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Potential hepatoprotective effect of combining vitamin C and L-Carnitine against acetaminophen induced hepatic injury and oxidative stress in rats.

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Abstract : Acetaminophen is one of the most popular OTC analgesics and antipyretics especially among women unfortunately; its miss use can result in serious hepatic injury. In the present study hepatoprotective activity of L-carnitine plus Vitamin C against acetaminophen induced hepatic damage in Adult Female Wistar albino rats was evaluated. L-carnitine at dose levels of 25 and 50mg/kg p.o. /day plus Vitamin C 100 mg/Kg p.o. /day were administered for 21 days. On day twenty-one; hepatic injury was induced by administering a single dose of 600mg/Kg body wt. p.o. of acetaminophen. Results revealed that combining L-carnitine and vitamin C reduced serum liver enzymes; Aspartate amino Transferase (AST) and Alanine amino Transferase (ALT), decreased cholesterol level and low density lipoproteins (LDL-Cholesterol), increased high density lipoproteins (HDL-Cholesterol), dropped interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα), hindered the progression of oxidative stress as foreseen by increasing glutathione (GSH) level and reducing malondialdehyde (MDA) and nitric oxide (NO_x) contents. In conclusion; we can recommend the use of vitamin C in combination with l-Carnitine to protect against adverse effects that could result from over dosage of acetaminophen.
Key words : L-Carnitine, Vitamin-C, InterLeukein-6, Acetaminophen, Liver injury.

Introduction

Among women, the primary headache form that is affected by the physiologic hormonal variations occurring through a woman's lifetime is migraine and the drug of choice for the symptomatic treatment of migraine during pregnancy is acetaminophen[1]. Acetaminophen is a widely used analgesic and antipyretic drug[2] that is available over the counter alone[3,4,5], with other medications such as caffeine[6], Aceclofenac[7], Nimesulide[8] or by prescription when combined with other drugs[9] such as opioids for example tramadol (37.5 mg tramadol/325 mg acetaminophen)[10]; regrettably no studies have ever been conducted on large patient samples to assess its safety during pregnancy and it is known to cross the blood/placental barrier and like all other NSAIDs, it can cause premature closure of ductus arteriosus, prolong pregnancy, and retard labor by inhibiting prostaglandin synthesis [1]. Moreover; the incidence of attempted suicide using this medication was high among women[11]. Acetaminophen can cause severe hepatotoxicity in overdose[12,13]. The pharmacokinetic and toxicological mechanisms of an acute over-exposure to acetaminophen have been well established [14]. Acetaminophen hepatotoxicity is dependent on cytochrome P450 (CYP) enzymes that metabolize

Acetaminophen to the reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI). At a therapeutic dose; around 90% of Acetaminophen undergoes glucuronidation and sulfation before excretion, while 5–10% of Acetaminophen is metabolized by CYP enzymes mainly the CYP2E1 subtype to form (NAPQI) ; which is normally detoxified and excreted in the urine by conjugation with glutathione[15]. An acute overdose of Acetaminophen saturates the glucuronidation and sulfation pathway leading to accumulation of the active metabolite, (NAPQI); which exceeds the detoxification capacity of glutathione forming active protein adduct[16]. Ben-Shachar et al. (2012), introduced a mathematical model that focusedon the detailed biochemical mechanisms of acetaminophen detoxification in therapeutic and overdose situations. They showed that a therapeutic dose of acetaminophen would lead to a 10% reduction in glutathione, which is used for the detoxification of (NAPQI). In addition, chronic therapeutic doses of Acetaminophen depleted glutathione by 30% in the liver and it took more than 2 days to retrieve glutathione to the normal level[17]. Excess (NAPQI) reacts with the sulfhydryl group on the cysteine of cellular protein, mainly the mitochondrial protein, which leads to mitochondrial dysfunction[18]. The subsequent mitochondrial dysfunction leads to the inhibition of mitochondrial respiration, ATP depletion and formation of reactive oxygen species (ROS) and peroxynitrite in the mitochondria; this,eventually triggers the opening of the mitochondrial membrane permeability transition pores, resulting in collapse of the mitochondrial membrane potential. Furthermore, fragmentation of mitochondrial DNA has been observed, which contributes to necrotic cell death from Acetaminophen toxicity[14].

Vitamin C, also called ascorbic acid, plays essential roles as an antioxidant[19], shares in biosynthesis of many important substances, such as cholesterol, catecholamines, amino acids, and certain peptide hormones, influences mitochondrial function by decreasing the generation of (ROS) through stimulation of the activity of superoxide dismutase and glutathione peroxidase and alteration of the activity of the electron transport chain[20], also has anti-inflammatory properties[21]. Pathogenic dysfunction of tissues owing to cell death via apoptosis is one of the important outcomes of oxidative stress that could be ameliorated by vitamin C[22]. Adikwu et al. (2013)**,** demonistrated that vitamin C has hepatoprotective effect which increases when co-administered with other agents precisely antioxidants[23].

L-Carnitine (4-*N*-trimethyl ammonium 3-hydroxybutyric acid) is an essential amino acid that could be synthesized from two other essential amino acids namely methionine and lysine in human liver, kidneys, and brain but principally obtained from diet which is involved in the energy metabolism[24]. It functions by transferring long-chain fatty acids across the mitochondrial membrane, enabling the oxidative release of energy. It has protective role against hepatotoxicity[25].

The present study aimed to evaluate the beneficial hepatoprotective and antioxidant activities of combining Vitamin C with L-Carnitine in ameliorating Acetaminophen -induced hepatic injury.

Material and Methods

Animals

Adult Female Wistar albino rats, 200-250 g were obtained from the animal house colony, National Research Centre, Giza, Egypt. All animals were housed in metal cages in a well-ventilated environment at (22 ± 3°C, 55 ± 5% humidity and 12h dark & light cycles); received standard rat food pellets and water was provided *ad libitum* throughout the experimental period. The animals were treated according to the national and international ethics guidelines stated by the ethics committee of NRC National Research Centre and all procedures and experiments were performed according to the protocol approved by it, and the earliest scientifically justified endpoint was used in this study to prevent pain or distress in the experimental animals..

Drugs

Vitamin C (VMD co, Belgium) provided as powder and was freshly solubilized in distilled water prior to ingestion. L-Carnitine (EMDOKA co, Belgium) nutritional liquid supplement. Acetaminophen (GSK co, EGYPT) provided as powder and was freshly solubilized in distilled water prior to ingestion.

Experimental Design

Study groups (8 female rats each) were treated as follows: Group (1) Control group (untreated group): Receiving oral distilled water ingestion (5ml/kg). Group (2) Acetaminophen group: Receiving oral distilled water ingestion (5ml/kg). Group (3): (25 LV): Receiving L-Carnitine at a dose of 25mg/kg/day p.o. and

Vitamin C at a dose of 100mg/kg/day p.o. Group (4): (50 LV): Receiving L-Carnitine at dose of 50mg/kg/day p.o. and Vitamin C at dose of 100mg/kg/day p.o. All groups received the corresponding drug treatments for twenty –one days. On day twenty-one all groups except the first group were administered Acetaminophen (600mg/kg p.o.). Twenty-four hours later blood samples were collected, allowed to clot, then centrifuged for 20 minutes at 3000 r.p.m. Serum was separated and stored into eppendroff tubes at – 20 °C to be used for determination of liver function parameters including (AST), (ALT), (HDL- Cholesterol), (LDL- Cholesterol), Total Cholesterol, (IL-6) and (TNFα). After collection of blood samples, rats were sacrificed by decapitation and their livers were immediately removed. Each liver was divided into 2 parts; the first part was kept at -80 °C for determination of (MDA), (GSH) and (NO_x) .The second part was preserved in phosphate buffered formalin 10% for further histopathological investigation.

A-Determination of liver function parameters:-

Determination of transaminases activity (ALT and AST):

Serum activities of (ALT) and (AST) were determined colorimetrically using kits of QCA® according to the method of [26]. The absorbance was measured at 546 nm.

B-Determination of lipid profile parameters:-

1-Determination of total cholesterol:

This test was performed using kits of Bio Diagnostic Company for the enzymatic colorimetric determination of total cholesterol activity at wave length 500 nm according to the method of and [27]

2-Determination of high density lipoproteins:

This test was performed using kits of Bio Diagnostic Company for the enzymatic colorimetric determination of (HDL-Cholesterol) activity at wave length 500 nm according to the method of [28].

3-Determination of low density lipoproteins:

This test was performed using kits of Bio Diagnostic Company for the enzymatic colorimetric determination of (LDL- Cholesterol) activity at wave length 500 nm according to the method of [29]

C-Determination of inflammatory markers:

1-Determination of serum Interleukein-6:

This test was performed using Rat Interleukin 6 ELISA kit of Glory Science Co. for the quantitative determination of rat IL-6 concentration at wave length 450 nm according to the method of [30].

2-Determination of tissue tumor necrosis factor-alpha:-

Serum levels of TNF-α were quantified as performed by [31] using an enzyme-linked immunosorbent assay (ELISA) kit Glory science co and read at 450 nm.

D-Determination of anti-oxidant activity, oxidative state and nitrosative stress:

1-Determination of malondialdehyde:

This test was performed using kits of Bio Diagnostic Company for the enzymatic colorimetric determination of (MDA) at wave length 534 nm according to the method of [32].

2- Determination of reduced glutathione:

This test was performed using kits of Bio Diagnostic Company for the enzymatic colorimetric determination of (GSH) at wave length 405 nm according to the method of [33].

3. Determination of hepatic nitric oxide:-

(NO_x) was determined in rat liver homogenate (20%) using a colorimetric method based on the Griess reaction according to the method of [34].

Histopathological examination:

For histopathological studies, autopsy samples were taken from the liver of rats from different groups and fixed in 10% formal saline for twenty four hours. Washing was done in tap water then serial dilution of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56^0 C in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin stain for routine examination then examination was done through the light electronic microscope[35].

Immunohistochemistry:

Demonstration of Bax and activated Caspase-3 immunostaining in liver sections of normal and treated rats, as apoptotic markers, was performed according to the method described by[36] . Rabbit anti-caspase-3 (diluted to 1:1000, Abcam, Ltd., USA) and Bax (1:200, Abcam, Ltd.,USA) were used as biotinylated primary antibodies. Colour intensity of positive immune-reactive cells was determined in 10 random low microscopic field (X10) using Image analyzer (Leica Qwin 500, Cambride, Engalnd). The image was transformed into a grey image [a grid of pixels each representing the intensity or brightness at that point by a range of numbers, typically from 0 (black) to 255 (white)]. A greyscale image is a colour mode that displays image using 256 shades of grey, referred to as 8-bit greyscale image. Each colour was defined as a value between 0 and 255, where 0 is the darkest (black) and 255 is the lightest (white).

Statistical Analysis:-

All results were expressed as mean ± standard error of the mean. Data analysis was achieved by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using software program Graph Pad Prism (version 5.00). Difference was considered significant when $P<0.05$.

Results:-

A-Effect on serum biochemical parameters:-

1-Effect on liver functions:-

Effect on serum ALT and AST:-

Administration of a single toxic dose of acetaminophen (600mg/ kg) induced acute significant increase in serum ALT and AST levels as compared to the normal control group (144.0 ± 4.29 vs. 36.93 ± 4.21) and (78.74 ± 1.11 vs. 42.47 ± 2.76) respectively at $p < 0.05$. Pretreatment of rats with Vitamin C (100 mg/kg) in combination with L-Carnitine (25 and 50 mg/kg) for 21 days before induction of hepatotoxicity exhibited hepatoprotective activity against acetaminophen intoxication revealed by a significant reduction in the elevated serum level of ALT (108.8 ± 5.05 and 76.58 ± 1.31 respectively vs. 144.0 ± 4.29) and AST (55.58 ± 2.82 and 43.43 ± 1.07 respectively vs. 78.74 ± 1.11) as compared to acetaminophen intoxicated group. (Figure1).

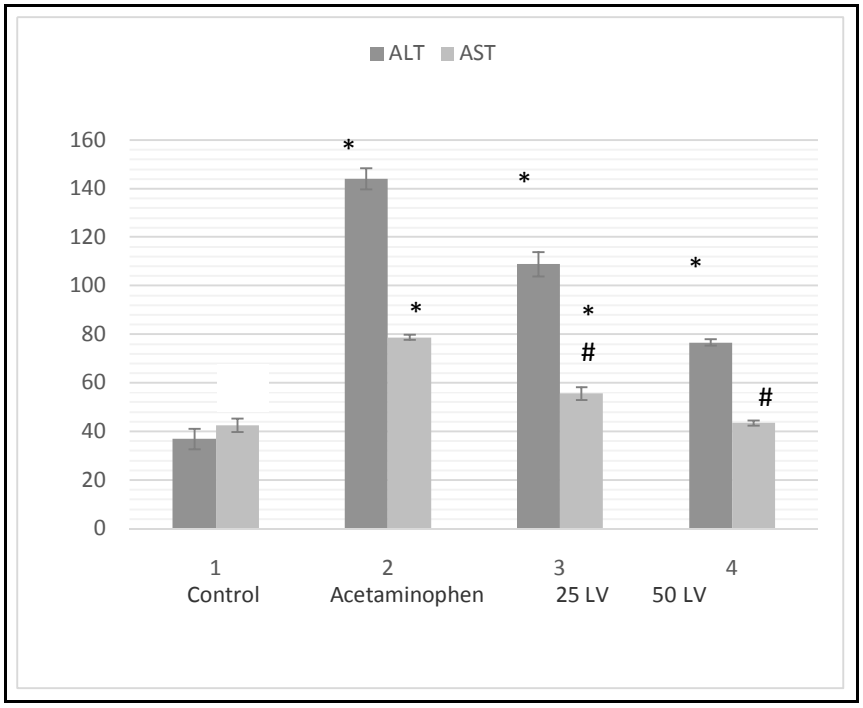

Fig.1: Effect of oral administration of *L-Carnitine plus Vitamin C* on serum AST and ALT levels. Data are presented as mean ± (standard error) S.E. (n=8 rats) for each group. Statistical analyses were carried out using one Way ANOVA followed by˝ Tukeys Multiple Comparison Test˙.

* Significantly different from normal control group, # significantly different from paracetamol intoxicated group ($P < 0.05$).

2. Effect on serum lipid profile.

Effect on serum total cholesterol, HDL- Cholesterol and LDL- Cholesterol.

Result revealed that ingestion of a single toxic dose of acetaminophen (600mg/ kg) induced acute significant increase in serum total cholesterol and LDL- Cholesterol levels and a prominent decrease in the HDL- Cholesterol level as compared to the normal control group. Pretreatment of rats with Vitamin C (100 mg/kg) in combination with L-Carnitine (25 and 50 mg/kg) for 21 days before induction of hepatotoxicity lowered both cholesterol and LDL levels. Moreover; Vitamin C (100 mg/kg) in combination with L-Carnitine (50 mg/kg) normalized HDL level as compared to acetaminophen group. (Table1).

(Table1):- Effect of oral administration of *L-Carnitine plus Vitamin C* on serum lipid profile.

Groups	HDL- Cholesterol (mg/dl)	LDL- Cholesterol (mg/dl)	CHOLESTEROL (mg/dl)
Control	**(33.66 ± 0.65)$^{\#}$**	**(24.06 ± 0.85)$^{\#}$**	**(82.40 ± 2.96)$^{\#}$**
Acetaminophen	**(18.31 ± 1.08)***	**(41.16 ± 0.32)***	**(144.5 ± 2.26)***
25 LV	**(22.73 ± 2.34)***	**(35.22 ± 0.37)$^{*\#}$**	**(55.31 ± 1.13)$^{*\#}$**
50 LV	**(27.27 ± 2.44)$^{\#}$**	**(35.99 ± 1.01)$^{*\#}$**	**(40.86 ± 0.99)$^{*\#}$**

Data are presented as mean ± (standard error) S.E. (n=8 rats) for each group. Statistical analyses were carried out using one Way ANOVA followed by˝ Tukeys Multiple Comparison Test˙.

* Significantly different from normal control group, # significantly different from paracetamol intoxicated group ($P < 0.05$).

3. Effect on serum inflammatory markers:

1-Effect on serum (IL-6):

A single toxic dose of acetaminophen (600mg/ kg) induced acute significant increase in serum IL-6 level when compared to the normal control group (134.1±8.837 vs. 41.13±6.802). Only pretreatment of rats with Vitamin C (100 mg/kg) in combination with L-Carnitine (50 mg/kg) for 21 days before induction of hepatotoxicity exhibited anti-inflammatory activity indicated by reducing the elevated serum level of IL-6 when compared to acetaminophen intoxicated group (76.38±2.275 vs. 134.1±8.837) . (Figure2).

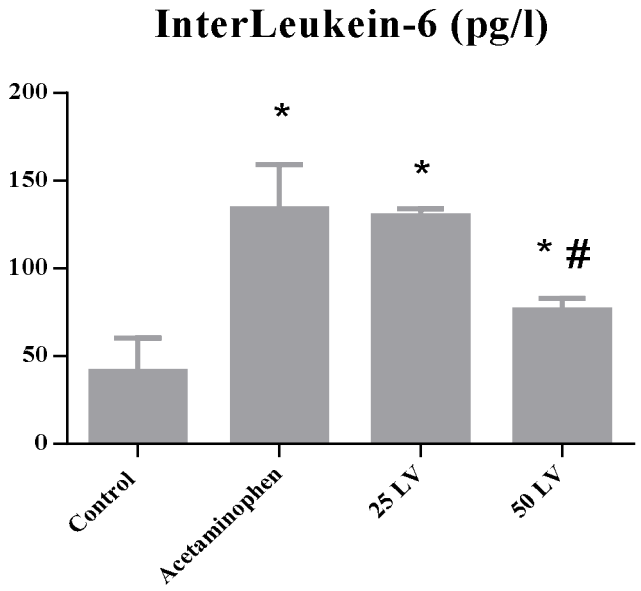

Fig.2: Effect of oral administration of *L-Carnitine plus Vitamin C* on serum IL-6.

Data are presented as mean ± (standard error) S.E. (n=8 rats) for each group. Statistical analyses were carried out using one Way ANOVA followed by˝ Tukeys Multiple Comparison Test˙.

* Significantly different from normal control group, # significantly different from paracetamol intoxicated group ($P < 0.05$).

2-Effect on tissue (TNF-α):-

A single toxic dose of acetaminophen (600mg/ kg) induced acute significant increase in TNFα level when compared to the normal control group (110.8 ±4.647vs. 30.66±0.2542). pretreatment of rats with Vitamin C (100 mg/kg) in combination with L-Carnitine (25 mg/kg) for 21 days before induction of hepatotoxicity exhibited anti-inflammatory activity indicated by reducing the elevated serum level of TNF-α when compared to acetaminophen intoxicated group (54.19± 5.325 vs. 110.8 ± 4.647). Also pretreatment of rats with Vitamin C (100 mg/kg) in combination with L-Carnitine (50 mg/kg) for 21 days before induction of hepatotoxicity normalized the TNF-α level (35.46± 1.869 vs. 30.66±0.2542) . (Figure3).

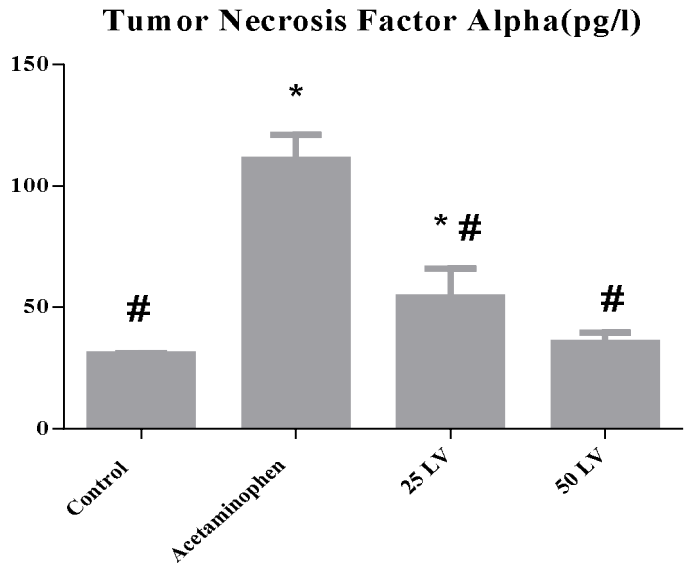

Fig.3: Effect of oral administration of *L-Carnitine plus Vitamin C* on tissue TNFα.

Data are presented as mean ± (standard error) S.E. (n=8 rats) for each group. Statistical analyses were carried out using one Way ANOVA followed by˝ Tukeys Multiple Comparison Test˙.

* Significantly different from normal control group, # significantly different from paracetamol intoxicated group ($P < 0.05$).

4-Effect on tissue anti-oxidant activity, oxidative state and nitrosative state:

Effect on tissue (MDA), (NO_x) and (GSH):

Acetaminophen (600mg/ kg) induced acute significant increase in tissue (MDA) and (NO_x) and significant decrease in (GSH) level as compared to the normal control group. Pretreatment of rats with Vitamin C (100 mg/kg) in combination with L-Carnitine (25 and 50 mg/kg) for 21 days before induction of hepatotoxicity displayed a pronounced anti-oxidant activity normalizing the levels of MDA, NO_x and GSH at the higher L-Carnitine dose combination group. (Table2).

(Table2):- Effect of oral administration of *L-Carnitine plus Vitamin C* on tissue anti-oxidant activity, oxidative state and nitrosative state.

Groups	GSH (µg/g tissue)	MDA (nmol//g tissue)	NO_x (µmol/l)
Control	$(64.83 \pm 4.42)^{\#}$	$(14.03 \pm 0.74)^{\#}$	$(20.63 \pm 0.58)^{\#}$
Acetaminophen	$(18.78 \pm 2.00)^{*}$	$(33.94 \pm 1.83)^{*}$	$(44.32 \pm 2.70)^{*}$
25 LV	$(76.46 \pm 3.20)^{\#}$	$(20.84 \pm 1.13)^{*\#}$	$(32.63 \pm 0.09)^{*\#}$
50 LV	$(86.76 \pm 4.03)^{*\#}$	$(13.49 \pm 0.31)^{\#}$	$(21.13 \pm 0.09)^{\#}$

Data are presented as mean ± (standard error) S.E. (n=8 rats) for each group. Statistical analyses were carried out using one Way ANOVA followed by˝ Tukeys Multiple Comparison Test˙.

* Significantly different from normal control group, # significantly different from paracetamol intoxicated group ($P < 0.05$).

Histopathology:-

Liver of control rats showed normal hepatic parenchyma with no evidence of histological abnormalities (figure 4a). Whereas liver of acetaminophen treated group revealed severe acute histopathological alterations characterized by ballooning degeneration of centrilobular hepatocytes associated with hepatocellular necrosis which is infiltrated by mononuclear cells (fig.4b). One of the most pathognomonic lesion demonstrated in nearly all examined sections was confluent massive centrilobular necrosis, with pyknotic and karryorrhectic nuclei (fig.4c), which extend to involve nearly all the hepatic lobules extending towered the portal triad with few preserved periportal hepatocytes, associated with disorganization of hepatic cords and dissociation of hepatocytes with subsequent sinusoidal dilatation and congestion as well as hemorrhage. Marked atrophy and dissociation of hepatocytes with nuclear pyknosis and sinusoidal congestion as well as hemorrhage (fig.4d) were characteristic lesions demonstrated in all examined sections in addition to abundant apoptosis (fig.4e). Portal triads, in some examined sections, revealed congestion of portal blood vessels with intra- and perivascular leukocytic cell aggregation. Periportal hepatocytes and hepatocytes at the periphery of the necrotic areas exhibited steatosis. Marked regression of these histopathological lesions was demonstrated in 50 LV pretreated group as the centrilobular hepatic cell necrosis was confined to the first three rows surrounding the central vein and infiltrated by mononuclear cells (fig.4f) with marked regression of apoptosis. On the other hand, mild improvement was recorded in 25 LV pretreated group compared to 50 LV pretreated one. Liver showed centrilobular necrosis infiltrated by mononuclear and oval cells associated with abundant apoptosis and sinusoidal leukocytosis (fig.4g), also showed focal hepatocellular necrosis (fig.1h).

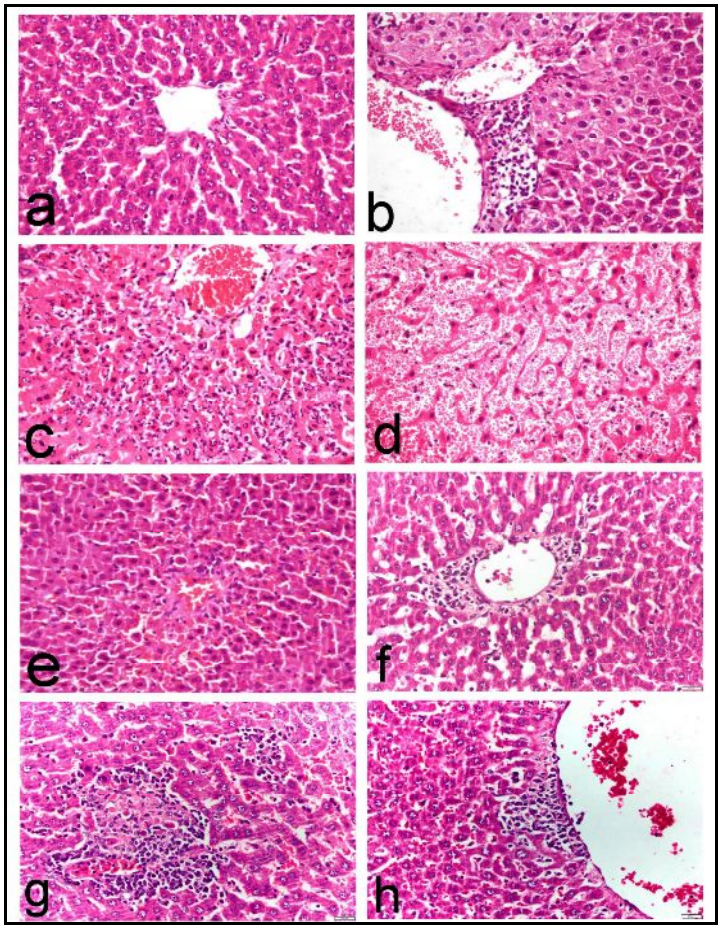

Fig 4. Histopathological investigation of liver tissues.

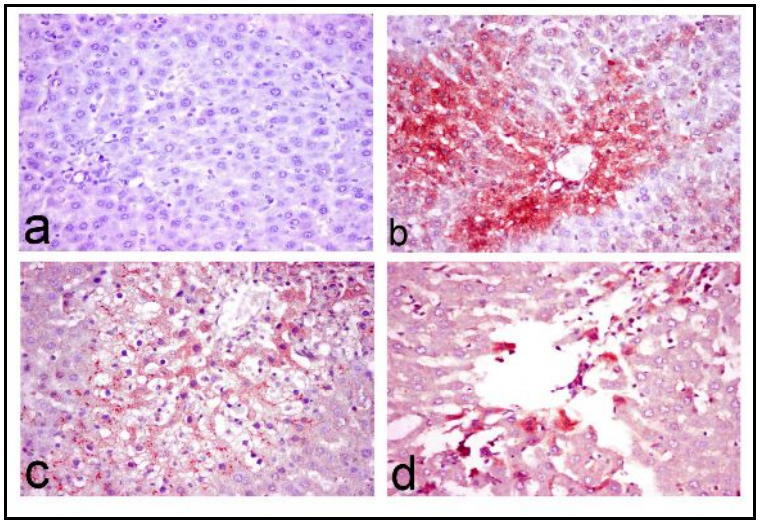

Fig 5. Bax-immunohistochemical staining of liver section of rats.

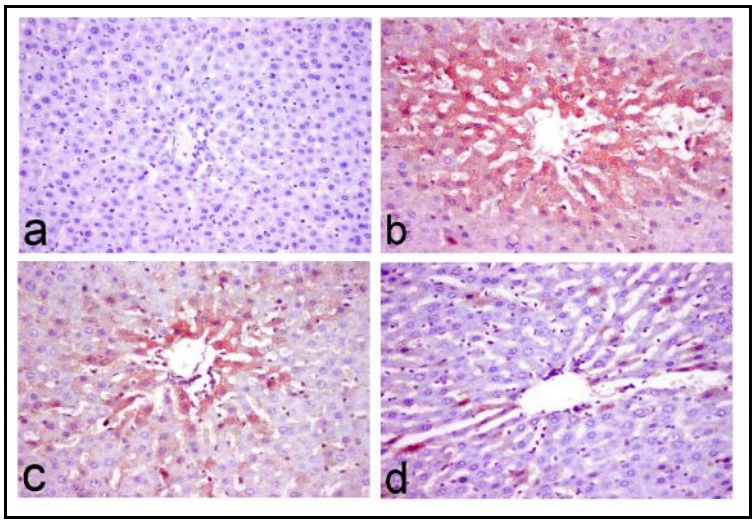

Fig 6. Caspase-3 immunohistochemical staining of kidney section of rats.

Immunohistochemistry:-

Liver sections of normal rats showing no immune-reactive cells of Bax and Caspase-3 (figure 5a & 6a, respectively) and (Table 3), Significant increase of Bax and Caspase-3 immune reactive cells were demonstrated in acetaminophen treated group (figure 5b & 6b, respectively) and (Table 3). 25 LV treated rats showing decreased numbers of immune-reactive cells of Bax and Caspase-3 (fig.5d & 6d, respectively) and (Table 3). These Bax and Caspase-3 immune reactive cells were markedly decreased in 50 LV pretreated group (fig.5c & 6c, respectively) and (Table 3). (Immunohistochemical staining of Bax and Caspase-3, X400).

(Table 3):- Illustrates the immunehistochemical findings recorded in the liver of different groups.

Caspase (count/ field)	Bax (count/ field)	Group
$(2.25 \pm 1.54)^{\#}$	$(2.00 \pm 1.08)^{\#}$	Control
$(66.75 \pm 16.23)^{*}$	$(85.25 \pm 9.65)^{*}$	Acetaminophen
$(8.50 \pm 7.17)^{\#}$	$(7.75 \pm 2.45)^{\#}$	50 LV group
$(13.00 \pm 1.68)^{\#}$	$(15.25 \pm 4.15)^{\#}$	25 LV group

Data are presented as mean ± (standard error) S.E. (n=8 rats) for each group. Statistical analyses were carried out using one Way ANOVA followed by˝ Tukeys Multiple Comparison Test˙.

* Significantly different from normal control group, # significantly different from paracetamol intoxicated group ($P < 0.05$).

Discussion:

Drug-induced liver disorders occur frequently and can be life threatening[37,22]. Acetaminophen toxicity is one of the most common causes of drug induced hepatotoxicity worldwide that leads to excessive treatment and hospitalization costs every year[18]. Moreover; is the most commonly used pain and fever medication during pregnancy[38].

The catastrophic free radical events related to acetaminophen such as lipid peroxidation, protein oxidation and DNA oxidation are hardly the cause of cell death in realistic in-vivo conditions. This is because the antioxidant defense in liver cells is capable of detoxifying free radicals and repair damage. However, when the antioxidant defense system is overcomed, free radicals may cause direct oxidative damage to cellular macromolecules, leading to cell death [22,39]. Nowadays supplementation with exogenous antioxidants improves the cellular defense system to prevent these effects.

N-acetyl cysteine amide is the treatment of choice for acute poisoning with acetaminophen. However, it also had some adverse side effects[40] Consequently, innovation of healthier treatments for acetaminophen toxicity is important.

The present study showed that a single dose of acetaminophen (600mg/kg.bw. p.o.) showed a marked increase in serum ALT, AST as compared to normal group which_indicates cellular leakage and loss of functional integrity of liver cell membrane. The extent of drug-induced hepatotoxicity is assessed by the release of these intracellular enzymes via the hepatocyte membrane into circulation[41]. This agrees with earlier reports by [42,43,44, 45,46,47] These authors opined that acetaminophen could be toxic to the hepatocytes and reported elevations in serum transaminases following a toxic overdose of acetaminophen.

Our study also shows that a single dose of acetaminophen (600mg/kg.bw. p.o.) resulted in a significant increase in liver content of MDA and NO_x and a marked decrease in hepatic content of GSH as compared to the normal group. This increased concentration of MDA in the liver of intoxicated rats suggests facilitated lipid peroxidation leading to tissue damage and failure of body's antioxidant defense mechanisms to prevent formation of excessive free radicals. It has been reported that acetaminophen caused significant increase in hepatic lipid peroxidation due to free radical injury in necrotic livers of rats[42]. Also inflammation activates Kupffer cells leading to release of numerous cytokines and signaling molecules including NO which react with superoxide anion generating peroxynitrite radicals causing further damage by oxidizing and nitrating cellular macromolecules and depleting GSH leading to high susceptibility to oxidative stress. The reduced amount of GSH in acetaminophen-treated rats is an obvious image of excessive formation of free radicals resulting in tissue damage. Several authors reported a significant change in hepatic malondialdehyde, NO and glutathione in rats subjected to acetaminophen hepatotoxicity[48,49,50,51].

Alongside; the lipid profile of our study revealed that a single dose of acetaminophen (600mg/kg.bw. p.o.) resulted in increase in total cholesterol and low density lipoproteins and decrease in high density lipoproteins. Acetaminophen produces metabolic changes in the liver, such as slight increase in fatty liver [52]. As mitochondrial β oxidation is the dominant oxidative pathway for the disposition of fatty acids under normal physiologic conditions[53], and mitochondrial dysfunction found in liver from patients treated with acetaminophen, a drug that inhibits mitochondrial respiratory chain activity and mitochondrial β oxidation. As the oxidative capacity of the mitochondria becomes impaired, cytosolic fatty acids accumulate [53].

Acetaminophen seems to cause impairment in lipoprotein metabolismand also alterations in cholesterol metabolism. The level of cholesterol and triglyceride were significantly increased in acetaminophen treated rats, when compared to control treated rats. That may be due to increased availability of free fatty acids, decreased hepatic release of lipoproteins and increase esterification of fatty acids[43].

One of the main important findings of our study that a single dose of acetaminophen (600mg/kg. bw. p.o.) showed a marked increase in serum level of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α). This comes in agreement with [54] who said that acetaminophen causes a massive production of intrahepatic TNF-α. Cytokines such as TNF-α is a key factor in liver inflammation so any increase in TNF-α is one of the early events in liver inflammation. Acute hepatitis due to viral, toxic, or autoimmune pathogenesis is characterized by an activation of macrophages and T cells with an increased production of cytokines that leads

to parenchymal liver damage and liver dysfunction[55]. Also elevated levels of proinflammatory factors such as proinflammatory cytokines IL-6 and TNF-α are found within the blood[56,55].

Besides; histopathological examinations were analogous to those of [57-59] who stated that liver injury was successfully induced by acute acetaminophen intoxication. After acetaminophen intoxicated dose and formation of NAPQI which act as a protein adduct factor, the initial stress of protein adduct formation is mitochondria. It is well established that the mitochondrial translocation of bax is a very early event. Bax together with Bak forms pores in the outer mitochondrial membrane that leads to the release of inter membrane proteins including cytochrome c, the second mitochondrial activator of caspase, endonuclease G and apoptosis-inducing factor which will translocate to the nucleus and contribute to the characteristic nuclear DNA fragmentation and cell death [60]

Vitamin C is an important free radical scavenger, trapping radicals and protecting biomembranes from peroxide damage as it effectively scavenges singlet oxygen, super-oxide, hydroxyl, water soluble peroxyl radical and hypochlorous acid. Also reported to be an excellent source of electrons and therefore can donate electrons to free radicals such as hydroxyl and super oxide radicals and quench their activity as an electron donor or reducing agent. Vitamin C has hepatoprotective effect which is attributed to its antioxidant property[23, 61]. Various studies approved the protective effects of vitamin C against acetaminophen induced hepatotoxicity vitamin C at a dose of 500 mg/ /kg body weight/ day for six successive days and orally given acetaminophen at a dose of 600 mg/kg body weight on the third and fourth days of administration[62, 63]. Orally dosed with single, daily 100, 200 and 500 mg/kg of ascorbic acid, respectively, 1 hour before intraperitoneal injection of 200 mg/kg acetaminophen for 14 days[64]. Moreover; vitamin C administration was Previously reported to provide protection against cyclosporine A -induced injury in rat liver function when (100mg/mL, 200 mg/kg/day) solution was given orally[65]. [66]Furthermore; [67]reported that vitamin C normalized levels of ALT, AST, alkaline phosphatase, blood hydro peroxide and MDA in liver of carbon tetrachloride in-toxicated rats. These advantageous effects increases when vitamin C is co-administered with other agents precisely antioxidants[23].

L-Carnitine is one of the nowadays nutraceuticals that has pleiotropic physiologic actions. L-Carnitine showed free radical scavenging activity and could also improve antioxidant status[68]. As L-Carnitine improves mitochondrial abnormalities associated with acetaminophen-induced liver injury in a rat model[40] , so providing such a compound which serves as a biofuel for mitochondria as well as antioxidant might preserve their bioenergetics and protects against acetaminophen overdose and since the chain reactions of acetaminophen generate free radicals we also used vitamin C as a potent antioxidant.

L-Carnitine had been used at the doses of 500, 300, 250, 200, 125, 100, and 50 mg/kg [69,42,70,71,72,73,74]. Carnitine administration prior to acetaminophen injection was reported to lower the enzyme activities of AST, ALT. It also reduced the serum TNF-α level, NO_x and liver MDA concentration and increased GSH content, also; the degree of pathologic alterations was less severe[25,75,40,69]. In non-alcoholic steatohepatitis patients, L-Carnitine reduced plasma activities of AST, ALT and inflammatory markers[76]. In addition, it enhances liver regeneration in rats after hepatectomy[70], also increase mitochondrial work action, decreasing apoptotic markers like caspase and bax[77]. Administration of carnitine may shift the metabolic bias of the liver away from esterification and synthesis of triglycerides toward the formation of acetyl-carnitines. This could decrease synthesis of triglycerides and VLDL also significantly decreased cholesterol with a significant decrease in LDL-Cholesterol concentrations and a significant increase in HDL-Cholesterol that indicate that this compound lessens oxidative stress in humans by increase mitochondrial b-oxidation of fatty acids[24, 76, 78].

In the present study, pretreatment with vitamin C (100mg/kg p.o. for 21 day) plus L-Carnitine (25mg/kg p.o. for 21 day) or vitamin C (100mg/kg p.o. for 21 day) plus L-Carnitine (50mg/kg p.o. for 21 day) significantly attenuated the elevation in serum ALT, AST, NO_x, LDL-Cholesterol, cholesterol, Il-6, TNF-α and MDA and increased the level of GSH and HDL-Cholesterol.

In conclusion, we can suggest that combination of Vitamin C and L-Carnitine could be favorable in ameliorating the hepato-deleterious effects induced by overdose of acetaminophen.

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