IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
www.iosrphr.org Volume 5, Issue 1 (January 2015), PP. -19-28

Whey protein products and their combination with L-methionine prevent liver fibrosis incidence in thioacetamide-toxicated rats

Somaia A. Nada[1], Noha F. Hassan [2], Ezz El-Din S. Eldenshary[3], Gehaad Abdel Reheem[1], Azza H. M. Hassan[4]

[1]*Pharmacology Department, National Research Centre, Dokki, Cairo, Egypt;*
[2]*Pharmacology and Toxicology Dept., Faculty of Pharmacy, Modern University for Technology and Information, Cairo, Egypt;*
[3]*Pharmacology and Toxicology Dept., Faculty of Pharmacy, Cairo University, Cairo, Egyp;*
[4]*Pathology Department, Faculty of Veterinary Medicine, Cairo University, Egypt.*

ABSTRACT: *effect of Beta-lactglobulin (β-LG), Lacprodan®alpha-10(LAC) and L-methionine as hepatoprotective agents against thioacetamide (TAA) rats' model was studied.* ***Method****: Group 1 control negative; Group2 control positive(TAA only) , the treated 3-9 groups received TAA (100mg/kg, i.p.) twice weekly ,group3,4 received daily β-LG (100mg/kg and 200 mg/kg), respectively. Group 5 received a combination of β-LG (50 mg/kg) and L-methionine (40 mg/kg).Groups 6,7 and 8 received the same as groups 3,4 and 5 except we use LAC instead of β-LG ,while group 9 received L-methionine (40 mg/kg) only* ***.Results****: TAA induced elevation in serum levels of alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin(TB) .Moreover, TAA induced oxidative stress indicated in elevation of malondialdehyde (MDA) and nitric oxide content(NO) as well as reduction in antioxidant enzymes(hepatic reduced glutathione concentration (GSH) and superoxide dismutase (SOD) activity). Administration of whey proteins and L- methionine concurrently with TAA attenuated hepatic fibrosis induced by TAA. WPs and L-methionine caused decline in serum AST, ALT, ALP and TB levels besides reduction of MDA and NO free radicals while increased GSH concentration, SOD activity.* ***Conc.:*** *whey proteins useful as pharmacological agent that suppress hepatic fibrosis.*

KEYWORDS-*Beta-lactglobulin,Lacprodan-alpha-10,Liverfibrosis,L-methionine, oxidative stress, Thioacetamide.*

I. INTRODUCTION

Liver fibrosis is a "wound-healing", multi step process resulting from the chronic effect of noxious elements of different nature [1, 2]. The mechanisms that participate in the induction of the fibrotic process are fairly constant. They include necrosis, apoptosis[3] inflammatory reactions and the activation of hepatic stellate cells (HSCs)[4] . Oxidative stress provokes and participates in all of these intermediate mechanisms[5]. The hepatic toxic chemical TAA has been widely used in the study of the hepatic fibrogenesis and the therapeutic effects of potential antifibrotic drugs. TAA is water soluble, and so can be easily administrated orally by being dissolved in drinking water [6], or utilized in other approaches such as intraperitoneal injection[7, 8] . Use of TAA in hepatic fibrosis animal models has many advantages, including highly specific hepatotoxicity, similar progression of human hepatic fibrosis development and damage regions of the liver to those observed in human hepatic fibrosis induced by chronic liver injury[9].TAA causes severe centrilobular necrosis and also induces apoptosis and periportal inflammatory cell infiltration in the liver. The initiation of the hepatotoxic effect of TAA requires metabolic activation which finally leads to oxidative stress [10-12]. Therapies for liver pathologies have potential adverse side-effects, especially if administered chronically or sub-chronically[13].Natural products with better effectiveness and safe profiles are needed as a substitute for chemical therapeutics. As oxidative stress plays a central role in liver pathologies and their progression, the use of antioxidants have been proposed as therapeutic agents, as well as drug co-adjuvant, to counteract liver damage[14]. Health benefits to humans of whey consumption have drawn increased attention, because whey is rich not only in nutrients but also in bioactive components such as proteins [15]. It has been reported that whey proteins exert several therapeutic effects on humans in a number of clinical trials as they are potential antioxidants, protect cells from ethanol damage and ROS-induced cell damage this protection includes their capacity to stimulate GSH synthesis as whey proteins are a cystine-rich protein source which is the rate-limiting step in GSH synthesis[16-18].The major proteins contained in whey are beta-lactglobulin (BLG), a-lactalbumin, immunoglobulin, and protease peptone 3[19].

BLG is a small globular protein composed of 162 amino acid residues (18.4 kDa) and the most abundant protein in bovine whey accounting for 50% of the total whey proteins[20]. BLG has been reported to have various biological effects [21, 22], While Lacprodan® alpha-10(LAC) is a whey protein with high concentration of alpha-lactalbumin (43 %).It is a food supplement produced by Arla Foods Ingredients amba, Denmark. Methionine is an essential amino acid that is required in the diet of humans and livestock. Plant proteins are frequently deficient in methionine and consequently an exclusively vegetable diet may fail to meet nutritional requirements. Methionine deficiency has been linked to development of various diseases and physiological conditions including toxemia, childhood rheumatic fever, muscle paralysis, hair loss, depression, schizophrenia, Parkinson's liver deterioration, and impaired growth [23]. In April 2000, the Complementary Medicines Evaluation Committee (CMEC) recommended that l-methionine is suitable for use as an ingredient in therapeutics and does not require any substance-specific restrictions on its use. Methionine is extensively used in the poultry and feedstock industry [24]. It possesses antioxidant activity on the basis of its sulfhdryl group, and is now being used clinically to decrease hepatic injuries after acetaminophen poisoning [25] the purpose of the current study is to evaluate the possible therapeutic effectiveness of LAC and β-LG versus L-methionine in preventing the occurrence of hepatic fibrosis induced by TAA.

II. MATERIALS AND METHODS

2.1.Chemicals:

Thioacetamide and L-methionine were obtained from El-Gomhouria Company for drug and chemicals, Cairo, Egypt. Lacprodan®alpha-10 was obtained from Arla food amba (Denmark).). beta-lactglobulin (β-LG) was obtained from Davisco Foods International, Inc.USA (11000 W. 78th St., Ste. 210 Eden Prairie, MN 55344), reduced glutathione and thiobarbituric acids were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals, used throughout the experiment, were of the highest analytical grade available. Kits used to measure serum ALT, AST, ALP, TB and SOD, were purchased from Spectrum Diagnostics (Cairo, Egypt).

2.2.Animals:

Sprague Dawley male rats weighing 150-160gm used throughout the experiments. Animals were housed under standard environmental conditions (23 ± 1ºC, 55 ± 5 % humidity and a 12-h light: 12-h dark cycle) and maintained with free access to water and a standard laboratory diet ad libitum. Animal care and the experimental protocols were approved by the National Research Centre Animal Care and Use Committee and are in accordance with the guidelines of the International Association for the Study of Pain Committee for Research and Ethical Issues [26].

2.3.Experimental design:

After an acclimatization period of one week, nine equal groups of seventy two Sprague Dawley rats (8 rats each). Group 1 kept as control and received saline only (10 ml/Kg) orally; groups 2-9 were injected with TAA (100mg/kg, i.p.) twice weekly for8 weeks, Group2 kept as non- treated control , while the treated 3-9 groups treated as follows incocomitant with TAA,group3-4 received daily β-LG (100mg/kg and 200 mg/kg given orally), respectively. Group 5 received a combination of β-LG (50 mg/kg body weight) and L-methionine (40 mg/kg body weight).Groups 6,7 and 8were received the same as groups 3,4 and 5 except we use LAC instead of β-LG ,while group 9 was received L-methionine (40 mg/kg body weight) only .At the end of the experimental period (8weeks), rats were sacrificed by cervical dislocation under diethyl ether anesthesia and liver samples were removed immediately for biochemical and histopathological examinations.

2.4.Collection of blood samples:

Blood samples were withdrawn from the retro-orbital vein of each animal, under light anesthesia by diethyl ether, according to the method described by [27]. Blood was allowed to coagulate and then centrifuged at 3000 rpm for 15 min. The obtained serum was used to estimate the activities of ALT, AST, ALP and TB enzymes.

2.5.Preparation of liver samples:

Immediately after blood sampling, animals were sacrificed by cervical dislocation and the liver tissues were rapidly removed, washed in ice-cooled saline, plotted dry and weighed. A weighed part of each liver was homogenized, using a homogenizer (Medical instruments, MPW-120, Poland), with ice-cooled saline (0.9%NaCl) to prepare 20% w/v homogenate. The homogenate was then centrifuged at 4000 rpm for 5 min. at 4°C Cusing a cooling centrifuge to remove cell debris (Laborzentrifugen, 2k15, Sigma, Germany). The aliquot was used for the assessment of reduced glutathione (GSH), lipid peroxidation (LPO) as malondialdehyde (MDA), level of nitric oxide (NO) and superoxide dismutase activity (SOD).

2.6. Measurement of serum liver function enzymes:

Hepatic dysfunction was assessed by measuring the elevation in serum levels of ALT, AST, ALP and TB using commercially available kits. The results were expressed in U/L except TB was expressed in mg/dl.

2.7.Measurements of lipid peroxidation and antioxidants status:

Lipid peroxidation, as an indicator of oxidative stress, was estimated by measuring thiobarbituric acid reactive substance (TBARS) that sometimes referred to as malondialdehyde (MDA) in hepatic homogenates following the method of [28]. Reduced glutathione (GSH) concentration in hepatic homogenate was determined according to the method described by[29]. Superoxide dismutase (SOD) activity was determined in rat liver homogenate (20%) using a commercial kit according to the method described by[30] this method is based on the ability of SOD to inhibit the autoxidation of pyrogallol expressed as U/mg protein, nitric oxide (NO) content[31].

2.8.Statistical analysis:

The degree in variability of results was expressed as means ± standard error of means (SEM). Data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. The level of significance was accepted at $P < 0.05$.

2.9.Histopathological examinations:

The specimens from the liver were taken and fixed immediately in 10% neutral buffered formalin, processed for light microscopy to get (5µm) paraffin sections and stained with: Hematoxylin & Eosin to verify histological details and Masson's trichrome staining to demonstrate the collagen fibers.

III. RESULTS:

3.1.Effect of whey proteins product as LAC and β-LG 100mg/kg and200mg/kg versus L-methionine 40mg/kg concurrently with TAA on serum ALT, AST, ALP and total bilirubin as shown in "Fig1":

The intoxication with TAA for 8 weeks produced mediocre liver damage characterized by significant increase in serum ALT and AST activities, compared with the control group. A significant decrease in serum aminotransferase was observed in all treated groups, compared with the group intoxicated with TAA for 8 weeks. Groups β -LG (50mg/kg) combined with L-methionine 40mg/kg, LAC (200mg/kg), LAC (50mg/kg) combined with L-methionine 40mg/kg concurrently with TAA showed insignificant change in ALT level as compared to the normal control group. Although, groups β-LG(100 and 200 mg/kg), LAC (200mg/kg) , L-methionine 40mg/kg concurrently with TAA significantly decreased ALT level compared with TAA group, they showed significant difference from normal control group. while in AST test ,all the treated groups showed no significant difference between each other. Except only group β-LG(100 mg/kg) , LAC (200mg/kg), concurrently with TAA show slight elevation in AST level, than the other groups. The mediocre liver damage produced because of TAA-administration for 8 weeks was further reconfirmed by significant elevation in serum ALP activity and total bilirubin compared with the control group. A significant decrease in serum ALP and TB was observed in all treated groups, compared with the group intoxicated with TAA for 8 weeks. In ALP test, group LAC (100 mg/kg) concurrently with TAA showed no significant difference from the normal control group. However, in the other side the rest of the groups can't reach to the normal value. While, in TB group's β-LG and LAC (100 mg/kg), LAC (50mg/kg) combined with L-methionine 40mg/kg and L-methionine 40mg/kg concurrently with TAA showed insignificant changes in TB level as compared to the normal control group. Although, groups β-LG and LAC (200 mg/kg), β-LG (50mg/kg) combined with L-methionine 40mg/kg concurrently with TAA significantly decreased TB level compared with TAA group, they showed significant difference from normal control group.

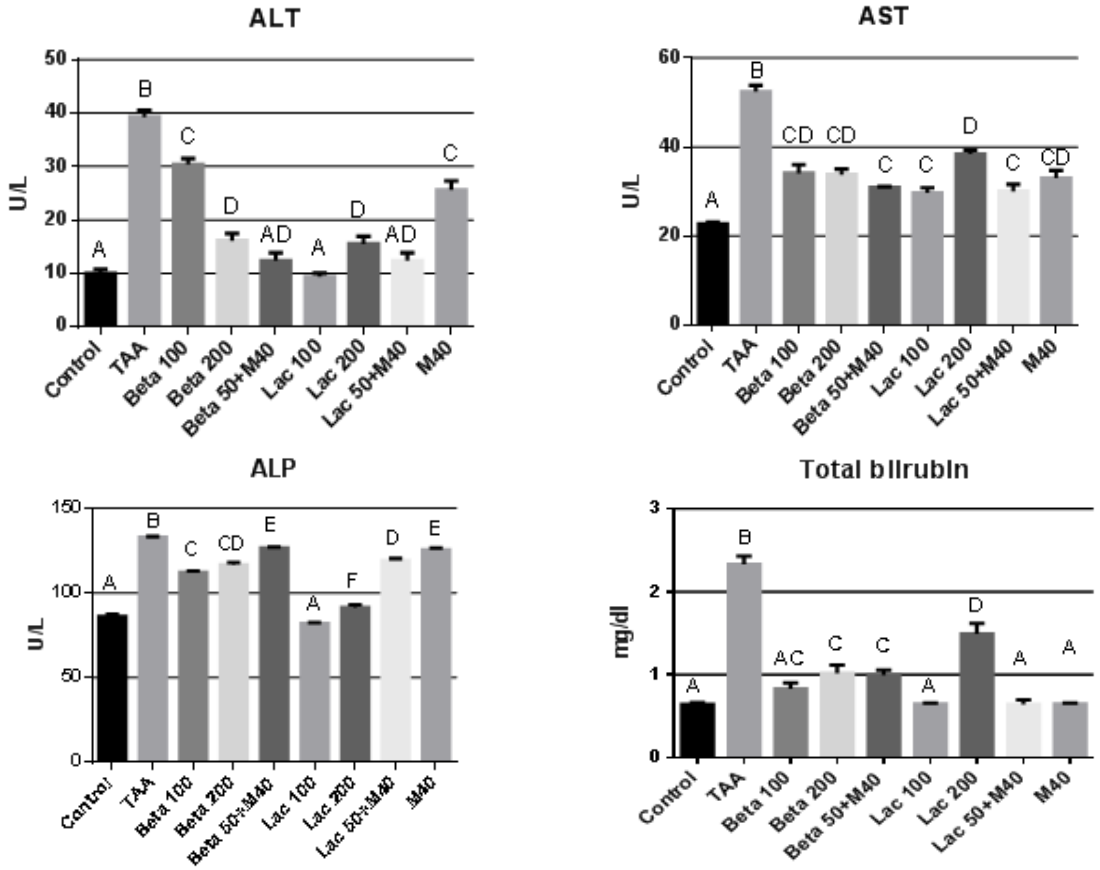

Fig.1: effect of whey proteins product as Lac and β-lg) 100mg/kg and200mg/kg versus L-methionine 40mg/kg concurrently with TAA on serum ALT,AST, ALP and total bilirubin.

3.2.Effect of whey proteins product as LAC and β-LG 100mg/kg and 200mg/kg versus L-methionine 40mg/kg concurrently with TAA on hepatic reduced GSH, MDA , NO and SOD activity as shown in "Fig2":

The administration of TAA for 8 weeks caused a mild increase in hepatic MDA, compared with the control group. A significant reduction in hepatic MDA was observed in all treated groups, compared with the group intoxicated with TAA for 8 weeks. There is no significant difference between each group. Except group LAC (200mg/kg) concurrently with TAA showed slight elevation in MDA level, than the other groups. Hepatic NO content was also found to be elevated significantly in rats intoxicated with TAA for 8 weeks, compared with the control group. TAA-intoxication induced elevation in hepatic NO content was reduced by all treated groups. All groups showed insignificant change in NO content as compared to the normal control group. Except group LAC (200mg/kg) concurrently with TAA showed significant difference from normal control group. Hepatic GSH content and SOD activity were significantly reduced due to TAA-intoxication for 8 weeks compared with the control group. Interesting, all treatments ameliorated efficiently the reduction in hepatic GSH content. Although β-LG (50mg/kg)combined with L-methionine 40mg/kg concurrently with TAA showed insignificant differences compared to the normal control group, group LAC (200mg/kg) concurrently with TAA showed insignificant differences compared to, thioacetamide treated group. On the other hand, TAA-induced depletion in hepatic SOD activity was counteracted by all treatments. Although group β-LG (50mg/kg) combined with L-methionine 40mg/kg , LAC (100 and 200mg/kg) concurrently with TAA insignificantly different from the normal control group, group β-LG(100 and 200mg/kg) , β-LG (50mg/kg) combined with L-methionine 40mg/kg , L-methionine 40mg/kg concurrently with TAA significantly increased SOD values.

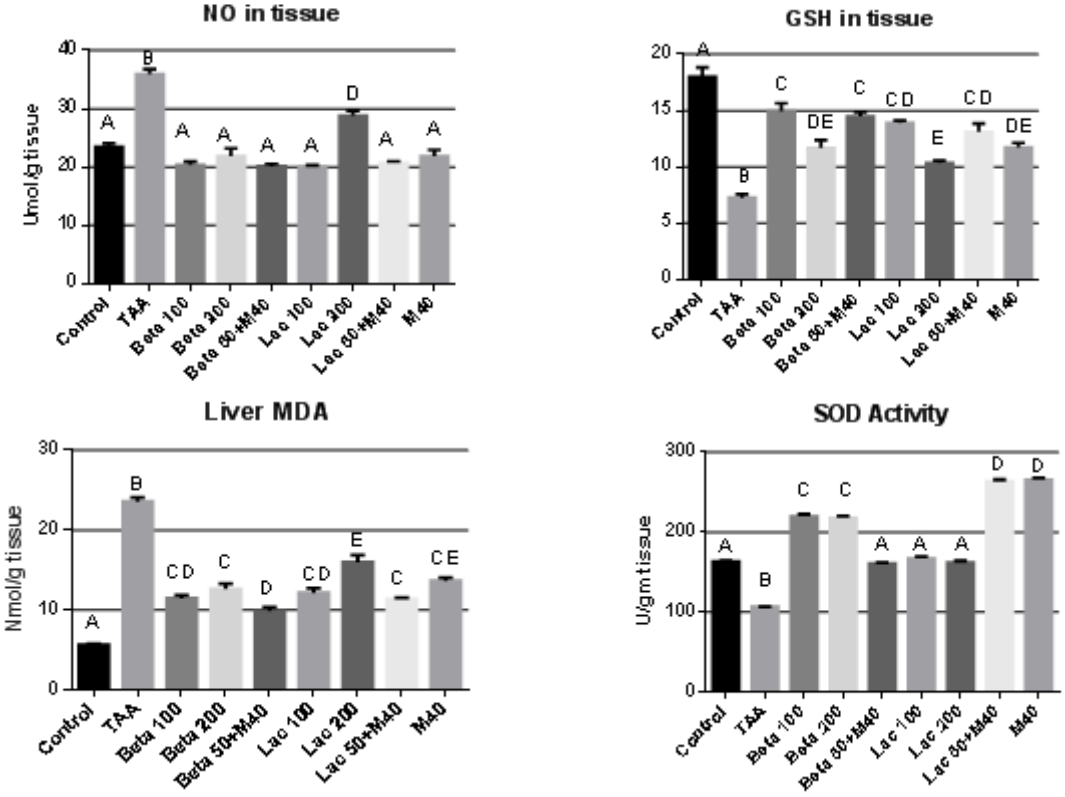

Fig. 2, Effect of whey proteins product as LAC and β-LG 100mg/kg and200mg/kg versus L-methionine 40mg/kg concurrently with TAA on hepatic reduced GSH, MDA, NO and SOD activity.

IV. HISTOPATHOLOGICAL STUDIES

Light microscopic examination of the liver of control rats revealed normal hepatic parenchyma with preserved and well organized lobular pattern with no evidence of fibrosis or inflammatory reaction "Fig.3a" confirmed by Masson's Trichrome stain which showed no blue stained fibrous tissue or collagen bundles "Fig.4a". TAA hepatocytes of this group showed altered and disorganized architecture with hepatic fibrosis that characterized by fibroblastic proliferation extending around the hepatic lobules "Fig.3b" that appeared blue In Masson's Trichrome stained liver section "Fig. 4b". Portal area showed marked dilatation of portal blood vessels, hyperplasia of biliary epithelium with formation of newly formed bile ductules, biliary cyst formation and intense fibroblastic proliferation which extend to infiltrate the surrounding hepatic parenchyma "Fig. 3 c &d" In L-methionine concurrently with TAA This group showed marked improvement with less tissue damage and liver fibrosis. Only delicate fibrous tissue could be demonstrated "Fig.3e" confirmed by Masson's Trichrome stain in which the fibrous tissue appeared faint blue "Fig.4c". Portal area appeared normal in most examined cases. In β-LG 100mg/kg concurrently with TAA this group significantly ameliorated liver fibrosis compared with thioacetamide treated one. Liver showed marked reduction of fibrosis "Fig.3f", with faint blue fibrous tissue in Masson's Trichrome stained liver section "Fig.4d". Portal area was infiltrated with few mononuclear cells. In β-LG 200mg/kg concurrently with TAA Liver showed slight improvement. Intense fibroblastic proliferation associated with mononuclear cell infiltration was demonstrated "Fig.3g".The fibrous tissue stained blue in Masson's trichrome stained liver section "Fig.4e". Portal area showed hyperplasia of biliary epithelium with formation of newly formed bile ductules. In β-LG 50mg/kg and L-methionine concurrently with TAA this group showed significant improvement compared with thioacetamide treated one, as the extent of fibrosis and cell injury were greatly reduced "Fig.3h" . Delicate strands of fibrous tissue stained blue in Masson's trichrome stained liver section "Fig.4f" was demonstrated Portal area appeared normal in most examined sections. In LAC 100mg/kg concurrently with TAA This group significantly ameliorated the thioacetamide induced liver fibrosis as evidenced by marked regression of the histopathological lesions compared with the thioacetamide intoxicated group. Liver showed cell preserved and organized hepatic lobules with very delicate fibrous tissue "Fig.3i" confirmed by Masson's Trichrome stained liver section "Fig.4G ". In LAC 200mg/kg concurrently with TAA Liver sections of this group showed no improvement, compared with other groups, with fibroblastic proliferation infiltrating the hepatic parenchyma "Fig.3j" that appeared blue in

Masson's Trichrome stained liver section "Fig.4h". In LAC 50mg/kg and L-methionine concurrently with TAA this group showed the same great extent resembles those of the control group in most of hepatic lobules.

Only mild fibroblastic proliferation infiltrating the histopathological alterations recorded in alpha 100 groups. Liver showed alleviated liver fibrosis "Fig. 3k". The hepatocytes to great extent resemble those of the control group in most of hepatic lobules. Only mild fibroblastic proliferation infiltrating the hepatic lobules stained blue in Masson's Trichrome stained liver section "Fig.4 i".

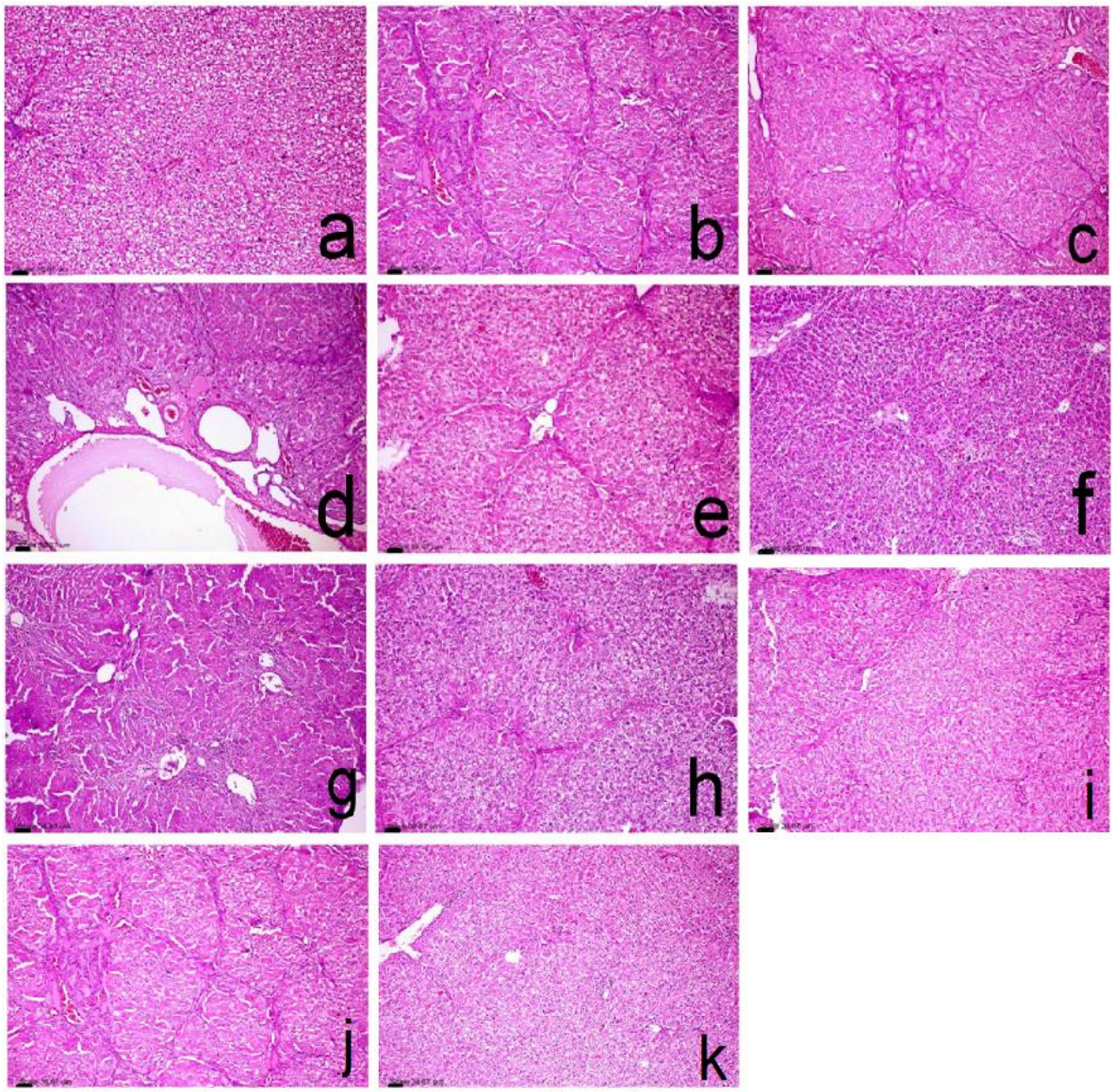

Fig. 3: Haematoxylin and eosin-stained liver sections. A) Normal control .B, C and D) TAA intoxicated group. E) L-methionine treated concurrently with TAA. F) β-LG 100mg/kg treated concurrently with TAA .G) β-LG 200mg/kg treated concurrently with TAA. H) β-LG 50mg/kg and L-methionine treated concurrently with TAA .I) LAC 100mg/kg treated concurrently with TAA .J) LAC 200mg/kg treated concurrently with TAA.K) LAC 50mg/kg and L-methionine treated concurrently with TAA.

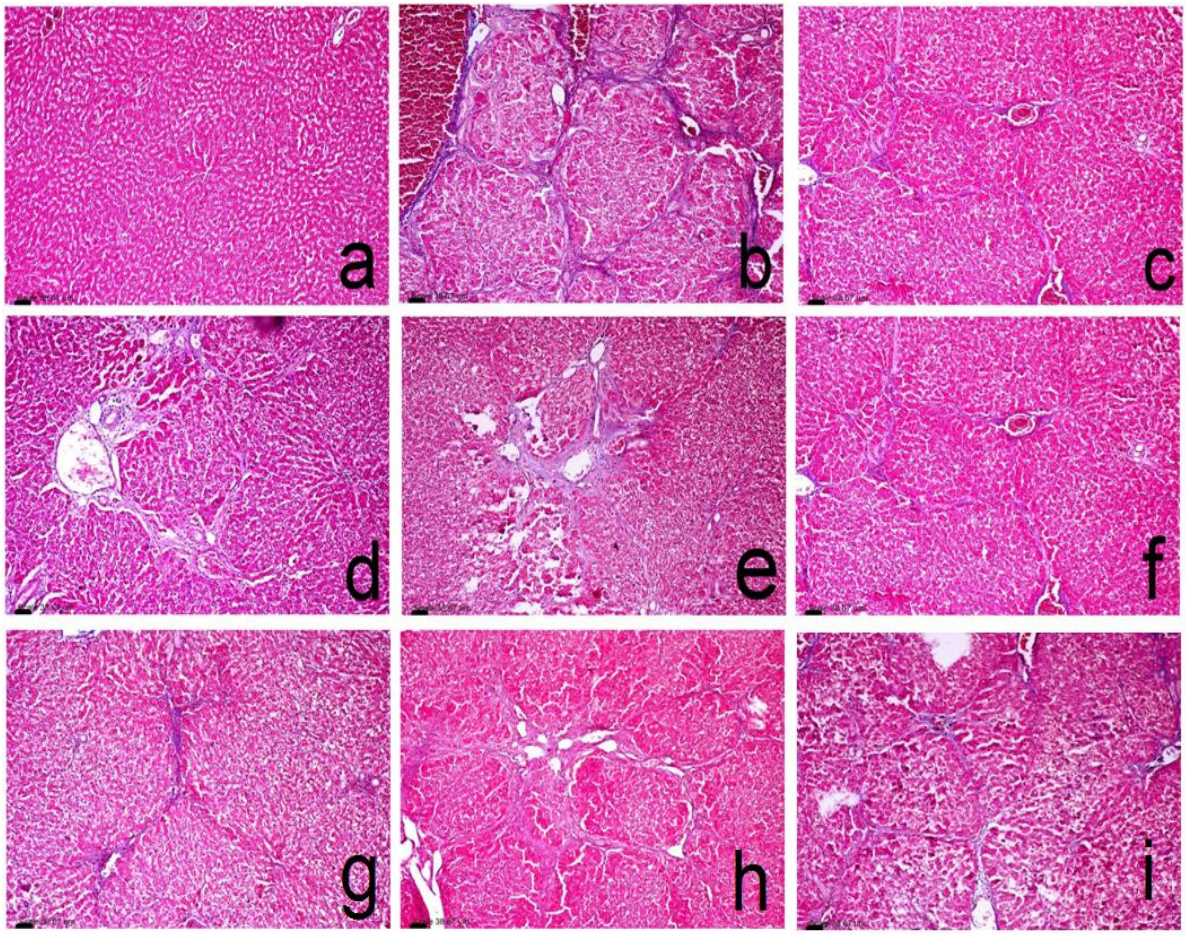

Fig.4: Liver sections with Masson's trichrome staining. A) Normal control .B) TAA intoxicated group. C) L-methionine treated concurrently with TAA. D) β-LG 100mg/kg treated concurrently with TAA .E) β-LG 200mg/kg treated concurrently with TAA. F) β-LG 50mg/kg and L-methionine treated concurrently with TAA .G) LAC 100mg/kg treated concurrently with TAA .H) LAC 200mg/kg treated concurrently with TAA.I) LAC 50mg/kg and L-methionine treated concurrently with TAA.

V. DISCUSSION

Thioacetamide (TAA) upon its Systemic administration is metabolized into TAA sulfoxide and TAA-S, S-dioxide, which covalently bind other intracellular molecules and damages the macromolecules of hepatocytes causing DNA damage , protein oxidation and per oxidation of the cell membrane biomolecules [10]. The consequences of TAA metabolism is oxidative stress which activate myofibroblasts that secrete fibrinogen and growth factors[32] Cell death triggered by increased oxidative stress induces an inflammatory response, activation of HSCs, and if this is left unregulated, eventually causes hepatic fibrosis[33]. Therefore, use of radical scavengers and antioxidative agents appear to be a good choice for preventing the hepatocellular injury and to prevent occurrence of fibrosis [34]. Regarding our biochemical analysis, TAA elevated hepatic MDA level suggests enhanced lipid peroxidation leading to tissue damage and failure in antioxidant defense mechanisms, leading to depletion of hepatic GSH and SOD activity [35] and causing irreversible inhibition in their activities [36]**.** TAA administration resulted also in increased NO free radical content in liver homogenate, the increased NO production occurred in response to inflammatory cytokines and enhancement over expression of inducible nitric oxide synthase (iNOS)[37]The excessive production of NO has deleterious effect on tissue function because of its ability to react with biomolecules or with other free radicals e.g., superoxide anion, yielding the highly reactive peroxynitrite radical capable of evoking the oxidation of important cellular biomolecules[38] .

During hepatic damage; cellular enzymes like AST, ALT and ALP present in the liver cells leak into the serum [39]. Liver toxicity by TAA elevated the AST levels in serum due to the extensive damage to the tissues producing acute necrosis since the mitochondrial AST is released only when the cells are severely disintegrated[40,41]. This result was confirmed by histopathological examination as TAA altered and disorganized the architecture of the hepatocytes. ALP levels elevated in plasma due to large bile duct obstruction by TAA induced oxidative stress [39] and this was appeared in the histopathological examination

which revealed hyperplasia of biliary epithelium with formation of newly formed bile ductules, biliary cyst formation and intense fibroblastic proliferation which extend to infiltrate the surrounding hepatic parenchyma. Kadir .,et al [42] Reported that total bilirubin elevation was a resultant effect of TAA chronic administration which caused hepatocytes disability to perform its role in bilirubin metabolism. This supported our results which revealed significant elevation of total bilirubin level in TAA-treated group

In response to groups which received β-LG and LAC (100mg/kg and 200 mg/kg), respectively, concurrently with TAA. LAC is a product from whey protein with high concentration of alpha-lactalbumin (43 %) which represent 20-25% of total whey protein and β-LG (50-55% of total whey protein) [16] attenuated almost all TAA deleterious effects as β-LG has two intermolecular disulfide bonds and one free thiol group, which plays an essential role in the antioxidant activities of β-LG [20]. It has been proposed that antioxidants which maintain the concentration of reduced GSH may restore the cellular defense mechanisms, block LPO and thus protect against the oxidative tissue damage [43]. Our results verify that LAC and β-LG attenuated the depletion of hepatic GSH compared to TAA intoxicated rats. This result is consistent with many investigators who confirmed the ability of whey protein to increase the level of GSH in rats liver [44].this depends on the fact that WP and alpha lactalbumin have a high content of cysteine and methionine [45], which are important antioxidants and are necessary for the glutathione synthesis that directly participates in the fight against inflammatory diseases[18]. Shaheen, et al [46] reported that LAC caused up regulation and enhancement of the production of more reduced GSH in liver while Mansour, et al [47] confirmed that β-LG treatment attenuated the depletion of hepatic GSH. It is remarkable that LAC and β-LG significantly decrease the hepatic MDA and thereby supported their antioxidant activity, which is mostly due to chelating activity for heavy metal ions that may catalyze formation of ROS[48] . Shaheen .,et al [46]Showed that LAC caused remarkable reduction in elevated MDA levels on the basis that LAC contributes to synthesis of potent antioxidants which render it reactive agent against number of ROS and has a potential effect in preventing further accumulation of free radicals as well as oxidative stress due to its anti-oxidant capacity and anti-inflammatory effect. While Mansour, et al [47] reported that β-LG significantly decrease the hepatic MDA level. Our data showed that LAC and β-LG significantly decreased TAA induced NO production and restore its normal level; this might be contributed to the attenuation of TAA-induced oxidative stress through enhancing hepatic GSH pool. Mansour, et al [47] concluded that β-LG treatment blocked peroxynitrite, a powerful and potent pro-apoptotic and pro-inflammatory mediator, which could be the reason for β-LG to exert significant reduction in NO level at both dose levels. In the same time Shaheen, et al [46] supported our results by reporting that LAC showed significant reduction in the NO level attributed to its high content of thiol compounds. Treatment with LAC and β-LG not only restored the decrease in hepatic SOD levels occurred by TAA to its normal level. But also, β-LG groups showed a significant increase when compared with the control group. Mansour, et al [47] Reported that α-LA and β-LG abrogated the reduction of SOD activity in hepatic tissues resulted from hepatotoxicity induction, its activity increased depending on increasing the time of the experiment. Estimation of serum liver function tests (ALT, AST) in those groups showed that LAC at the dose level 100 mg/kg restored the increased level of ALT enzyme to its normal level. The protective effect of LAC and β-LG and their ability to resist the elevation in ALT and AST levels by retaining them in the hepatocytes which appeared with preserved and organized hepatic lobules concerning the histopathological examination. This may be due to whey proteins are rich in cysteine, glutamate and methionine (cysteine donor), these two amino acids are precursors to the tripeptide glutathione. On the light of our results, Treatment with LAC at the dose level 100 mg/kg attenuated the increased levels of the serum ALP, and caused absolute recovery towards normalization. Bilirubin is one of the most clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocytes .LAC and β-LG treatment succeed to restore TB to its normal control level [49]. In response to group received L-methionine (40 mg/kg), concurrently with TAA. L-methionine caused elevation in GSH and SOD activity as reported by Nagib, Al-Masri. [50, 51] and it reduced the susceptibility of lipid peroxidation by restoration of the level of free radical scavengers. [52] Corroborated our results and reported that methionine is a known precursor of glutathione by its conversion to cysteine, the main limiting step to the synthesis of glutathione in terms of SH-group donor and can also serve as a free radical scavenger by scavenging superoxide ions, increasing reduced glutathione levels and maintaining the energy state of mitochondria. by increasing glutathione levels, methionine helps the liver to effectively neutralize toxins besides, its beneficial influence on the morphological picture of the liver which suggest that methionine act as antioxidants that may be effective in reversing the toxic changes [53] and this can interpenetrate our results which reveal a subsequent recovery towards normalization almost like that of normal control group in serum ALT, AST, ALP and total bilirubin as concerning the histopathological examination hepatocytes of this group showed marked improvement with less tissue damage and liver fibrosis was resulted , Methionine attenuated all biochemical changes induced by TAA, by preventing either the depletion of GSH or the binding to protein sulfhdryl groups [54] . Nagib, Al-Masri [50,51] reported that L-methionine administration caused reduction in MDA level and NO content in liver tissue, it also elevated GSH and SOD activity, which means complete suppression of oxidative stress induced by

TAA and therefore it could prevent hepatic fibrosis occurrence. The complicated pathogenesis of liver fibrosis suggests that combination therapy may have greater benefits in protecting from incidence of liver fibrosis than monotherapy; consequently, we hypothesized that a combination between whey protein products and l-methionine could reverse fibrosis induced by TAA concomitant administration and potentiate the antioxidant effects of each other .this obviously happened in group which rats were received daily β-LG and LAC , respectively, at very low dose 50 mg/kg combined with l-methionine(40mg/kg) , concurrently with TAA. The augmentation in their antioxidant activity versus l-methionine group appeared in more than one parameter as in ALT and NO tests in which they could preserve their normal levels, besides; they have been reached to one of the highest reduced glutathione levels compared to all groups .on the other side LAC and L-methionine showed superior antioxidant activity than β-LG and L-methionine and this appeared in more than one parameter like total bilirubin, ALP, SOD and MDA besides the histopathology which revealed that hepatocytes in LAC and L-methionine group to great extent resemble those of the control group in most of hepatic lobules. Only mild fibroblastic proliferation infiltrating the hepatic lobules while in β-LG and L-methionine group the extent of fibrosis and cell injury were greatly reduced and Portal area appeared normal in most examined sections.

VI. CONCLUSION

Our results demonstrated that administration of LAC , β-LG and L-methionine concurrently with TAA can protect hepatocytes against oxidative damage which finally lead to hepatic fibrosis induced by TAA this evidenced by decreased liver marker enzymes (ALT, AST and ALP) ,TB, MDA, NO, increased the antioxidant enzymes(GSH and SOD). And eventually, WPs and their combination with L-methionine improved hepatic histopathological picture. These effects could be due to their antioxidant nature, and the potentiating of antioxidant property due to combination which may include free radical scavenging properties and its anti-oxidant promoting activity.

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