaxseed controlby 16.8% and 35.4% respectively as comparing to control group. High density lipoprotein-cholesterol (HDL-cholesterol) showed a significant decrease in liver cirrhotic rats.

Discussion

Liver cirrhosis is a major disease associated with various pathological processes including progressive fibrosis, portal hypertension and carcinoma [13]. Free radical generation, mitochondrial dysfunction and depletion of antioxidants lead to the progression of fibrosis and cirrhosis [14]. Thioacetamide is an organic solvent with thiono-sulfur componentswhich have been used widely to induce liver cirrhosis [15–19]. In the present study, the prolonged administration of TAA to the rats caused visual and quantifiable responses, which were recognizable by the alterations in the body and organ weights.TAA contributes to the development of cirrhosis through multiple mechanisms of action [20] like the oxidation of its metabolic products [21, 22], oxidative stress [23, 24], and decreased antioxidant defenses and lipid peroxidation. During the five-week-long study, the hepatotoxic rats lacked in gaining body weight as compared with the controls (figure1). Using the same experimental model of cirrhosis, the previous studies reported the same and attributed this outcome to the lower levels of nutrient absorption, energy utilization, and metabolic efficiency as the major factors affecting the inability of the rats to gain weight after being exposed to TAA [25]. Factoring the reduced body weight into the calculation yielded significantly high ratios of liver , spleen , kidney ,testis and heart-to-body weight. The hepatocyte proliferation is a critical determinant for the survival of liver from an injury [24]. Based on this, the upregulation of the hepatocyte activity in response to the exposure to TAA toxicity is likely to be the cause of the recorded increase in the organ weights (figure2).

In the cirrhotic group, we detected elevated levels of serum ALAT, ASAT, ALP, LDH and Arginaseconcentrations(figure3), which were typically measured for assessing the liver function. The increase in serum enzymatic activities is related to hepatic parenchymal damage since ALAT is released from mitochondrial and cytosolic localization from membranal sites, and cellular rupture allows the enzyme to escape into the blood [26]. The raised serum liver enzymes such as ALAT, ASAT, ALP, LDH and Arginasein intoxicated rats compared to normal indicates necrosis of hepatocytes that results in the leakage of transaminase and the elevation of serumALAT, ASAT, ALP, LDH and Arginase .

On the other hand, TAA group exhibited higher values of cellular lipid peroxidation which measured through the malonialdehyde level(MDA) than did the control livers [27].

The toxicity of TAA results from its bioactivation in the liver to active metabolites, causing the production of ROS responsible for oxidative stress [28,29].These events are followed by glutathione depletion, a reduction in SH-thiol groups and oxidation of cell macromolecules, including lipids [30,31]. Our results are in agreement with these findings (figure 4). Glutathione plays a key role in detoxification of ROS and reactive electrophilic compounds [32, 33] . In tissues, glutathione occurs in a reduced (GSH) and oxidized form (GSSG). More than 99% of the total glutathione occurs as GSH (34, 35). In our experiments control hepatocytes contained about 85% of the total glutathione in the form of GSH. This could be explained by the fact that, in vitro experiments, cells are artificially exposed to hyperoxia, thus increasing oxidative stress [36].

Silymarin is a well-established plant-based formulation and a clinical drug with proven capacity to guard liver from harmful hepatotoxins. Such pharmacological power was attributed to silymarin's inherent constituents with antioxidant, anti-inflammatory, and diuretic properties, as in othermedicinalplantsin nature [37]. In addition, silymarin has the capacity as antilipid peroxidation and induced detoxification system, protector of cell against employed glutathione, reducer of leukotiene formation from unsaturated free acid, enhancer of protein synthesis, stabilizer of mast cells and regulator of immune functions. It inhibits cytoP450 detoxi- fication system and prevents metabolism of toxic compound such asTAA [38].

In our study, we reconfirmed that silymarin played substantial role against the progression of cirrhosis induced by TAAinrats. It significantly reduced the liver pathology indicated by the declined levels of ALAT, ASAT, ALP as compared to the hepatotoxic rats. The readings for these biochemicals were the best achievable values and therefore set the baseline for comparisons of therapeutic improvements attained with the flaxseed [39]

Flaxseedlignan is a precursor ofthemammalian lignans, enterodiol [ED; 2,3-bis[(3-hydroxy-phenyl)methyl]-1,4-Butanediol;MW302]and enterolactone[EL; trans-dihydro-3,4-bis[(3-hydroxyphenyl)methyl]-2(3H)- Furanone; MW 298] via the activity of colonic facultative aerobes (Clostridia sp.). During this conversion, SDG first undergoes hydrolysis to yield the aglycone plant lignansecoisolariciresinol (SECO); [R-(R*0,R*)-2,3-bis[(4-hydroxy-3- methoxy phenyl)methyl]-1,4-Butanediol; MW 362], which is then dehydroxylated and demethylated to yield ED; ED can then be oxidized to EL .The mammalian lignans differ fromthe plant precursors due to the presence of phenolic hydroxyl group moieties only in the meta position on the aromatic rings, compared to the 3-methoxy-4-hydroxyl substituents on the A and B rings of the parent molecules SDG and SECO [40].

The flaxseed lignan and its mammalian metabolites have been reported to exert protective effects against diet-related chronic diseases through a variety of mechanisms including phytoestrogenic and antioxidant effects from in vitro and in vivo studies [41, 45].

The flax-induced increase in the liver enzyme GSH, which we propose to play a role in flax-induced hepatoprotection, is due to the lignan, likely SDG, and not the oil component of flaxseed [46].

Conclusion

All the observations made and measurements collected in this study provided preliminary evidence that the progression of the liver cirrhosis induced by TAA in rats can be intervened using the flaxseed. Specifically, this natural seed has power to protect the liver by preventing the actions of the harmful events associated with the TAA toxicity from taking place.

By preservation of GSH levels in the normal and injured. The effects are comparable to those of silymarin and the capability of the flaxseed to preserve the liver's status quo of property, structure, and function against toxic exposure is encouraging and warrants further studies exploring the significance of its pharmacologic potential in treating the liver cirrhosis by mapping the molecular pathways of action.

References

1.Pavla Sk, Otto K , Halka L, Tomáš R, René E andZuzana C. (2010):"The toxic effect of thioacetamide on rat liver in vitro".Toxicology in Vitro, 24: 2097–210.

2.Grattagliano I , Bonfrate L , Catia V D, Wang HH, Wang D Q H and Portincasa P, (2009):"Biochemical mechanisms in drug-induced liver injury". World Journal of Gastroenterology ,15: 4865–4876.

3.Al-Bader A , Mathew T , Abul H , Al-Mosawi M , Dashti H, Kumar D and Singal P

. (1999): “Thioacetamide induced changes in trace elements and kidney damage”. J. Trace Elem. Exp. Med., 12: 1–14.

4.Chun-Nan Y,AnirbanM,KamF , Yi-YinJ andMiin-Fu Ch.(2004): “Thioaceatamide-induced intestinal-type cholangio carcinoma in rat: an animal model recapitulating the multi stage progression of human cholangiocarcinoma,” Carcinogenesis,25(4): 631–636.

5.Zhang W, Xiaobing W, Liu Y, Tian H, Flickinger B, Mark W, Sam E, Sun Z. (2008): “Dietary flaxseed lignan extract lowers plasma cholesterol and glucose concentrations in hypercholesterolaemic subjects”. Bri. J. Nutr., 99:1301–09.Doi: 10.1017/ S0007114507871649.

6.Eliasson C, Kamal-Eldin A, AnderssonR, Aman P.(2003):“High-performance liquid chromatographic analysis ofsecoisolariciresinoldiglucoside and hydroxycinnamic acid glucosides in flaxseed by alkaline extraction”. J Chromatogr A., 1012:151–59.

7.Harris W. (2003):“ ω-3Fatty acids and serum lipoproteins: “human studies”. Am J ClinNutr. , 65:1645S–54S.

8.Ball R and KowdleyK V. (2005): “A review of silybummarianum(milk thistle) as a treatment for alcoholic liver disease,” Journal of Clinical Gastroenterology, 39(6): 520–528.

9. Bruck R, Shirin H, Aeed H, Matas Z, Hochman A , Pires, M. and Avni, Y.(2001): “ Prevention of hepatic cirrhosis in rats by hydroxyl radical scavengers”.J Hepatol. , 35(4):457-64.

10. ChattopadhyayR (2003):“Possible mechanism of hepatoprotective
activity of azadirachtaindica leaf extract part” Journal of Ethnopharmacology, 89(2-3): 217–219,.

11. Rastogi R, Arvind K and Anil K R :“Long term effect of aflatoxin B1 on lipid peroxidation in rat liver and kidneyeffect of picroliv and silymarin”phytotherapy Res,15:307-310 .

12.PhilipG, ForrestH andGeorge C.(1997): **“**Components of the AIN-93 diets as improvements in the AIN-76A diet**”**The Journal of nutrition, 127: (838S-841S).

13. Friedman S. L. (2003) : “Liver fibrosis—from bench to bedside” Journal of Hepatology,38(1) : S38–S53.

14. NatarajanS ,Thomas S, RamamoorthyP et al . 2006 : “Oxidative
stress in the development of liver cirrhosis: a comparison of two different experimental models,” Journal of Gastroenterology and Hepatology, 21(6): 947–957.

15. SatoM,Kakubari M, Kawamura M, Sugimoto J, Matsumoto K
andIshiiT.(2000): “The decrease in total collagen fibers in the liver by hepatocyte growth factor after formation of cirrhosis induced by thioacetamide” Biochemical Pharmacology, 59(6): 681–690.

16. Galisteo M, arez A Su,Montilla M P , Fernandez M I , Gil A, and
Navarro M(2006):“Protectiveeffects of rosmarinustomentosus ethanol extract on thioacetamide-induced liver cirrhosis in rats,” Phytomedicine, 13(1-2):101–108.

17. Bruck R,Genina O, Aeed H et al., (2001): “Halofuginone to prevent and treat thioacetamide-induced liver fibrosis in rats,” Hepatology, 33(2): 379–386.

18. Kumar G, BanuG,Pappa P ,Sundararajan M, andPandianM (2004): “Hepatoprotective activity of trianthemaportulacastruml. Against paracetamol and thioacetamide intoxication in albino rats,” Journal of Ethnopharmacology, 92(1):37–40.

19.Madani H, Talebolhosseini M, Asgary S, and NaderiG,(2008): “Hepatoprotective activity of silybummarianum and cichoriumintybus against thioacetamide in rat,” Pakistan Journal of Nutrition, 7(1) :172–176.

20.SunithaS,Nagaraj M, and Varalakshmi P, (2001): “Hepatoprotective effect of lupeol and lupeollinoleate on tissue antioxidant defence system in cadmium-induced hepatotoxicity in rats,” Fitoterapia, 72(5): 516–523,.

21. LeeJ,ShinK, LeeM et al., (2003):“Role of metabolism byflavin-containing monooxygenase in thioacetamide-induced immunosuppression,” Toxicology Letters, 136(3):163–172,.

22.Chilakapati J,KorrapatiM , Hill R , WarbrittonA,LatendresseJ and MehendaleH (2007):“Toxicokinetics and toxicity of thioacetamidesulfoxide: a metabolite of thioacetamide,” Toxicology, 230(2-3): 105–116.

23. Balkan J,Dog˘gru-Abbaso˘ glulS, ¨ OKanbaglil, ¸evikbasU,ykac ¸-TokerGand UysalM, (2001): “Taurine has a protective effect against thioacetamide-induced liver cirrhosis by decreasing oxidative stress,” Human and Experimental Toxicology, 20(5): 251–254,.

24. Sun F, HayamiS, OgiriY et al., (2000) : “Evaluation of oxidative stress based on lipid hydroperoxide, vitamin c and vitamin e during apoptosis and necrosis caused by thioacetamide in rat liver,” Biochimica et BiophysicaActa, 1500(2) :181–185,.

25. Alshawsh M,Abdulla M , Ismail S, and Amin Z,(2011) "Hepatoprotective effects of orthosiphonstamineus extract on thioacetamide-induced liver cirrhosis in rats," Evidence-Based Complementary and Alternative Medicine., 2011:103039-103045

26.GalisteoM, Su´ arez A,MontillaM , Fernandez M , Gil Aand Navarro M.(2006)"Protectiveeffects of rosmarinustomentosus ethanol extract on thioacetamide-induced liver cirrhosis in rats," Phytomedicine, 13(1-2): 101–108.

27.Gressner O A, Weiskirchen R and GressnerA M ,(2007): "Biomarkers of liver fibrosis: clinical translation of molecular pathogenesis or based on liver-dependent malfunction tests," ClinicaChimicaActa, 381(2):107–113.

28.Pallottini V, Martini C, Bassi A , Romano P, Nanni G andTrentalanceA, (2006) "RatHMGCoAreductase activation in thioacetamide-induced liver injury is related to an increased reactive oxygen species content". Journal of Hepatology, 44: 368–374.

29.Okuyama H , Nakamura H , Shimahara Y, ArayaS, Kawada N, Yamaoka Y and Yodoi J. (2003)"Overexpression of thioredoxin prevents acute hepatitis caused by thioacetamide or lipopolysaccharide in mice". Hepatology, 37: 1015–1025.

30. Sanz N, Diez-Fernandez C, Andres D, Cascales M, (2002)"Hepatotoxicity and aging: endogenous antioxidant systems in hepatocytes from 2-, 6-,12-, 18- and30-month-old rats following a necrogenic dose of thioacetamide" . BiochimicaetBiophysicaActa,1587:12–20.

31. Diez-Fernandez C, Sanz N, CascalesM.(1996) " Intracellular calcium concentration impairment in hepatocytes from thioacetamide-treated rats. Implications for the activity of Ca(2+)-dependent enzymes". Journal of Hepatology,24:460–467.

32. Han D, Canali R, Rettori D, Kaplowitz N.(2003):"Effect of glutathione depletion on sites and topology of superoxide and hydrogen peroxide production in mitochondria". Molecular Pharmacology ,64: 1136–1144.

33. DeLeve L.D , Kaplowitz N . (1991) "Glutathione metabolism and its role in hepatotoxicity" Pharmacology and Therapeutics, 52: 287–305.

34. Meister A, (1988): "Glutathione metabolism and its selective modification". The Journal of Biological Chemistry, 263: 17205–17208.

35. Dickinson D, Forman H .(2002):"Cellular glutathione and thiols metabolism". Biochemical Pharmacology, 64: 1019–1026.

36.Gnaiger E, Mendez G, Hand S .(2000):"High phosphorylation efficiency and depression of uncoupled respiration in mitochondria under hypoxia". Proceedings of the National Academy of Sciences of the United States of America, 97: 11080–11085.

37. KiruthigaP ,Shafreen R ,PandianS K, ArunS, Govindu S and Devi K P. (2007)"Protective effect of silymarin on erythrocyte haemolysate against benzo(a)pyrene and exogenous reactive oxygen species (H2O2) induced oxidative stress," Chemosphere, 68(8): 1511–1518.

38. PapettiA,Daglia M and GazzaniG,.(2002): "Anti- and pro-oxidant activity of water soluble compounds in cichoriumintybus var. silvestre (treviso red chicory)," Journal of Pharmaceutical and Biomedical Analysis, 30(4) : 939–945,.

39.ZahraA ,Mehmet B , Mohammed A, Hapipah M , Hamid A andMahmood A. (2012):"Protective Role of Phyllanthusniruri Extract against Thioacetamide-Induced Liver Cirrhosis in RatModel "Evidence-Based Complementary and Alternative Medicine, 2012: 241583–241592

40.Chun Hu, Yvonne V, David D.(2007) "Antioxidant activities of the flaxseed lignansecoisolariciresinoldiglucoside, its aglyconesecoisolariciresinol and the mammalian lignansenterodiol and enterolactone in vitro".Food and Chemical Toxicology ,45: 2219–2227.

41.Kitts D, Yuan Y, WijewickremeA, Thompson L, (1999): "Antioxidant activity of the flaxseed lignansecoisolariciresinoldiglycoside and its mammalian lignan metabolites enterodiol and entero lactone". Mol. Cell. Biochem., 202: 91–100.

42.YuanY, Rickard S, Thompson L, (1999):"Short-term feeding of flaxseed or its lignan has minor influence on in vivo hepatic antioxidant status in young rats". Nutr. Res, 19: 1233–1243.

43.Prasad K.(2000): "Antioxidant activity of secoisolariciresinoldiglucoside- derived metabolites, secoisolariciresinol, enterodiol, and enterolactone". Int. J. Angiol., 9: 220–225.

44.Pool-ZobelB, Adlercreutz H , Glei M, Liegibel U, Sittlingon J, RowlandI, Wa ¨ha ¨la ¨K, RechkemmerG,. (2000):"Isoflavonoids and lignans have different potentials to modulate oxidative genetic damage in human colon cells". Carcinogenesis, 21: 1247–1252.

45.Thompson L, .(2003):"FFlaxseed in Human Nutrition, seconded. AOCS Press".Champaign., 194–222.

46. ZelkisA, Zak B.(1969):"Study of a new cholesterol reagent". Anal Biochem; 29:143-47.

Table (1): AIN-93M diet formulated for maintenance of adult rodent

Dietary constituents	Group(1) Group(4) Group(5)	Group(2) Group(6)	Group(3) Group(7)
	g/kg diet	g/kg diet	g/kg diet
Cornstarch	465.692	465.692	465.692
Flaxseed	0.000	50.000	100.000
Casein (>85% protein)	140.000	140.000	140.000
Dextrinized cornstarch	155.000	155.000	155.000
Sucrose	100.000	100.000	100.000
Soybean oil	40.000	40.000	40.000
Fiber2	50.000	50.000	50.000
Mineral mix (AIN-93M-MX)	35.000	35.000	35.000
Vitamin mix (AIN-93-VX)	10.000	10.000	10.000
L-Cystine	1.800	1.800	1.800
Choline bitartrate (41.1% choline)	2.500	2.500	2.500
Tert-butylhydroquinone	0.008	0.008	0.008

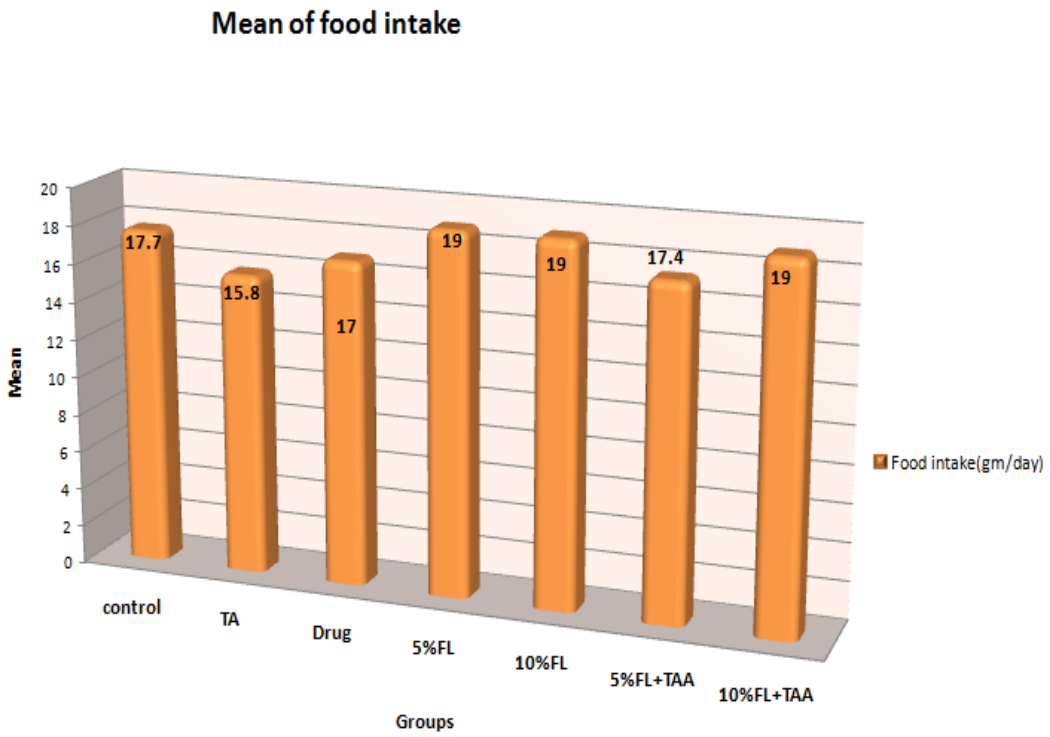

Fig (1): Mean of food intake of intake in experimental groups.

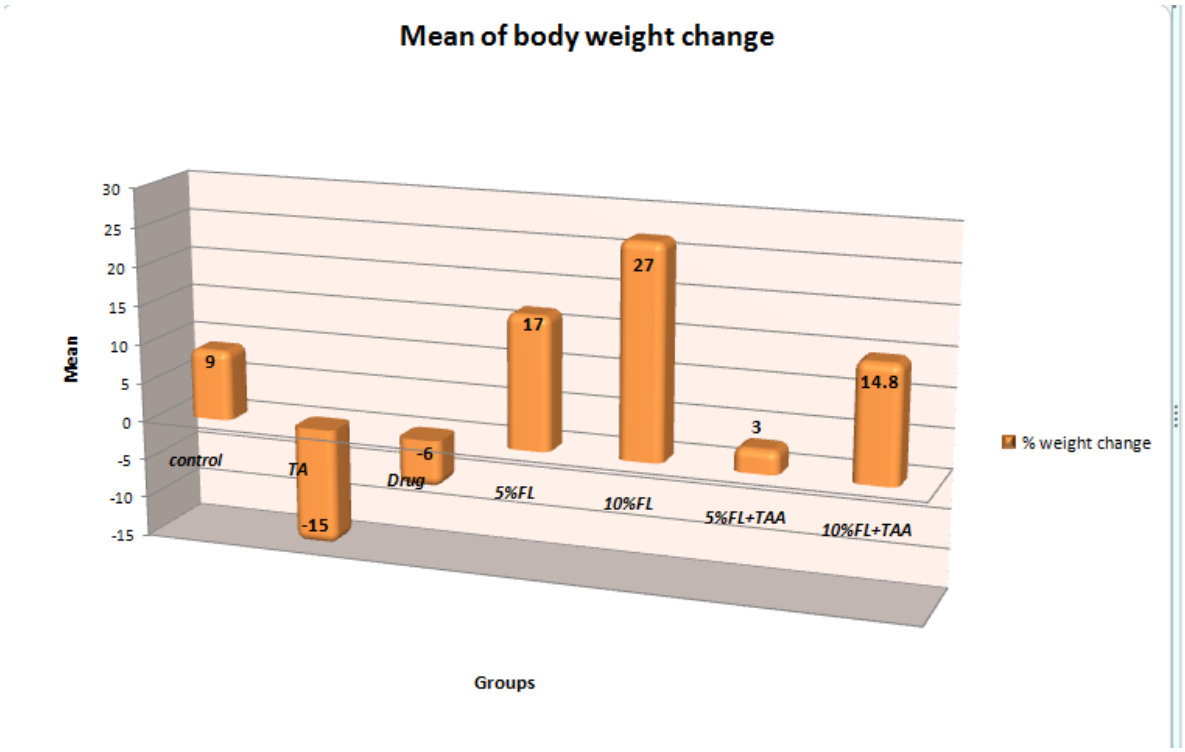

Fig (2): Mean of body weight change in the experimental groups.

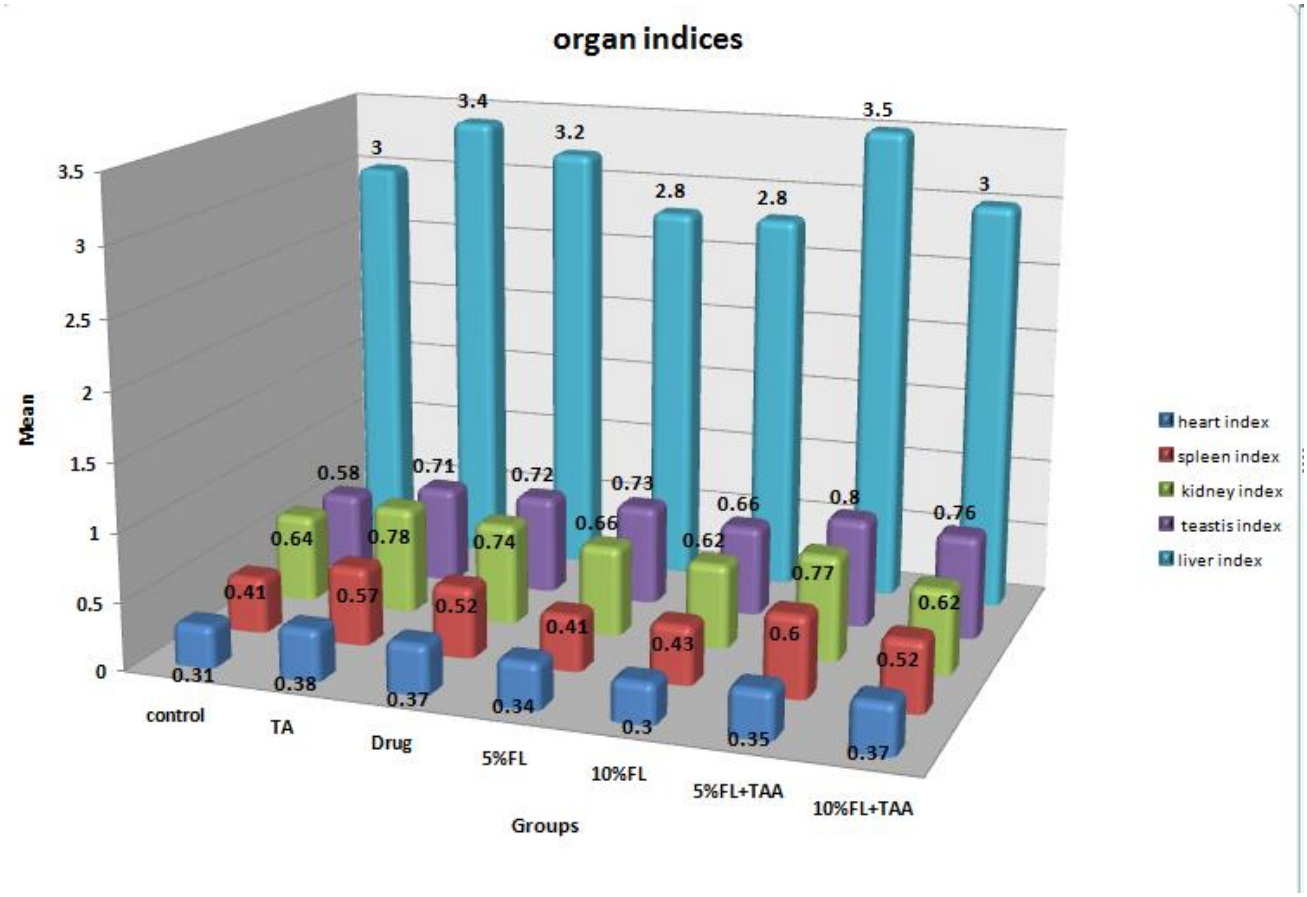

Fig (3): Mean of organs indices in the experimental groups.

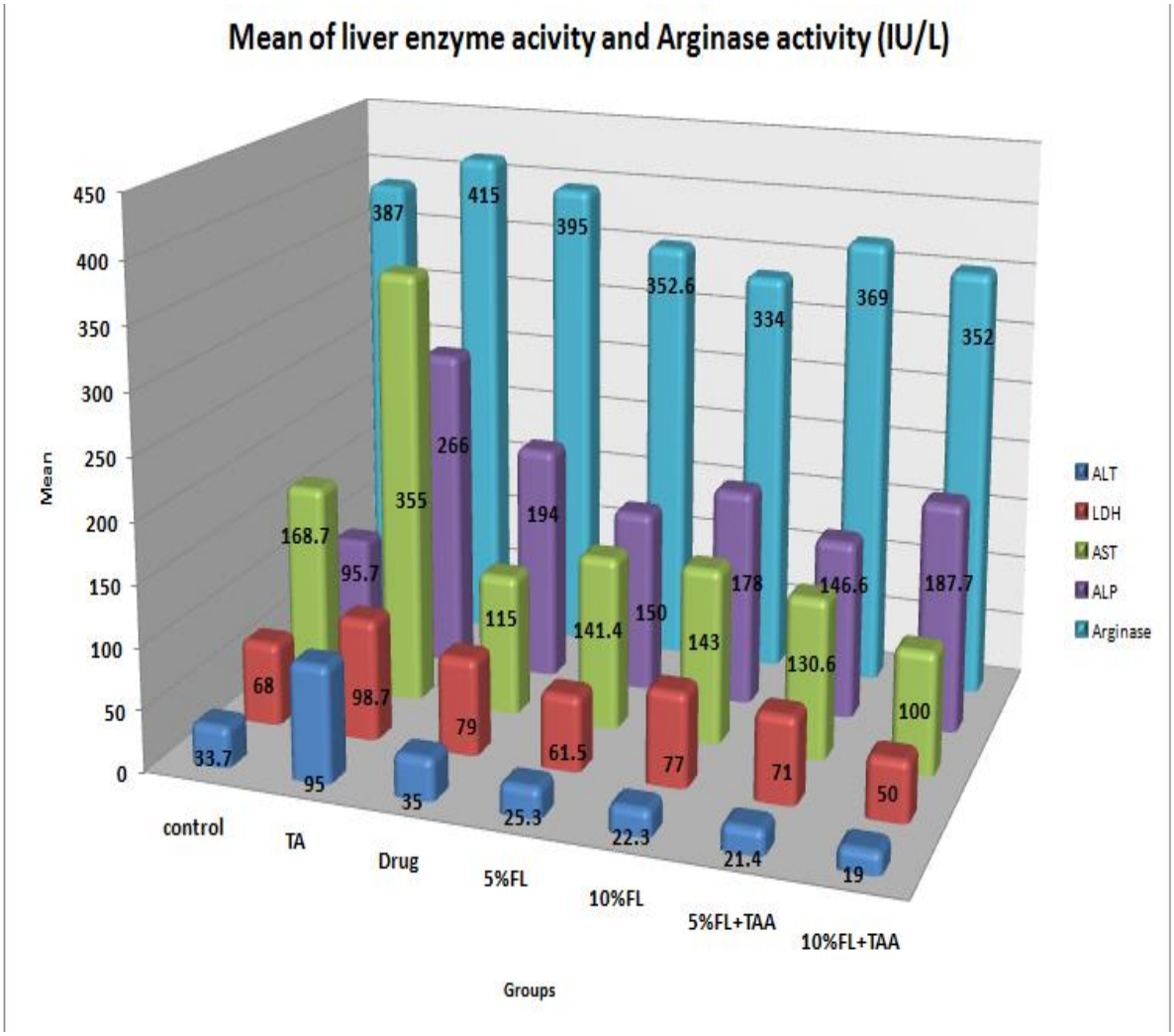

Fig (4): Mean ofserum liver enzyme activity and Arginase activity in the experimental groups.

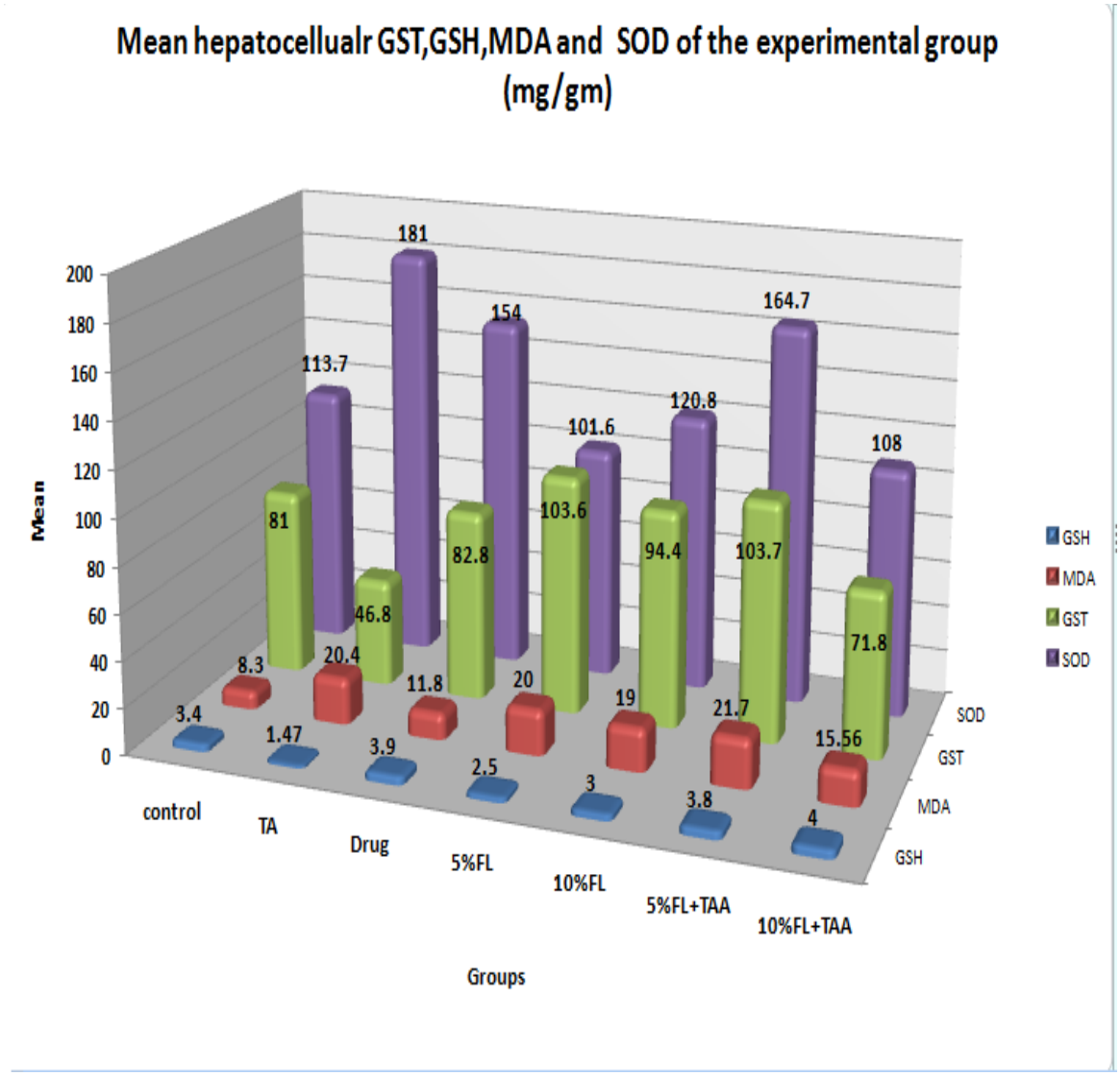

Fig (5): Mean of hepatocellualr GST, GSH, MDA and SOD in the experimental groups.

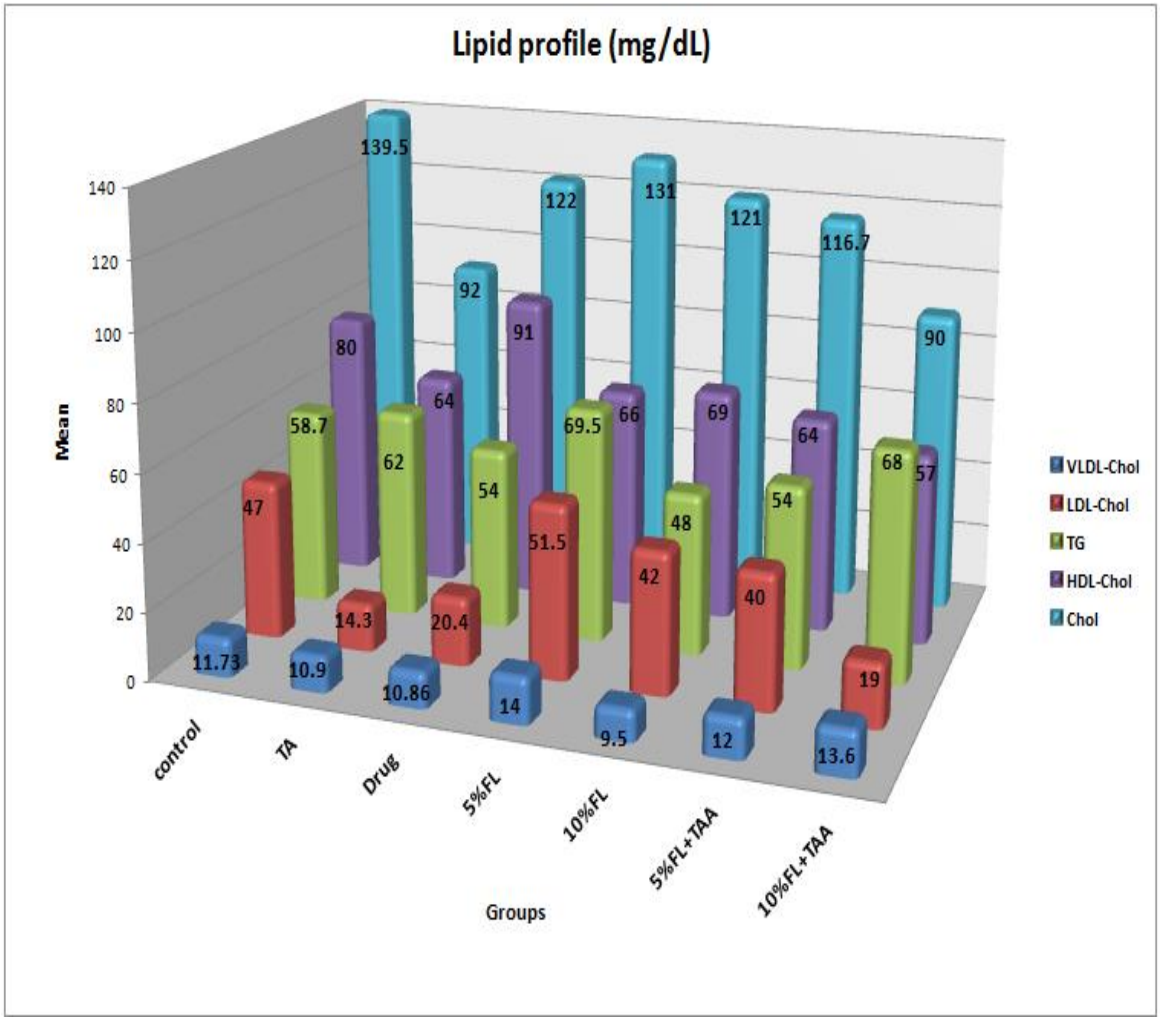

Fig (6): Mean of serum lipid profile in the experimental group