Melatonin Versus N-Acetylcysteine In Acetaminophen Induced Hepatotoxicity & Nephrotoxicity and in Metabolic Syndrome (Experimental Study)

Samah B. Ebaed, Sherif A. Shaltoot, Mary Boutros, Ahmed S. Mohamed, Mohanad M. Shehab.

Department of Pharmacology & Therapeutics, Benha Faculty of Medicine, Benha University, Egypt.
samahbahi2005@yahoo.com

Abstract: Acetaminophen is a widely used over-the-counter analgesic and antipyretic drug. Although considered safe at therapeutic doses, at higher doses, acetaminophen produces hepatotoxicity and nephrotoxicity. The present study is divided into two main parts: part I is devoted to investigate the contribution of free radical formation in pathogenesis of the main pathological conditions associated with acute overdose of single dose of acetaminophen (900 mg IP) in mice namely hepatotoxicity and nephrotoxicity after 4 and 24 hours of acetaminophen administration and the possible protective effect of single dose of melatonin 10 mg/kg i.p or single dose of N-acetylcysteine150mg/kg i.p or in combination. Metabolic syndrome (MetS) is a cluster of pathophysiological abnormalities including obesity, insulin resistance, impaired glucose tolerance, dyslipidemia and high blood pressure. Part II of the present study was designed to investigate the implication of free radical formation in pathogenesis of experimentally fructose induced metabolic syndrome in rats as well as the possible protective effect of melatonin (25ug/ml/ day) versus N-acetylcystein (2g/kg/day). The obtained results in the current work in part I revealed that induction of hepatoxicity and nephrotocity by acetaminophen resulted in significant increase of the oxidative stress with increase in the hepatic and renal functions while administration of single dose of melatonin or N-acetylsysteine decrease the oxidative stress parameters and decrease both hepatic and renal function while combination between both drugs give significant improvement rather than each drug alone. Result of part II revealed that induction of metabolic syndrome by 10% fructose for 8 weeks resulted in significant increase body weight, arterial blood pressure, insulin level, fasting blood glucose, HOMA-IR index and lipid profiles at the end of the study, while administration of melatoninor N-acetylcysteine significantly decrease all metabolic parameters with the upper hand to melatonin.

[Samah B. Ebaed, Sherif A. Shaltoot, Mary Boutros, Ahmed S. Mohamed, Mohanad M. Shehab. **Melatonin Versus N-Acetylcysteine In Acetaminophen Induced Hepatotoxicity & Nephrotoxicity and in Metabolic Syndrome(Experimental Study).** *J Am Sci* 2016;12(4):88-103]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 12. doi:10.7537/marsjas12041612.

Keywords: acetaminophen; glutathione; lipid peroxidation; metabolic syndrome; fructose; dyslipidemia; hypertension; obesity; melatonin, N-acetylcysteine

1. Introduction:

Acetaminophen (paracetamol, N-acetyl-p-aminophenol; APAP) (AA) is an analgesic and antipyretic effects similar to those of aspirin and ibuprofen. Unlike these other drugs, acetaminophen has only weak anti-inflammatory properties **(Jaeschke and Bajt, 2006).** According to the US Food and Drug Administration, each week approximately 50million adults in the United States take acetaminophen-containing products **(*Litovitz et al.*, 2005).**

Although considered safe at therapeutic doses, at higher doses, acetaminophen produces a centrilobular hepatic necrosis that can be fatal. Acetaminophen poisoning accounts for approximately one-half of all cases of acute liver failure in the United States and Great Britain today. The direct costs of acetaminophen overdose have been estimated to be as high as US$87 million annually **(Larson *et al.*, 2005).**

Renal insufficiency occurs in approximately 1–2% of patients with APAP overdose. Data in a retrospective case series of pediatric patients with acetaminophen poisoning suggests that associated nephrotoxicity may be more common in children and adolescents **(Boutis and Shannon, 2005).**

Glutathione (GSH) is one of the most important molecules in the cellular defense against chemically reactive toxic compounds or oxidative stress. At sufficiently high doses of AA, GSH becomes depleted, leaving NAPQI free to bind to possibly critical cellular proteins and to cause hepatic necrosis. The toxicity of AA is, therefore, a function of the amount of NAPQI formed and the availability of hepatic GSH for detotixification of this toxic metabolite **(Dimova *et al.*, 2007).**

APAP may also cause hepatotoxicity by mechanisms leading to the formation of reactive oxygen species (ROS), such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (HO), reactive nitrogen species (RNS), such as nitric

oxide and peroxynitrite (ONOO-), and eroxidation reaction products **(Reid *et al.*, 2006).**

Eventually, oxidative damage causes an increase in tissue levels of malondialdehyde (MDA) which are the end products of lipid peroxidation **(Erdemir *et al.*, 2011).** Current evidence suggests that oxidative stress with increased generation of reactive oxygen species, depletion of reduced glutathione (GSH) and lipid peroxidation play a crucial role in the development of acetaminophen-induced renal damage **(Liu *et al.*, 2011).**

Metabolic syndrome(MetS) is a cluster of pathophysiological abnormalities including obesity, insulin resistance, impaired glucose tolerance, dyslipidemia and high blood pressure **(Cai and Liu, 2012).** Among adults, the prevalence of MetS was recently estimated at 30% in the US, whereas a recent study using National Health and Nutrition Examination Survey (NHANES) 2001–2006 data collected on adolescents estimated MetS prevalence at 6.9% among boys and 3.9% among girls based on National Cholesterol Education Program Adult Treatment Panel III criteria **(Ganji *et al.*, 2011).**

Although it is generally accepted that the main pathogenic mechanism underlying the first level of metabolic changes in patients with the metabolic syndrome relies on insulin resistance, an abundance of evidence demonstrating a close link among the metabolic syndrome, a state of chronic low-level inflammation and oxidative stress as second-level abnormalities had emerged **(Bonomini *et al.*,2015).**

Melatonin is synthesized in the pineal gland **(Reiter, 1991c).** After entering circulation melatonin acts as endocrine factor and a chemical messenger of light and darkness (circadian and circannual pacemaker) (**Reiter *et al.*, 2001)**. Melatonin receptors, MT1 (Mel1A) and MT2 (Mel1B), were cloned from humans **(Dubocovich *et al.*, 1998, 2010).** In vertebrates, the roles of melatonin are numerous and include: regulating circadian rhythm and acting as a neuromodulator, hormone, cytokine and biological response modifier **(Man *et al.*, 2011).**

It also affects brain, immune, gastrointestinal, cardiovascular, renal, bone and endocrine functions, and acts as an oncostatic and anti-aging molecule **(Leja-Szpak *et al.*, 2010; Celinski *et al.*, 2011).**In the gut it seems that melatonin plays significant roles in regulating intestinal motility, the immune system, GI secretion, and the release of peptides involved in energy balance such as peptide YY **(Aydin *et al.*, 2008**). Melatonin was also shown to protect the colon in different pathophysiological conditions; frequently these protective effects involve activation of antioxidative mechanisms.

Melatonin also acts through non-receptor mediated mechanisms, for example serving as a scavenger for reactive oxygen species and reactive nitrogen species **(Gomez-Moreno *et al.*, 2010)**. Melatonin, in addition to being a broad-spectrum antioxidant, can also activate cytoprotective enzymes **(Rodriguez *et al.*, 2004).** At both physiological and pharmacological concentrations melatonin attenuates or counteracts oxidative stress, and regulates cellular metabolism **(Korkmaz *et al.*, 2009**). In addition to these direct interactions with ROS, melatonin may induce up regulation of the activity of antioxidants and antioxidant enzymes, such as superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx) and glutathione reductase (GSR), in the environment of oxidative stress **(Tomás-Zapico and Coto-Montes, 2005).**

N-acetylcysteine (NAC) is a low molecular weight compound (163.2 g/mol) administered to neutralize the deleterious effects of free radicals. NAC raises the intracellular concentration of cysteine and hence of reduced glutathione (GSH), which acts as an important endogenous antioxidant. Moreover, NAC has direct scavenging properties in vitro against hydroxyl radicals and hypochlorous acid **(Brunet *et al.*, 2006).**

N-acetylcysteine has an anti-inflammatory and antioxidant properties already used in the clinical setting, for example in acetaminophen intoxication, idiopathic pulmonary fibrosis, bronchitis, ischemia-reperfusion injury, cardiac injury and doxorubicin cardiotoxicity **(Samuni *et al.*, 2013).**

Animals and Experimental Design

Mice: 42 male, adult locally breading mice, weighing between 25-30g at the beginning of the study were used. Rats: 30 male adult albino rats, weighing 180-250g at the beginning of the study were used. They were obtained from animal house of college of veterinary medicine, Benha University. Before starting the experiment all animals were left for 1 week to acclimatize. They were caged (6 per cage) in fully ventilated room at room temperature in pharmacology department, Benha faculty of Medicine. They were allowed free access to food (balanced diet) and water. They were used for vivo experiments.

Part I:

Animal groups: each group contained 6 rats.

Group (I): The control group receive distilled water intraperitoneal in comparative volume to the tested drugs (n=6). **Group (II):** (acetaminophen-4h): In this group, acetaminophen toxicity was induced in mice by single dose of acetaminophen 900mg/kg intraperitoneal (i.p) (n=6). **Group (III)**: (acetaminophen+melatonin-4 h): In this group melatonin (10 mg/kg i.p) was administrated 30 minutes before induction of acetaminophen toxicity. **Group (IV):** (acetaminophen+N-acetylcysteine-4h):

In this group N-acetylcysteine (150mg/kg i.p) was administrated 30 minutes before induction of acetaminophen toxicity. **Group (V):** (acetaminophen+ melatonin +N-acetylcysteine-4h): Both were administrated 30 minutes before induction of acetaminophen toxicity. After 4 hours of acetaminophen administration, all animals were sacrificed by decapitation 1 ml blood sample was collected from the site of decapitation and from puncture of the retrobulbar sinus in glass test tubes. All animals were dissected for liver and kidney. The dissected organs were washed by saline. One kidney and 1 g piece of liver were preserved in 10% formalin to be used for histopathology. 0.2 g pieces of liver and kidney were mixed with 5 ml of phosphate buffer; they were used for formation of tissue homogenates for determination of tissue GSH, MDA. **Group VI:** (acetaminophen-24h): Animals will be given the same drug as group II. **Group VII**: (Acetaminophin+ melatonin-24h): Animals will be given the same drugs as group III. **Group VIII**: (Acetaminophen+N-acetylcysteine-24h): Animals will be given the same drugs as group IV. **Group IX:** (Acetaminophen+ melatonin +N-acetylcysteine-24h): Animals will be given the same drugs as group V. Animals of groups VI &VII & VIII&IX were subjected to the same treatments as previously described in groups II&III &IV &V except that the obtaining of blood samples and scarification were performed after 24 hours of acetaminophin administration.

2. Parameters of the experiment: Serum aspartate aminotransferase (AST)(U/L), serum alanine aminotransferase (ALT)(U/L), Blood urea nitrogen (BUN) (mg/dl), creatinine concentrations(mg/dl), Measurement of hepatic & renal tissue homogenate content of malondialdehyde (MDA) (nmol/g.tissue) &glutathione(GSH)(mmol/g.tissue),
histopathological examination of the liver, kidney sections.

Part II:

Group (I): Normal control group: rats were allowed free access to standard animal chow and tap water for 8 weeks. **Group (II):** (Fructose-induced metabolic syndrome): This group received 10% fructose in drinking solution for 8 weeks. **Group (III):** (melatonin- treated group): This group received melatonin (25 ug/ml/ day) with 10% fructose in the drinking solution for 8 weeks. **Group (IV):** (N-acetylcysteine-treated group): This group received N-acetylcysteine (2g/kg/day in drinking solution) with 10% fructose in the drinking solution for 8 weeks. The body weight of the rats was measured at the start and at the end of the experiment (8 weeks), at the end of the 8 weeks blood pressure was measured for all rats. In the following morning, rats were over night fasted for12-hours all animals were anesthetized with diethyl ether inhalation and 5 ml blood sample was obtained from retroorbital plexus.

Parameters of the experiment: Measurement of body weight (g), systolic & diastolic arterial blood pressure (mmHg), fasting serum insulin (µIU/mL), fasting blood glucose levels (F.B.G) (mg/dl), Calculation of HOMA IR index, cholesterol level (mg/dL), triglyceride level (mg/dL), high density lipoprotein (HDL) (mg/dL), low density lipoprotein (LDL) (mg/dL), in the pancreatic tissue homogenate of malondialdehyde& reduced glutathione.

Statistical analysis:

Data are presented as Mean □□SE and analyzed by one way ANOVA followed by Tuckey Kramer post –test using Graph Pad prism software. The p value of less than 0.05 was considered to be statistically significant.

3.Results:

Result of part I:

Data in table (1,2): Showed that intraperitoneal injection of single dose acetaminophen (900 mg/kg) resulted in deterioration of the liver functions evidenced by significant increase in the serum concentration of AST & ALT at 4h, 24h from acetaminophen administration concomitant with a significant rise of the oxidative stress evidenced by significant elevation in the malondialdehyde (MDA) hepatic tissue homogenate with deterioration of the level of natural antioxidant activity in the liver as evidenced by significant decrease in the reduced glutathione (GSH) in comparison to control group. Administration of single dose of melatonin (10 mg/kg i.p) or N-acetylcystein (150 mg/kg i.p) 30min before induction of acetaminophen hepatoxicity partially improved acetaminophen hepatotoxicity as evidenced by significant reduction of AST, ALT with significant reduction of the oxidative activity evidenced by significant reduction in MDA concentration and significant elevation of the GSH at 4h &24h from acetaminophen administration in comparison to acetaminophen non- treated group but without significance difference between both drugs. Pretreatment of acetaminophen non treated group with combination of both drugs melatonin (10 mg/kg i.p) +N-acetylcysteine (150 mg/kg i.p) 30 min before giving them a single injection of AA significantly reduced AST, ALT, MDA concentration with significant elevation of the GSH in comparison to treatment with each drug alone.

Data in table (3): Showed the effect of single intraperitoneal injection of acetaminophen (900 mg/kg) on average concentration of serum concentration (AST, ALT), (MDA& GSH) in the hepatic tissue homogenate in normal adult mice at 4h, 24h of acetaminophen administration which revealed that acetaminophen induced hepatotoxicity is more prominent after 4 hours and relatively ameliorated after 24 hours in untreated acetaminophen administrated mice.

Data in table (4,5): Showed that intraperitoneal injection of single dose acetaminophen (900 mg/kg) resulted in deterioration of the kidney functions evidenced by significant increase in the serum concentration of urea & creatnine at 4h, 24h from acetaminophen administration concomitant with a significant rise of the oxidative stress evidenced by significant elevation in the malondialdehyde (MDA) renal tissue homogenate with deterioration of the level of natural antioxidant activity in the kidney as evidenced by significant decrease in the reduced glutathione (GSH) renal tissue homogenate in comparison to control group. Administration of single dose of melatonin (10 mg/kg i.p) or N-acetylcystein (150 mg/kg i.p) 30min before induction of acetaminophen nephrotoxicity partially improved acetaminophen nephrotoxicity as evidenced by significant reduction of urea, creatnine concentrations with significant reduction of the oxidative activity evidenced by significant reduction in MDA concentration and significant elevation of the GSH concentration at 4h &24h from acetaminophen administration in comparison to acetaminophen non-treated group but without significance difference between both drugs. Pretreatment of acetaminophen non treated group with combination of both drugs melatonin (10 mg/kg i.p) + N-acetylcysteine (150 mg/kg i.p) 30 min before giving them a single injection of AA significantly reduced urea, creatnine, MDA concentration with significant elevation of the GSH in comparison to treatment with each drug alone.

Data in table (6): Showed the effect of single intraperitoneal injection of acetaminophen (900mg/kg) on average concentration of serum (urea &creatnine),(MDA&GSH)in renal tissue homogenate in normal adult mice at 4h, 24h after AA administration which revealed that on the contrary to acetaminophen induced hepatotoxicity nephrotoxicity was more prominent after 24h than 4h of drug administration.

Result of part II:

There were insignificant changes in the body weight during acclimatization period among all animals participating in this experiment. The average total body weight of the normal control animals was 203.3±25.8g. This value was not changed significantly neither by induction of metabolic syndrome by administration of 10% fructose in drinking water for 8 weeks nor by administration of the tested drugs(figure 17).

Data in table (7): Induction of metabolic syndrome significantly elevated SBP, DBP. Treatment with melatonin (25ug/ml/day) normalized the blood pressure; On the other hand, N-acetylcysteine treatment (2g/kg/day) partially improved the hypertension induced by fructose administration. Comparing the effect of melatonin and N-acetylcysteine on fructose induced hypertension revealed that the former is more potent in ameliorating fructose induced systolic hypertension by significant value of 6.4%

Data in table (8):showed that administration of (10% L-fructose solution in drinking water for 8 weeks resulted in significant increase in serum insulin level, fasting blood glucose with consequence increase in the HOMA-IR index which significantly decreased after administration of melatonin (25ug/ml/day) or N-acetylcysteine (2g/kg/day). This indicate that either melatonin or N-acetylcysteine partially ameliorated the hyperinsulimeia and hyperglycemia induced by fructose administration without significant difference between them.

Data in table (9): showed that administration of (10% L-fructose solution in drinking water for 8 weeks resulted in significant increase in the lipid profiles. Either melatonin or N-acetylcysteine treatment partially improved lipid profile without significant difference between them.

Data in table (10): showed that administration of (10% L-fructose solution in drinking water for 8 weeks resulted in significant rise of pancreatic tissue homogenate (MDA) with decrease in pancreatic tissue (GSH). Melatonin or N-acetylcysteine administration decrease the pancreatic concentration of MDA significantly while they increase glutathione significantly in compared to non-treated fructose group without significance difference to the normal values or between both drugs.

Table (1): Effect of prophylactic single dose of melatonin (10 mg/kg i.p) and N-acetylcysteine (150mg/kg i.p) either singly or in combination on serum concentration of (aspartate & alanine transaminase), hepatic tissue homogenate (malondialdehyde& reduced glutathione) 30 min before induction of hepatotoxicity by single dose of acetaminophen (900 mg/kg i.p) in normal adult mice at 4h after acetaminophen administration (n=6):

Parameter / Group	AST(U/L) (Mean ± SE)	ALT (U/L) (Mean± SE)	MDA in the hepatic tissue homogenate (nmol/g.tissue) (Mean ± SE)	GSH in the hepatic tissue homogenate (mmol/g.tissue) (Mean ± SE)
Control group	109.2±3.5	47.3±3.2	1.4±0.07	37.8±1.2
Acetaminophen non-treated group. % Change to control group.	388.8±29.4* ↑256.1%	130.5±18.3* ↑175.9%	8.7±0.8* ↑521.4%	13.8±1.2* ↓63.5%
Melatonin treated group. % Change to control group % Change to acetaminophen non- treated group.	259.7±4.1* + ↑137.8% ↓33.2%	81.7±4.9* + ↑72.7% ↓37.4%	5.1±0.7*+ ↑264.3% ↓41.4%	20.7±1.4* + ↓45.2% ↑50%
N-acetylcysteine treated group. % Change to control group. % Change to acetaminophen non- treated group. % Change to melatonin treated group.	264.7±12.6*+ ↑142.4% ↓31.9% ↑1.9%	82.7±4.3* + ↑74.8% ↓36.6% ↑1.2%	6.1±0.6* + ↑335.7% ↓29.9% ↑19.6%	24.3±0.8*+ ↓35.7% ↑76.1% ↑17.4%
Melatonin+ N-acetylcysteine treated group. % Change to control group. % Change to acetaminophen non- treated group. % Change to melatonin treated group. % Change to N-acetylcysteine treated group.	232.2±6.9*+ ¥£ ↑112.6% ↓40.3% ↓10.6% ↓12.3%	59.8±3.9*+ ¥£ ↑26.4% ↓54.2% ↓26.8% ↓27.7%	3.1±0.5*+ ¥ £ ↑121.4% ↓64.4% ↓39.2% ↓49.2%	29.5±1.1* + ¥ £ ↓22% ↑113.8% ↑42.5% ↑21.4%

Data represented as Mean ± SE (n = 6):
* Significant difference with control group at p<0.05.
+ Significant difference with acetaminophen non-treated group at p<0.05.
¥ Significant difference with melatonin treated group at p<0.05.
£ Significant difference with N-acetylcysteine treated group at p<0.05.

Table (2): Effect of prophylactic single dose of melatonin (10mg/kg i.p) and N-acetylcysteine (150mg/kg i.p) either singly or in combination on serum concentration of (aspartate & alanine transaminase), on hepatic tissue homogenate (MDA& GSH) 30 min before induction of hepatotoxicity by single dose of acetaminophen (900 mg/kg i.p) in normal adult mice at 24h after acetaminophen administration (n=6):

Parameter / Group	**AST(U/L) (Mean±SE)**	**ALT (U/L) (Mean±SE)**	**MDA in the hepatic tissue homogenate (nmol/g.tissue) (Mean±SE)**	**GSH in the hepatic tissue homogenate (mmol/g.tissue) (Mean±SE)**
Control group	109.2±3.5	47.3±3.2	1.4±0.07	37.8±1.2
Acetaminophen non-treated group. % Change to control group.	285.3±39.7* ↑161.3%	106.8±5.2* ↑125.8%	3.9±0.15* ↑178.6%	20.3±1.6* ↓46.3%
Melatonin treated group. % Change to control group % Change to acetaminophen non- treated group.	133.8±4.02* + ↑22.5% ↓53.1%	67±4.3* + ↑41.7% ↓37.3%	2.3±0.13*+ ↑64.3% ↓41.1%	29.3±1.2* + ↓22.5% ↑44.3%
N-acetylcysteinetreated group. % Change to control group. % Change to acetaminophen non- treated group. % Change to melatonin treated group.	135.7±3.9* + ↑24.3% ↓52.4% ↑1.4%	68.8±4* + ↑45.5% ↓35.6% ↑2.7%	2.7±0.14* + ↑92.9% ↓30.8% ↑17.4%	33.3±2.2* + ↓11.9% ↑64% ↑13.7%
Melatonin+ N-acetylcysteinetreated group. % Change to control group. % Change to acetaminophen non- treated group. % Change to melatonin treated group. % Change to N-acetylcysteinetreated group.	117.2±6.8+ ¥ £ ↑7.3% ↓58.9% ↓12.4% ↓13.6%	54±3.9+ ¥ £ ↑14.2% ↓49.4% ↓19.4% ↓21.5%	1.5±0.15+ ¥ £ ↑7.1% ↓61.5% ↓34.8% ↓44.4%	36±1.4+ ¥ £ ↓4.8% ↑77.3% ↑22.9% ↑8.1%

Data represented as Mean ± SEM (n = 6)
***** Significant difference with control group at *p*<0.05.
+ Significant difference with acetaminophen administrated group at *p*<0.05.
¥ Significant difference with melatonin treated group at *p*<0.05.

£ Significant difference with N-acetylcysteine treated group at $p<0.05$.

Table (3): Effect of single intraperitoneal injection of acetaminophen (900 mg/kg) on average (Mean ± SE) concentration of serum concentration of (AST& ALT), (MDA & GSH) in the hepatic tissue homogenate in normal adult mice at 4h, 24h of acetaminophen administration:

Parameter / Group/Time	AST (U/L) (Mean ± SE)	ALT (U/L) (Mean ± SE)	MDA in the hepatic tissue homogenate (nmol/g.tissue) (Mean ± SE)	GSH in the hepatic tissue homogenate (mmol/g.tissue) (Mean ± SE)
Control group	109.2±3.5	47.3±3.2	1.4±0.07	37.8±1.2
Acetaminophen non-treated group at 4h of acetaminophen administration.	388.8±29.4*	130.5±7.3*	8.7±0.8*	13.8±1.2*
% Change to control group	↑256.1%	↑175.9%	↑521.4%	↓63.5 %
Acetaminophen non -treated group at 24h of acetaminophen administration.	285.3±19.7*$	106.8±5.2*$	3.9±0.15*$	20.3±1.6*$
% Change to control group.	↑161.3%	↑125.8%	↑178.6%	↓46.3%
% Change to acetaminophen non-treated group at 4h of acetaminophen administration	↓26.6%	↓18.2%	↓55.2%	↑47.1%

Data represented as Mean ± SE (n = 6)

***** Significant difference with control group at p<0.05.

$ Significant difference with acetaminophen non- treated group in which parameters measured after 4h from acetaminophen administration at p<0.05.

Histological examination of the hepatic tissue of different groups:

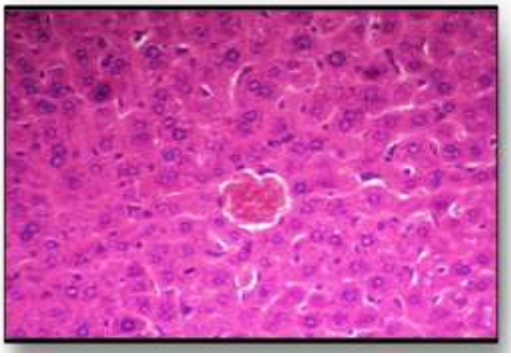

Figure (1): A photomicrograph of asection in the liver of a control mice showing central veins with columns of normal hepatocytes having abundant esinophilic cytoplasm, central rounded nuclei separated by blood sinusoids) (H&Ex40)

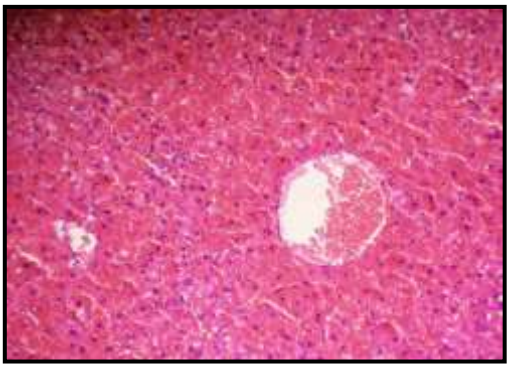

Figure (2): A photomicrograph of a section in the liver of acetamenophin toxicity group after 4h from acetaminophen administration in a dose of (900mg/kg/i.p) showing extensive central vein dilation and congestion, inflammatory foci, hemorrhage (H&E X20).

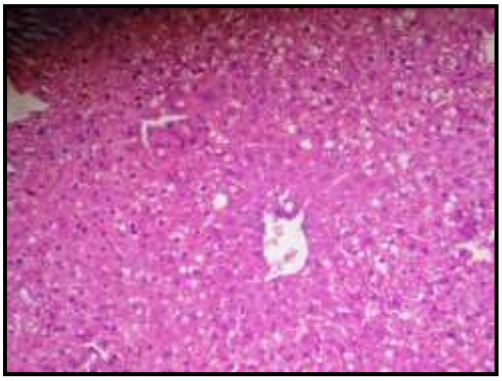

Figure (3): A photomicrograph of a section in the liver of melatonin treated group after 4h from acetaminophen administration in a dose of (900mg/kg/i.p) showing decreased in the central vein congestion, no hemorrhage, decreased degeneration of hepatocytes, no inflammatory foci (H&E X20).

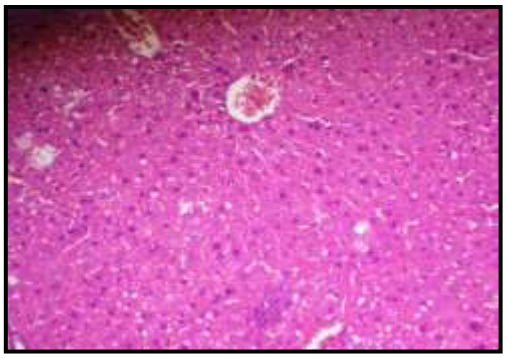

Figure (4): A photomicrograph of a section in the liver of N-acetylcystein treated group after 4h from acetaminophen administration in a dose of (900mg/kg/i.p) showing moderate central vein congestion, no hemorrhage, decreased degeneration of hepatocytes &minimal inflammatory foci (H&E X20).

Figure (5): A photomicrograph of a cut section in the liver of melatonin + N-acetylcystein treated group after 4h from acetaminophen administration in a dose of (900mg/kg/i.p) showing mild central vein congestion, no hemorrhage, minimal degeneration of hepatocytes & minimal inflammatory foci (H&E X20).

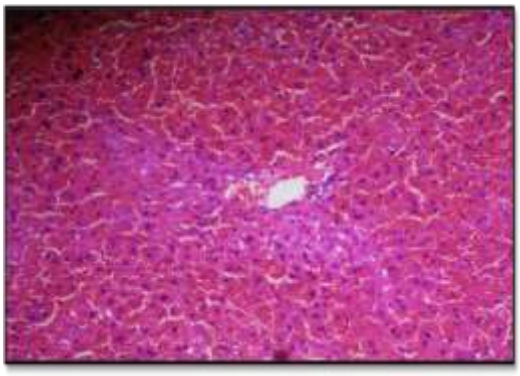

Figure (6): A photomicrograph of a section in the liver of acetamenophin toxicity group after 24h from acetaminophen administration in a dose of (900mg/kg/i.p) showing moderate central vein dilation and congestion, moderate amount of inflammatory foci, hemorrhage, degeneration of hepatocytes (H&E X20).

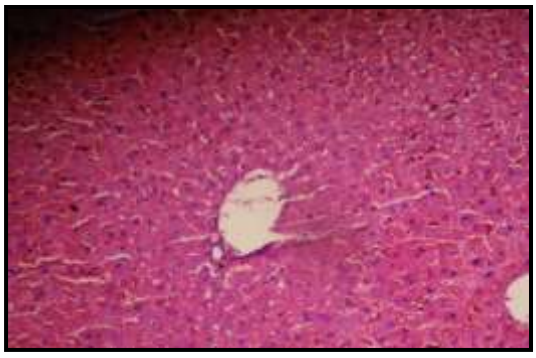

Figure (7): A photomicrograph of a section in the liver of melatonin treated group after 24h from acetaminophen administration in a dose of (900mg/kg/i.p) showing decreased central vein congestion, decreased hemorrhage), decreased degeneration of hepatocytes, no inflammatory foci(H&E X20).

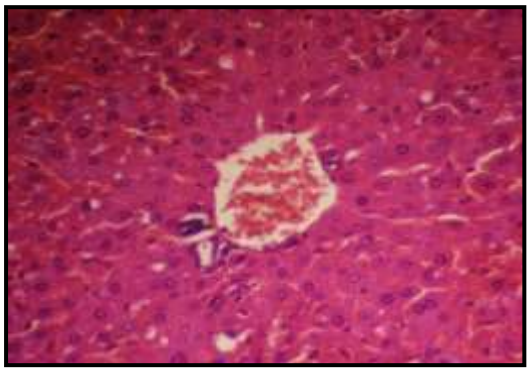

Figure (8): A photomicrograph of a section in the liver of N-acetylcystein treated group after 24h from acetaminophen administration in a dose of (900mg/kg/i.p)showing showing portal tract with decreased portal vein congestion, decreased hemorrhage, decreased degeneration of hepatocytes (H&E X40).

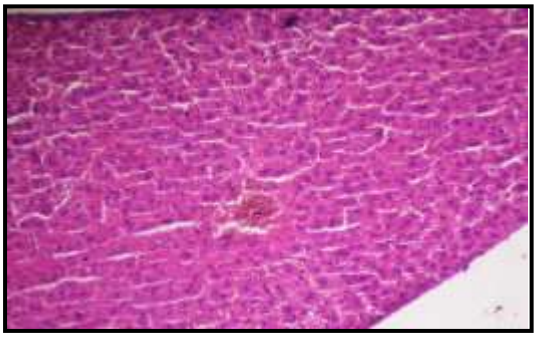

Figure (9): A photomicrograph of a section in the liver of melatonin +N-acetylcystein treated group after 24h from acetaminophen administration in a dose of (900mg/kg/i.p) showing minimal central vein congestion, no hemorrhage, minimal vacculation of hepatocytes & no inflammatory foci (H&E X20)

Histological examination of the renal tissue of different groups:

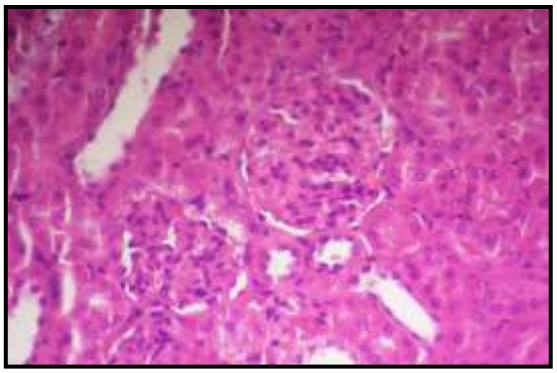

Figure (10): A photomicrograph of a transverse section in the kidney of a normal mice showing central glomeruli surrounded by Bowman's space, normal renal tubules lined by one layer of cuboidal epithelial cells (H&E X40).

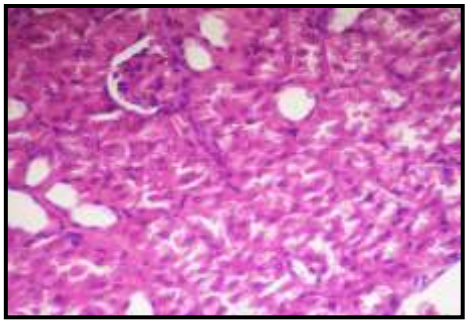

Figure (11): A photomicrograph of a transverses section in the kidney of acetaminophen toxicity group after 4h from acetaminophen administration in a dose of (900mg/kg/i.p) showing marked tubular vacculation (H&E X40).

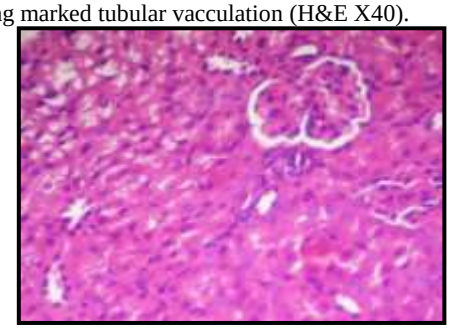

Figure (12): A photomicrograph of a transverse section in the kidney of melatonin treated group- after 4h from acetaminophen administration in a dose of (900mg/kg/i.p), showing decreased tubal vacculation (H&E X40).

Figure (13): A photomicrograph of a transverse section in the kidney of N-acteylcystein treated group after 4h from acetaminophen administration in a dose of (900mg/kg/i.p) showing mild decreased tubal vacculation & tubular necrosis (H&E X40).

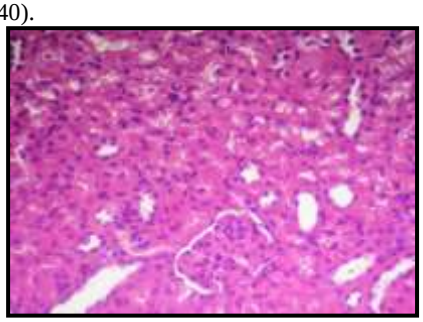

Figure (14): A photomicrograph of a tranverse section in the kidney of melatonin + N-acteylcystein treated group after 4h from acetaminophen administration in a dose of (900mg/kg/i.p) showing moderate decrease tubular vacculation (H&E X40).

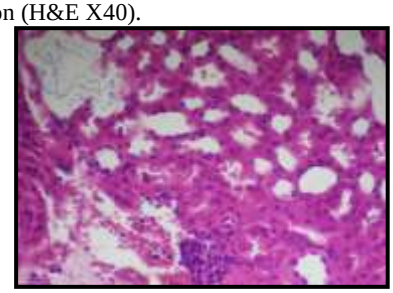

Figure (15): A photomicrograph of atransverse section in the kidney of acetaminophen toxicity group after 24h from acetaminophen administration in a dose of (900mg/kg/i.p) showing kidney with marked tubular necrosis &interstitial congestion & inflammation (H&E X40)

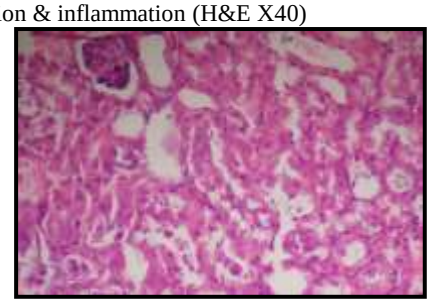

Figure (16): A photomicrograph of a transverse section in the kidney of melatonin treated group after 24h from acetaminophen administration in a dose of (900mg/kg/i.p) showing mild decreased tubal vacculation, tubular sloughing necrosis (B)(H&E X40)

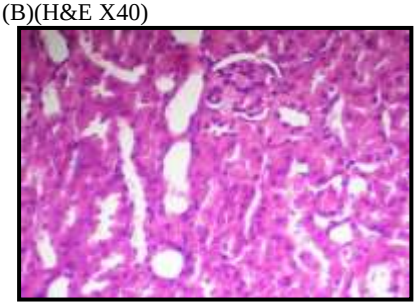

Figure (17): A photomicrograph of a transverse section in the kidney of N-acteylcystein treated group after 24h from acetaminophen administration in a dose of (900mg/kg/i.p) showing mild decreased tubal vacculation (A) (H&E X40)

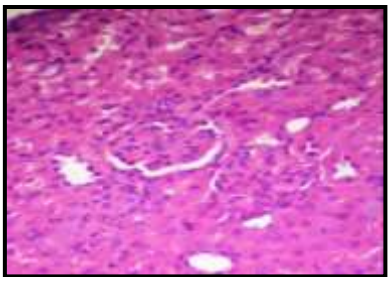

Figure (18): A photomicrograph of a transverse section in the kidney of melatonin + N-acteylcystein treated group after 24h from acetaminophen administration in a dose of (900mg/kg/i.p) showing marked decreased tubular vacculation, no congestion or inflammation (H&E X40)

Table (4): Effect of prophylactic single dose of melatonin (10 mg/kg i.p) and N-acetylcysteine (150mg/kg i.p) either singly or in combination on renal tissue homogenate (malondialdehyde& reduced glutathione), serum concentration of (urea &creatinine) 30min before induction of hepatotoxicity by single dose of acetaminophen (900 mg/kg i.p) in normal adult albino mice at 4h after acetaminophen administration (n=6):

Parameter / Group	Urea (mg/dl) (Mean ± SE)	Creatinine (mg/dl) (Mean ± SE)	MDA in the renal tissue homogenate (nmol/g.tissue) (Mean ± SE)	GSH in the renal tissue homogenate (mmol/g.tissue) (Mean ± SE)
Control group	37.5±2.9	0.43±0.02	0.99±0.09	17.5±1.1
Acetaminophen non-treated group.	71.2±3.5*	0.9±0.03*	2.9±0.11*	9.8±1.2*
% Change to control group.	↑89.9%	↑109.3%	↑192.9%	↓44%
Melatonin treated group.	55.2±4.5*+	0.55±0.03*+	1.9±0.07* +	12.2±0.6*+
% Change to control group	↑47.2%	↑27.9%	↑92%	↓30.3%
% Change to acetaminophen non- treated group.	↓22.5%	↓38.9%	↓34.5%	↑24.5%
N-acetylcysteine treated group.	59.3±5.5* +	0.58±0.02*+	2.1±0.05* +	14±0.9* +
% Change to control group.	↑58.1%	↑34.9%	↑112.1%	↓20%
% Change to acetaminophen non- treated group.	↓16.7%	↓35.6%	↓27.6%	↑42.8%
% Change to melatonin treated group.	↑7.4%	↑5.5%	↑10.5%	↑14.8%
Melatonin+ N-acetylcysteine treated group.	39.7±3.1+ ¥ £	0.45±0.01+ ¥ £	1.2±0.08+ ¥ £	17.2±0.98+ ¥ £
% Change to control group.	↑5.9%	↑4.7%	↑21.2%	↓1.7%
% Change to acetaminophen non- treated group.	↓44.2%	↓50%	↓58.6%	↑75.5%
% Change to melatonin treated group.	↓28.1%	↓18.2%	↓36.8%	↑41%
% Change to N-acetylcysteine treated group.	↓33.1%	↓22.4%	↓42.9%	↑7.5%

Data represented as Mean ± SEM (n = 6)

***** Significant difference with control group at $p<0.05$.

\+ Significant difference with acetaminophen administrated group at $p<0.05$.

¥ Significant difference with melatonin treated group at $p<0.05$.

£ Significant difference with N-acetylcysteine treated group at $p<0.05$.

Table (5): Effect of prophylactic single dose of melatonin (10 mg/kg i.p) and N-acetylcysteine (150mg/kg i.p) either singly or in combination on renal tissue homogenate (malondialdehyde& reduced glutathione), serum concentration of (urea &creatinine) 30min before induction of hepatotoxicity by single dose of acetaminophen (900 mg/kg i.p) in normal adult albino mice at 24h after acetaminophen administration (n=6):

Parameter / Group	Urea (mg/dl) (Mean ± SE)	Creatnine (mg/dl) (Mean ± SE)	MDA in the renal tissue homogenate (nmol/g.tissue) (Mean ± SE)	GSH in the renal tissue homogenate (mmol/g.tissue) (Mean ± SE)
Control group	37.5±2.9	0.43±0.02	0.99±0.09	17.5±1.1
Acetaminophen non-treated group.	95.7±13.5*	1.2±0.07*	3.8±0.12*	6±1.1*
% Change to control group.	↑155.2%	↑179.1%	↑283.8%	↓65.7%
Melatonin treated group.	64.2±3.9* +	0.64±0.01*+	2.5±0.19* +	11.7±1.2* +
% Change to control group	↑71.2%	↑48.8%	↑152.5%	↓ 33.1%
% Change to acetaminophen non- treated group.	↓32.9%	↓46.7%	↓34.2%	↑95%
N-acetylcysteinetreated group.	66.5±5.7* +	0.68±0.03* +	2.8±0.13* +	13.5±0.15* +
% Change to control group.	↑77.3%	↑58.1%	↑182.8%	↓22.9%
% Change to acetaminophen non- treated group.	↓30.5%	↓43.3%	↓26.3%	↑125%
% Change to melatonin treated group.	↑3.6%	↑6.3%	↑12%	↑15.4%
Melatonin+ N-acetylcysteinetreated group.	47.1±3.3*+ ¥ £	0.56±0.02*+ ¥ £	1.6±0.1*+ ¥ £	15.3±0.88* + ¥ £
% Change to control group.	↑25.6%	↑30.2%	↑61.6%	↓12.6%
% Change to acetaminophen non- treated group.	↓50.8%	↓53.3%	↓57.9%	↑155%

% Change to melatonin treated group.	↓26.6%	↓12.5%	↓36%	↑30.8%
% Change to N-acetylcysteinetreated group.	↓29.2%	↓17.6%	↓42.9%	↑13.3%

Data represented as Mean ± SEM (n = 6)
***** Significant difference with control group at $p<0.05$.
+ Significant difference with acetaminophen administrated group at $p<0.05$.
¥ Significant difference with melatonin treated group at $p<0.05$.
£ Significant difference with N-acetylcysteine treated group at $p<0.05$.

Table (6): Effect of single intraperitoneal injection of acetaminophen (900 mg/kg) on average (Mean ± SE) concentration of malondialdehyde & reduced glutathione in renal tissue homogenate, serum concentration of urea &creatnin in normal adult albino mice at 4h, 24h after acetaminophen administration:

Parameter / Group/Time	Urea (mg/dL) (Mean ± SE)	Creatinine (mg/dL) (Mean ± SE)	MDA in the renal tissue homogenate (nmol/g.tissue) (Mean ± SE)	GSH in the renal tissue homogenate (mmol/g.tissue) (Mean ± SE)
Control group	0.99±0.09	17.5±1.1	37.5±2.9	0.43±0.02
Measured at 4h from the acetaminophen injection	2.9±0.11*	9.8±1.2*	71.2±3.5*	0.9±0.03*
% Change to control group	↑193%	↓44%	↑90%	↑110%
Measured at 24h from the acetaminophen injection	3.8±0.12*+	6±1.1*+	95.7±23.5*+	1.2±0.07*+
% Change to control group.	↑283.8%	↓65.7%	↑155.2%	↑179%
%Change to acetaminophen administrated group after 4h.	↑31.1%	↓38.8%	↑34.5%	↑33.4%

Data represented as Mean ± SE (n = 6)

* Significant difference with control group at p<0.05.

$ Significant difference with acetaminophen non-treated group in which parameters measured after 4h from acetaminophen administration at p<0.05.

Result of Part II:
1- The effect on body weight:

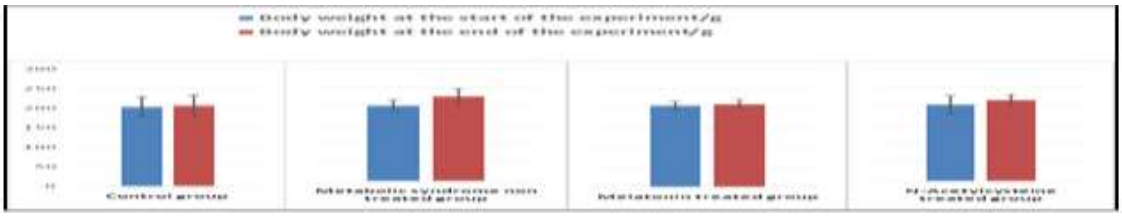

Figure (19): Showing the average (body weight /g) of the control group, metabolic syndrome induced group, melatonin treated group, N-acetylcysteine-treated group at the start and at the end of the experiment (after 8 weeks)

Table (7): Effect of melatonin (25ug/ml/day) and N-acetylcysteine (2g/kg/day) administration for 8 weeks on arterial blood pressure in fructose induced metabolic syndrome in rats by administration of (10%L-fructose solution in drinking water for 8 weeks).

Parameter / Groups	Systolic blood pressure (mmHg) (Mean ± SE)	Diastolic blood pressure (mmHg) (Mean ± SE)
Control group	116.2±5.4	78.3±5.7
Metabolic syndrome -non treated group	151.8±6.2*	108.7±9.5*
% Change to control group	↑30.6%	↑38.8%
Melatonin treated group	125.8±2.3+	88.2±5.1+
% Change to control group	↑8.3 %	↑12.6%
% Change to Metabolic syndrome non-treated group	↓17.1%	↓18.9%
N-acetycysteine treated group	133.8±2.04*+ ¥	89.7±4.5*+
% Change to control group	↑15.1%	↑14.6%
% Change to Metabolic syndrome non- treated group	↓11.9%	↓17.5%
% Change to melatonin treated group	↑6.4%	↑1.7%

Data represented as Mean ± SE (n = 6)

* Significant difference with control group at p<0.05
+ Significant difference with metabolic syndrome non treated group at p<0.05
¥ Significant difference with melatonin treated group p<0.05 -

Table (8): Effect of melatonin (25ug/ml/day) and N-acetylcysteine (2g/kg/day) administration for 8 weeks on hyperinsulinemia parameters in fructose-induced metabolic syndrome in rats by administration of (10%L-fructose solution in drinking water for 8 weeks).

Parameter / Group	Serum insulin level (µIU/ml) (Mean ± SE)	Fasting blood glucose (mg/dl) (Mean ± SE)	HOMA-IR Index (Mean ± SE)
Control group	37.7±0.8	77±2.1	7.2±0.4
Metabolic syndrome non- treated group	79±2.5*	152.2±4.7*	29.7±1.6*
% Change to control group	↑ 109.5%	↑ 97.7%	↑ 312.5%
Melatonin treated group	41.7±1.8* +	126.7±5.8*+	13.1±0.9*+
% Change to control group	↑10.6%	↑64.5%	↑81.9%
% Change to Metabolic syndrome non -treated group	↓ 47.2%	↓16.8 %	↓55.9%
N-acetycysteine treated group	44±2* +	136±4.8*+	14.8±0.4*+
% Change to control group	↑16.7%	↑76.6%	↑105.6%
% Change to Metabolic syndrome non- treated group	↓ 44.3%	↓10.6%	↓50.2%
% Change to melatonin treated group	↑5.5%	↑7.3%	↑13%

Data represented as Mean ± SEM (n = 6)

***** Significant difference with control group at p<0.05
+ Significant difference with metabolic syndrome non treated group at p<0.05
¥ Significant difference with melatonin treated group p<0.05 -

Table (9): Effect of melatonin (25ug/ml/day) and N-acetylcysteine (2g/kg/day) administration for 8 weeks on lipid profiles parameters in fructose- induced metabolic syndrome in rats by administration of (10% L-fructose solution in drinking water for 8 weeks).

Parameter / Group	Total cholesterol(mg/dl) (Mean±SE)	Triglyceride (mg/dl) (Mean±SE)	LDL (mg/dl) (Mean±SE)	HDL (mg/dl) (Mean±SE)
Control group	106.8±2.1	86.2±2.1	47.6±2.9	42±1.4
Metabolic syndrome -non treated group	229±3.2*	162.3±4.7*	147.4±3.9*	22.2±2.04*
% Change to control group	↑114.4%	↑ 88.3%	↑ 209.7%	↓ 47.1%
Melatonin treated -fructose administrated group	132.3±3.3*+	103.7±1.8* +	79.2±2.5* +	26.7±1.4* +
% Change to control group	↑23.9%	↑20.3	↑66.4%	↓36.4%
% Change to Metabolic syndrome non- treated group	↓42.2%	↓36.1%	↓46.3%	↑20.3%
N-acetycysteine treated -fructose administrated group	126.7±1.9*+	111.7±4.1* +	84.9±3.6*+	25.2±1.2*+
% Change to control group	↑18.6%	↑29.6%	↑78.4	↓40%
% Change to Metabolic syndrome non -treated group	↓44.7%	↓31.2%	↓42.4%	↑13.5%
% Change to melatonin treated - fructose administrated group	↓4.2	↑7.7%	↑7.2%	↓5.6%

Data represented as Mean ± SEM (n = 6)

* Significant difference with control group at p<0.05
+ Significant difference with metabolic syndrome non treated group at p<0.05
¥ Significant difference with melatonin treated group p<0.05 -

Table (10): Effect of melatonin (25ug/ml/day) and N-acetylcysteine (2g/kg/day) administration for 8 weeks on pancreatic tissue homogenate content of malondialdehyde and reduced glutathione in fructose- induced metabolic syndrome in rats by administration of (10%L-fructose solution in drinking water for 8 weeks).

Parameter / Groups	MDA in the pancreatic tissue homogenate (nmol/g.tissue) (Mean±SE)	GSH in pancreatic tissue homogenate (mmol/g.tissue) (Mean±SE)
Control group	7.8±1.2	1.4±0.2
Metabolic syndrome non- treated group	14.7±1.03*	0.8±0.1*
% Change to control group	↑88.5%	↓42.9%
Melatonin treated group	8.3±1.2+	1 ±0.2+
% Change to control group	↑ 6.4%	↓28.6%
% Change to Metabolic syndrome -non treated group	↓43.5%	↑25%

N-acetycysteine treated group	10.3±1.27+	1.3±0.2+
% Change to control group	↑32.1%	↓7.1%
% Change to Metabolic syndrome non-treated group	↓29.9%	↑62.5%
% Change to melatonin treated group	↑24.1%	↑30%

Data represented as Mean ± SE (n = 6)
***** Significant difference with control group at p<0.05
+ Significant difference with metabolic syndrome non treated group at p<0.05
¥ Significant difference with melatonin treated group p<0.05.

4. Discussion:

Excessive use of paracetamol can damage multiple organs, especially the liver and the kidney because the former is the site of formation of the toxic metabolites and the latter is the site of clearance ***(James et al., 2005).***

In the first part of this work, the effect of melatonin and N-acetylcysteine were investigated on acetaminophen hepatotoxicity and nephrotoxicity in male adult albino mice. Such experimental animal model was chosen in this work because it is available, cheap, easy to handle and more sensitive for induction of hepato& renal toxicity by acetaminophen than other animal models ***(Walker et al., 1975).*** Furthermore, recent work has shown that the mechanisms of AA toxicity in humans are similar to mice with early data demonstrated that rats are resistant to AA toxicity ***(McGill et al., 2012).***

The result in the first part of this study showed that the serum levels of the hepatic enzymes AST &ALT significantly increased following administration of toxic dose of acetaminophen (900 mg /kg i.p) as single dose, which demonstrating deterioration of the liver due to rupture of liver cells and release of such enzymes in blood stream. These results support the findings of several experimental studies demonstrating the hepatotoxic effect of acetaminophen on mice model ***(Carrasco et al., 2000; Abd-Allah et al., 2002*** and ***Ayman et al., 2003;*** and on humans as ***(Mahadevan et al., 2008).***

Hepatoxicity is believed to involve reaction between the unconjugated NAPQI and the critical cellular components namely proteins, DNA, membrane lipids after saturation of the glucuronide pathway and depletion of the glutathione reserve ***(Kala et al., 2015).***

APAP-induced liver damage might involve free radicals. Several studies have indicated that APAP might induce oxidative injury, including tissue lipid peroxidation, enzyme inactivation, and changes in cellular non-enzymatic and enzymatic antioxidant defense systems and glutathione (GSH) status ***(Gu et al., 2006).***

The above mentioned changes in the hepatic biochemical parameters in non treated acetaminophen administrated mice were associated with significant elevation of malondialdehyde and reduction of reduced glutathione level in hepatic tissue homogenate. Such changes clearly reflect a state of oxidative stress in the context of reduction of natural antioxidant and elevated reactive free radicals ***(Negre-Salvayreet al., 2010).***

This result in line with ***Abd-Allah et al. (2002)*** and ***Ayman et al. (2003)*** who demonstrated the elevated level of MDA in paracetamol hepatotoxicity and ***Reid et al. (2006)*** in which levels of lipid peroxidation products were increased from 40 to 100% above the basal values in the acetaminophen toxicity.

The deterioration of the liver is supported in this work by the microscopic pictures which showed that administration of toxic dose of acetaminophen produced histological picture similar to that of acute hepatitis. It caused profound hydropic degeneration of hepatocytes with severe congestion in the central vein with multiple areas of hemorrhage and lymphocytic infiltration. This was in line with ***Moffit et al. (2007); Arakawa et al. (2012); Zhang et al.(2013); Naguib et al.(2014).***

It is worthy to mention that acetaminophen induced hepatotoxicity is more prominent after 4 hours and relatively ameliorated after 24 hours in untreated acetaminophen administrated mice. This may imply that acetaminophen hepatotoxicity depends mainly on dose of acetaminophen which is converted to its main toxic metabolite NAPQI within short time after its administration in toxic dose. It is spontaneously

partially improved after 24 hours as a result of disposition of toxic dose through renal excretion and detoxification by reduced glutathione which showed marked elevation after 24 hours compared with 4 hours of acetaminophen administration.

The first part of this work also revealed that toxic dose of acetaminophen (900mg/kg/i.p) induced nephrotoxicty which was reflected on renal function tests as significant elevation of serum urea and creatinine. These results are in line with ***Sener et al. (2003); Ilbey et al. (2009) and Molinas et al. (2010).***

The mechanism of acetaminophen nephrotoxicity is a point of much debate. Traditionally, it is attributed to formation of toxic metabolites namely NAPQI by extrahepatic metabolism of acetaminophen by renal cytochrome p450 oxidase which is present in both renal and hepatic tissues ***(Bessems and Vermeulen, 2001).***

Another potential mechanism of acetaminophen toxicity is related to prostaglandin endoperoxide synthase (PGES), although its effect may be more substantial in the chronic rather than the acute setting. PGES is an enzyme found in the kidney that activates APAP into toxic metabolites, most likely NAPQI. This process is more pronounced in the medulla of the kidney, whereas the cytochrome P450 plays a more important role in the cortex ***(Mazer and Perrone, 2008).***

Such assumption was confirmed in this part of this study by the observed significant elevation of malondialdehyde in the renal tissue homogenate which is one of the end products of free radical chain reaction and depletion of reduced glutathione which is the master natural human antioxidant and this also may explain the observed microscopic changes in the form of hydropic degeneration of the tubular epithelium, tubular necrosis, biochemical changes in the form of elevated serum urea and creatinine.

On the contrary to acetaminophen induced hepatotoxicity nephrotoxicity was more prominent after 24 hours than 4 hours of drug administration. This confirmed the clinical case report of ***Von Mach et al. (2005) and Şener et al. (2006)*** who showed that acetaminophen induced nephrotoxicity had slower course than hepatotoxicity.

Pretreatment with either melatonin or N-acetylcysteine produced partial protection against acetaminophen induced hepatotoxicity and nephrotoxicity represented by significant reduction of serum level of hepatic enzymes aspartate and alanine transaminases , urea & creatinine with significant reduction in the hepatic , renal tissue concentration malondialdehyde and increase in that of reduced glutathione , compared with non treated acetaminophen administrated group.

Concomitant administration of both tested antioxidant agents produced more marked protective effect than either drug singly. This may be attributed to the fact that either drugs acted by different mechanisms. Melatonin acted by direct free radical scavenging effect, indirect portion of glutathione synthesis and activation of antioxidant enzymes. N-acetyl cysteine acted by anti-free radical effect through neutralization of free radicals and increase synthesis of reduced glutathione as well as independent anti-inflammatory effect.

In the second part of this work, the possible protective effects of the tested drugs were investigated in fructose induced metabolic syndrome in male albino adult rats.

10% fructose administration in drinking water for 8 weeks resulted in production of pathological condition in the tested animals more or less similar to metabolic syndrome. There were significant increase in fasting blood glucose, fasting insulin levels which is reflected as increase in HOMA- IR ratio, marked hyperlipidemia manifested as increase serum total cholesterol, triglycerides, LDL cholesterol concentration and lowering of HDL cholesterol concentration but total body weight showed insignificant increase.

High fructose intake in this study resulted in significant increase in both systolic and diastolic blood pressure suggested that fructose may raise BP via several mechanisms inhibition of endothelial nitric oxide synthase system ***(Steinberg et al., 1994),*** including stimulation of uric acid (***Johnson et al., 2009),*** or by directly increasing sodium absorption in the gut ***(Soleimani and Alborzi, 2011)*** or by stimulation of the sympathetic nervous system ***(De et al., 2012).***

This study also highlighted an association of fructose induced metabolic syndrome and oxidative stress. This was evidenced by marked increase in pancreatic concentration of the malondialdehyde which may indicate increase oxidative free radical formation and decrease in reduced glutathione concentration which may represent consumption of natural antioxidants.

This is in agreement with ***Punitha et al. (2005); Kumar and Anandan(2007);Reddy et al. (2009); Miller and Adeli, (2010)*** who demonstrated an association between fructose catabolism and free radical formation.

Regarding blood pressure tracing, pretreatment with either melatonin or N-acetylsyteine partially attenuated the hypertensive effect of fructose administration compared with non treated animal groups. Such values were significantly more than the normal value. Melatonin was more effective in decreasing the systolic hypertension than N-acetylcysteine

The central effect of melatonin may contribute in its hypotensive through increase the release of GABA from suprachiasmatic nucleus via stimulation of ML1 & ML2 receptors.

Regarding parameters of glycemic control, either melatonin or N-acetylcysteine decreased fasting serum concentration of insulin, blood glucose and decrease HOMA -IR index compared with non treated fructose administrated group. This may imply that melatonin partially improved fructose induced insulin resistance .This is in agreement with ***(Claustrat et al., 2009)*** who suggests that the melatonin signal is critical for glucose regulation in blood and maintaining homeostasis.

Regarding the effect of N-acetylcysteine on the tested parameters of glycemic control, similar effect to that of melatonin was elicited both quantitatively and qualitatively. This effect may be attributed to previously explained anti-free radical effect of the tested drug which augments the synthesis of glutathione (master natural antioxidant).

Regarding the effect of melatonin on the lipid profiles, the present study revealed that it markedly decreased concentration of total cholesterol, LDL cholesterol and triglycerides compared with non treated metabolic syndrome animal model

Melatonin may reverse the hyperinsulinemia which up regulate sterol regulatory element binding protein. The latter acts as transcription factor required for synthesis of enzymes involved in sterol biosynthesis ***(Yokoyama et al., 1993).***

On the other hand, HDL-cholesterol increasing effect of melatonin may be mediated by increase the esterification of cholesterol inside HDL particle by insulin dependent lecithin acyl coA reductase enzyme ***(Fossati and Romon-Rousseaux, 1982).***

Regarding the effects of N-acetylcystine on the parameters of lipid profiles, they were similar to that of melatonin both quantitatively and qualitatively. This effect is strongly correlated with both antioxidant and insulin resistance ameliorating effects observed in this study. Similar results were obtained by ***Korou et al. (2014)*** who showed that the beneficial effects of N-acetylcysteine on the lipid profiles was more pronounced in old and high fat fed animals which may be explained by high oxidative stress in such groups.

The above mentioned hemodynamic and metabolic changes under the effect of pretreatment of the tested drugs were associated with marked improvement in oxidative stress

The previously explained antioxidant effect of the tested drug may explain the observed hypotensive, hyoglygemic and hypolipidemic effects through maintaining the integrity of vascular endothelium, B islet cells and insulin receptors against free radicals liberated from excessive fructose metabolism.

References:

1. Abd-Allah AR, Al-Majeed AA, Mostafa AM, Al-Shabanah OA, Din A G, Nagi MN.(2002): Protective effect of arabic gum against cardiotoxicity induce by doxorubicin in mice: a possible mechanism of protection. J Biochem Mol Toxicol. 16:254–259.
2. Arakawa M, Ushimaru N, Osada N, Oda T, Ishige K and Ito Y. (2012): N-acetylcysteine selectively protects cerebellar granule cells from 4-hydroxynonenal-induced cell death. Neurosci. Res. 55, 255–263.

3. Aydin M, Canpolat S, Kuloğlu T, Yasar A, Colakoglu N and Kelestimur H. (2008): Effects of pinealectomy and exogenous melatonin on ghrelin and peptide YY in gastrointestinal system and neuropeptide Y in hypothalamic arcuate nucleus: immunohistochemical studies in male rats. Regul Pept.;146:197–203.
4. Ayman M, Gamal el-din AM, Mostafa OA, Al-Shabanah AM, Al- Bekairi MN.(2003): Protective effect of Arabic gum against acetaminophen –induced hepatotoxicity in mice. Pharmacological research. 48: 631-635.
5. Bessems JG and Vermeulen NP. (2001): Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues, and protective approaches. Rev Toxicol; 31:55–138.
6. Bonomini F, Rodella LF, and Rezzani R (2015): Metabolic Syndrome, Aging and Involvement of Oxidative Stress. Aging Dis. Mar; 6(2): 109–120.
7. Boutis, K and Shannon M (2005): Nephrotoxicity after acute severe acetaminophen poisoning in adolescents. Clin Toxicol;39:41–445.
8. Brunet J, Boily MJ, Cordeau S, Des Rosiers C. (2006): Effects of N-acetylcysteine in the rat heart reperfused after low-flow ischemia: evidence for a direct scavenging of hydroxyl radicals and a nitric oxide-dependent increase in coronary flow. Free RadicBiol Med.; 19(5): 627–638.
9. Cai D and Liu T (2012): Inflammatory cause of metabolic syndrome via brain stress and NF-κB. Aging (Albany NY), 4(2):98–115.
10. Carrasco R, Perez-Mateo M, Gutierrez A, Esteban A, Mayol MJ, Cartula, J, Ortiz, P (2000): Effect of different doses of S-adenosyl-L-methionine on paracetamol hepatotoxicity in a mouse model. Methods Find Exp Clin Pharmacol. 22(10): 737-745.
11. Celinski K, Konturek SJ, Konturek PC, Brzozowski T, Cichoz-Lach H, Slomka M, Malgorzata P, Bielanski W and Reiter RJ (2011): Melatonin or L-tryptophan accelerates healing of gastroduodenal ulcers in patients treated with omeprazole. J Pineal Res.; 50:389–394.
12. Claustrat B, Brun J and Chazot G. (2009): The basic physiology and pathophysiology of melatonin. Sleep Med Rev.; 9 (1):11-24.
13. De AK, Senador DD, Mostarda C, Irigoyen MC and Morris M. (2012): Sympathetic over activity precedes metabolic dysfunction in a fructose model of glucose intolerance in mice. American Journal of Physiology; 302(8):R950–R957.
14. Dimova S, Hoet PHM and Nemery B (2007): Paracetamol (acetaminophen) citotoxicity in rat type II pneumocytes and alveolar macrophages in vitro. Biochem Pharmacol; 59:1467-1475.
15. Dubocovich ML, Cardinali DP, Delagrange P, Krause DN, Strosberg D, Sugden D, et al. (2010): Melatonin Receptors. The IUPHAR Compendium of Receptor Characterization and Classification. IUPHAR Media: London; pp. 270–277.
16. Erdemir F, Atilgan D, Firat F, Markoc F, Parlaktas BS, Sogut E(2014): The effect of sertraline, paroxetine, fluoxetine and escitalopram on testicular tissue and oxidative stress parameters in rats. ntBraz J Urol.;40(1):100-8.
17. Fossati P and Romon-Rousseaux M. (1982): Insulin and HDL-cholesterol metabolism. Diabete Metab. ; 13 (3 Pt 2): 390 - 4.
18. Ganji V, Zhang X, Shaikh N, Tangpricha V. (2011): Serum 25-hydroxyvitamin D concentrations are associated with prevalence of metabolic syndrome and various cardiometabolic risk factors in US children and adolescents based on assay-adjusted serum 25-hydroxyvitamin D data from NHANES 2001-2006. Am J Clin Nutr.;94(1):225-33.
19. Gomez-Moreno G, Guardia J, Ferrera MJ, Cutando A and Reiter RJ (2010): Melatonin in diseases of the oral cavity. Oral Dis.;16:242–247.
20. Gu J, Cui H, Behr M, Zhang L, et al. (2006): In vivo mechanisms of tissue-selective drug toxicity: effects of liver-specific knockout of the NADPH-cytochrome P-450 reductase gene on acetaminophen toxicity in kidney, lung, and nasal mucosa. Mol Pharmacol; 67 : 623–630.
21. Ilbey YO, Ozbek E, Cekmen M, Somay A, Ozcan L, Otünctemur A, Simsek A and Mete F. (2009): Melatonin prevents acetaminophen-induced nephrotoxicity in rats. nt Urol Nephrol.; 41 (3) : 695 - 702.
22. Jaeschke H and Bajt ML (2006): Intracellular signaling mechanisms of acetaminophen-induced cell death. Toxicol Sci, 89:31-41.
23. James LP, McCullough SS, Lamps LW and Hinson JA. (2005): Effect of N-acetylcysteine on acetaminophen toxicity in mice: relationship to reactive nitrogen and cytokine formation. Toxicol Sci.; 75:458–467.
24. Johnson RJ, Perez-Pozo SE, Sautin YY, Manitius J, Sanchez-Lozada LG and Feig D.(2009): Hypothesis: could excessive fructose intake and uric acid cause type 2 diabetes? Endocr Rev.; 30(1):96-116.
25. Kala C, Ali SS, Mohd A, Rajpoot S and Khan NA. (2015): "Protection against FCA Induced Oxidative Stress Induced DNA Damage as a Model of Arthritis and In vitro Anti-arthritic Potential of Costus speciosus Rhizome Extract". Pharmacognosy Res.7 (2): 383–389.
26. Korkmaz A, Topal T, Tan DX and Reiter RJ (2009): Role of melatonin in metabolic regulation. Rev Endocr Metab Disord.;10:261–270.
27. Korou LM, Agrogiannis G, Koros C, et al. (2014): Impact of N-acetylcysteine and sesame oil on lipid metabolism and hypothalamic-pituitary-adrenal axis homeostasis in middle-aged hypercholesterolemic mice.28; 4: 6806
28. Kumar HS and Anandan R (2007): Biochemical studies on the cardioprotective effect of glutamine on tissue antioxidant defense system in isoprenaline-induced myocardial infarction in rats. J Clin Biochem Nutr.; 40: 49 – 55.
29. Larson AM, Polson J, Fontana RJ, et al. (2005): Acetaminophen induced acute liver failure: results of a United States multicenter prospective study. Hepatology;42:1364–1372.
30. Leja-Szpak A, Jaworek J, Pierzchalski P and Reiter RJ (2010): Melatonin induces pro-apoptotic signaling pathway in human pancreatic carcinoma cells (PANC-1) J Pineal Res.;49:248–255.

31. Litovitz TL, Klein-Schwartz W, Rodgers GC, Jr, Cobaugh DJ, Youniss J, Omslaer JC, May ME, Woolf AD, Benson BE (2005): Annual report of the American Association of Poison Control Centers Toxic Exposure Surveillance System. Am J Emerg Med.;20:391–452.
32. Liu YT, Lu BN and Peng JY (2011): Hepatoprotective activity of the total fla-vonoids from Rosa laevigata Michx fruit in mice treated by acetaminophen. Food Chemistry; 125: 719 – 725.
33. Mahadevan T, Williams AP, Philips G O, Al- assaf S, Baldwin TC (2008): New Insights into the Structural Characteristics of the Arabinogalactan-Protein (AGP) Fraction of Gum Arabic. J. Agric. Food Chem. 56, 9269–9276.
34. Man GC, Wong JH, Wang WW, Sun GQ, Yeung BH, Ng TB, Lee SK, Ng BK, Qiu Y and Cheng JC (2011): Abnormal melatonin receptor 1B expression in osteoblasts from girls with adolescent idiopathic scoliosis. J Pineal Res.; 50:395–402.
35. Mazer M and Perrone J. (2008): Acetaminophen-Induced Nephrotoxicity: Pathophysiology, Clinical Manifestations, and Management. J Med Toxicol.;4 (1):2-6.
36. McGill MR, Williams DC, Xie Y, Ramachandran A and Jaeschke H. (2012): Acetaminophen-induced Liver Injury in Rats and Mice: Comparison of Protein Adducts, Mitochondrial Dysfunction, and Oxidative Stress in the Mechanism of Toxicity. Toxicol Appl Pharmacol. 1; 264(3): 387–394.
37. Miller A and Adeli K. (2010): Dietary fructose and the metabolicsyndrome. Curr Opin Gastroenterol; 24: 204-209.
38. Moffit JS, Koza-Taylor PH, Holland RD, Thibodeau MS, Beger RD, Lawton MP and Manautou JE. (2007): Differential gene expression in mouse liver associated with the hepatoprotective effect of clofibrate. Toxicol Appl Pharmacol. 15; 222(2) : 169-79.
39. Molinas SM, Rosso M, Wayllace NZ, Pagotto MA, Pisani GB, Monasterolo LA and Trumper L. (2010): Heat shock protein 70 induction and its urinary excretion in a model of acetaminophen nephrotoxicity. Pediatr Nephrol. ; 25(7): 1245 - 53.
40. Naguib YA, Azmy RM, Rehab M, Samaka RA and Salem MF.(2014): Pleurotus ostreatus opposes mitochondrial dysfunction and oxidative stress in acetaminophen-induced hepato-renal injury. BMC Complement Altern Med.; 14: 494.
41. Negre-Salvayre A, Auge N, Ayala V, et al.,(2010): "Pathological aspects of lipid peroxidation". Free Radical Research 44 (10): 1125–1171.
42. Punitha IS, Rajendran K, Shirwaikar A and Shirwaikar A. (2005): Alcoholic stem extract of Coscinium fenestratum regulates carbohydrate metabolism and improves antioxidant status in streptozotocin-nicotinamide induced diabetic rats. Evid Based Complement Alternat Med.; 2 : 375–81.
43. Reddy SS, Ramatholisamma P, Karuna R and Saralakumari D (2009): Preventive effect of Tinosporacordifolia against high-fructose diet-induced insulin resistance and oxidative stress in male Wistar rats. Food Chem Toxicol.; 47: 2224–9.
44. Reid AB, Kurten RC, McCullough SS, Brock RW and Hinson JA (2006): mechanisms of hepatotoxcicity induced by acetaminophen: role of oxidative stress and mitochondrial permeability transition in freshly isolated mouse hepatocytes. J Pharmacol Exp Ther. 312(2):509-16.
45. Reiter RJ (1991c): Melatonin: clinical relevance. Best Pract Res Clin Endocrinol Metab.;17:273–285.
46. Reiter RJ, Tan DX and Manchester LC, QIW (2001): Biochemical reactivity of melatonin with reactive oxygen and nitrogen species. Cell Biochem Biophys; 34:237-256.
47. Rodriguez C, Mayo JC, Sainz RM, Antolin I, Herrera F, Martin V and Reiter RJ (2004): Regulation of antioxidant enzymes: a significant role for melatonin. J Pineal Res.;36:1–9.
48. Samuni Y, Goldstein S, Dean OM and Berk M (2013): The chemistry and biological activities of N-acetylcysteine. Biochim Biophys Acta. 1830; 4117–4129.
49. Şener G, Şatiroglu H, Kabasakal L, Arbak S, Öner S, Ercan F, Keyer-Uysal M (2006): The protective effect of melatonin on cisplatin nephrotoxicity. Fundamental and Clinical Pharmacology; 14 (6): 553-560.
50. Sener G, Sehirli AO and Ayanoğlu-Dülger G. (2003): Protective effects of melatonin, vitamin E and N-acetylcysteine against acetaminophen toxicity in mice: a comparative study. J Pineal Res. ; 35(1):61-8.
51. Soleimani M and Alborzi P. (2011): The Role of Salt in the Pathogenesis of fructose-Induced Hypertension. Int J Nephrol.; :392708.
52. Steinberg HO, Brechtel G, Johnson A, Fineberg N, and Baron AD. (1994): Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. J Clin Invest.; 94(3): 1172–1179.
53. Tomás-Zapico C and Coto-Montes A (2005): A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes. J Pineal Res.; 39:99–104.
54. Von Mach MA, Hermanns-Clausen M, Koch I et al. (2005): Experiences of a poison center network with renal insufficiency in acetaminophen overdose: an analysis of 17 cases. Clin Toxicol, ;43:31–37.
55. Walker RM, Racz WJ and McElligott TF. (1985): Acetaminophen-induced hepatotoxic congestion in mice. Hepatology.;5 :233–240.
56. Yokoyama C, Wang X, Briggs MR, Admon A, Wu J, Hua X, Goldstein JL and Brown MS. (1993): "SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene". Cell 75 (1): 187–97.
57. Zhang YH, An T, Zhang RC, Zhou Q, Huang Y and Zhang J.(2013): Very high fructose intake increases serum LDL-cholesterol and total cholesterol: a meta-analysis of controlled feeding trials.;143(9): 1391-8.