RELATIONSHIP BETWEEN INSULIN SENSITIVITY, GLUCOSE LEVEL AND ADIPONECTIN LEVEL IN LIVER DISEASED RATS

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ABSTRACT
Introduction: The liver has an important role in carbohydrate metabolism since it is responsible for the balance of blood glucose levels by means of glycogenogenesis and glycogenolysis. Aim of the Study: Evaluation of the effect of the single and combined administration of insulin and N-acetyl cysteine on glucose metabolism in hepatotoxic rats. Materials and Methods: This study enrolling 5 groups of adult male albino rats. Hepatotoxicity was induced in group II, III, IV &V using carbon tetrachloride, while group I was left as control. Group II received no treatment, group III received N-acetyl cysteine, group IV received insulin while group V received insulin and N-acetyl cysteine. Samples were taken and measured liver function tests, insulin, glucose and adiponectin level as well as histopathological studies analysis of the liver specimens were measured and non-parametric multicompasion test was applied. Results: Liver function tests, group II and group IV showed higher significant statistical difference than group I and III. Also group II showed higher significant statistical difference than other groups as regarding blood glucose level and adiponectin level. The histopathological results revealed that the untreated group showed significant liver damage when compared to the control group, while treatment with NAC alone and treated with insulin and NAC showed significant reduction of all histopathological changes, whereas treatment with insulin alone significantly reduced all the histopathological changes except lobular inflammation. Conclusion: Good control of carbohydrate metabolism including insulin level, insulin resistance and adiponectin level helped in improving the prognosis of liver dysfuction diseases.

KEYWORDS: Carbon Tetrachloride, Hepatotoxicity, N-Acetylcystiene, Insulin, Adiponectin, Insulin Resistance.

INTRODUCTION
The liver has an essential role in carbohydrate metabolism since it is responsible for the balance of blood glucose levels by glycogenogenesis and glycogenolysis.¹ In the presence of hepatic disease, the metabolic homeostasis of glucose is compromised as a result of disorders such as glucose intolerance, insulin resistance and diabetes mellitus (DM).²

Up to 96% of patients with liver cirrhosis could be glucose intolerant and 30% could be clinically diabetic.³ The diabetes which has resulted as a complication of cirrhosis is known as “hepatogenous diabetes”.⁴

Treatment of diabetes in the cirrhotic patient is complex because of the presence of liver damage. Moreover, the hepatotoxicity induced by some oral hypoglycemic drugs may complicate such treatment. Accordingly, the pharmacological therapy must be closely monitored.⁵

It is known that from the early stages of chronic liver disease, insulin resistance and glucose intolerance may be present in most of these patients. Diabetes manifests clinically as the liver function deteriorates, thus hepatogenous diabetes can be considered as an pointer of advanced liver disease.⁶

On the other hand, several studies submitted that DM may have an etiological role in chronic liver disease and hepatocellular carcinoma (HCC) regardless of alcohol and viruses. The incidence of non-alcoholic chronic liver disease and HCC was significantly higher in diabetic patients compared with non-diabetic patients. This risk was 2-fold greater and was independent of alcoholic liver disease, viral hepatitis and demographic factors.⁷

MATERIALS AND METHODS
Materials
I. Animals used: Adult male albino rats (200-250 g.) have been used. Rats were purchased from animal house, Faculty of Medicine, Assuit University. The animals were housed under standardized environmental conditions, fed with standard diet, water and left to aclimatize to the environment for one week prior to
inclusion in the experiment. All the animal experiments were conducted in accordance with the guide for the care and use of laboratory animals of the National Institutes of Health (NIH 1985).

2. Chemicals used: Carbon tetrachloride (CCl4) was purchased from Koch-Light Laboratories LTD (England), N-acetyl cysteine (NAC) was purchased from Sigma Company (USA), Insulin mixtard 70/30 was purchased from Medical Union Pharmaceutics (Egypt), Liver function test kits (AST, ALT, ALP and TP) were purchased from Spectrum (Egypt), Adiponectin kits was purchased from Elabsscience Biotechnology Inc. (USA), glucose detection using On- Call Ez glucometer was purchased from ACON laboratories, Inc. (USA) with its specific glucose strips and insulin ELISA kit was purchased from Immunospec Corporation (USA). Normal saline solutions and pentobarbital were obtained in pure grade from El Naser Company.

Methods

Induction of hepatotoxicity: Hepatotoxicity is induced in rats using CCl4. It was diluted 1:1 v/v in olive oil. Each rat received intraperitoneal (IP) injection of CCl4 0.5 mL/kg, twice per week, for one month. Development of hepatotoxicity in rats was confirmed by presence of abnormalities in liver function tests (AST, ALT, ALP and total protein).

To confirm hepatic affection: Blood samples were collected from the retro-orbital plexus of rats using a non-heparinized capillary tube. In each collection about 0.5 mL of blood was taken into a non-heparinized tube and centrifuged at 1000-2000 rev./min for 10 min. Serum was then collected carefully and used for estimation of enzymes’ levels stored at temperature -20 °C until assayed. Animals showed raised liver function tests were considered having hepatic dysfunction. Preliminary experiments were conducted to adjust the dose.

At the end of the experiment: each animal was sacrificed by decapitation. Blood samples from each animal were collected into centrifuge tubes, incubated in an upright position at room temperature for 30-45 minutes to allow clotting, and then centrifuged at 1000-2000 rev./min. for 10-15 minutes. Serum was then collected carefully and used for estimation of enzymes’ levels stored at -20 °C until assayed. Then, the liver of each animal was taken for histopathological evaluation.

Experimental design

Animal groups

Animals were randomly divided into five groups (10 animals in each)
- **Group I:** Rats were injected with equivalent volume of the solvent (olive oil) and served as negative control group.
- **Group II:** Rats were injected IP with CCl4 (0.5 mL/kg body weight, 1:1 v/v mixture of CCl4 and olive oil) twice a week for 4 weeks and served as the positive control group of chemically induced hepatic dysfunction model.
- **Group III:** Chemically induced hepatic dysfunction model of rats were injected IP with NAC (200 mg/Kg in 0.5 mL of normal saline) for three consecutive days.
- **Group IV:** Chemically induced hepatic dysfunction model of rats were injected once with insulin (0.5U/Kg, IP).
- **Group V:** Chemically induced hepatic dysfunction model of rats were injected IP by NAC (200 mg/Kg in 0.5 mL of normal saline) for three consecutive days. Then insulin will be injected in the third day (0.5U/Kg, IP).

Experimental Procedures

1) Liver function tests: Marker enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and Level of total serum protein (TSP) were estimated.\(^9\)

2) Measuring the serum level of glucose and insulin: Serum glucose levels were determined using On- Call Ez glucometer (ACON laboratories, Inc., USA) with its specific glucose strips. Serum insulin levels were determined using a rat/mouse insulin ELISA kit (Millipore) according to the manufacturer’s protocol.\(^10\)

3) Measuring adiponectin: Adiponectin level were determined using a rat/mouse adiponectin ELISA kit according to the manufacturer’s protocol. This ELISA kit for adiponectin is a so-called Sandwich-Assay using two specific and high affinity antibodies.

6) Histopathology: The excised livers were fixed in 10% buffered formalin for 24 h. At least three different sections were taken per liver. Paraffin blocks were cut into 4µm thick section and stained with hematoxylin & eosin (H&E) and Masson’s trichrome according to standard protocols. A pathologist blinded to the animal grouping evaluated the slides and scored each liver tissue specimen using a semi-quantitative score for inflammation, necrosis, hydropic degeneration, sinusoidal dilatation, venous congestion and steatosis. An arbitrary score was given to each microscopic field viewed at magnifications of ×40. At least 5 fields were scored per liver section to obtain the mean value.

Data management and statistical analysis: Mean and standard deviation (SE) were used to describe the distribution of variables and compare cases and controls. The Mann–Whitney U test was used to compare values between groups. All statistical analyses were performed using SPSS software v20.0 (SPSS, Chicago, IL, USA). P < 0.05 was considered significant.
RESULTS

1) Effect of CCl4 alone and in combination with NAC and insulin on liver function tests

Table 1: Liver function tests among the studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CCl4</th>
<th>CCl4+NAC</th>
<th>CCl4+ insulin</th>
<th>CCl4 + insulin +NAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>30.71±1.71</td>
<td>397.27±39.20**</td>
<td>41.26±1.07</td>
<td>119.01±12.05*</td>
<td>29.44±2.18</td>
</tr>
<tr>
<td>ALT</td>
<td>36.9±0.88</td>
<td>370.94±40.11**</td>
<td>40.06±0.35</td>
<td>126.04±6.12*</td>
<td>32.23±2.10</td>
</tr>
<tr>
<td>ALP</td>
<td>370.59±41.38</td>
<td>785.49±20.21**</td>
<td>335.63±25.14</td>
<td>460.2±22.24</td>
<td>405.21±34.16</td>
</tr>
<tr>
<td>Total protein</td>
<td>6.52±0.28</td>
<td>4.39±0.33**</td>
<td>6.35±0.25</td>
<td>5.59±0.25</td>
<td>5.85±0.25</td>
</tr>
</tbody>
</table>

Data represents mean± SE of 10 observations in each group.

*Statistically significant difference (p<0.05).

**Highly statistically significant difference (p<0.01) between control group and the other groups.

A) AST level in the five tested groups: After injection of CCl4 IP in a dose of 0.5 ml/kg, twice per week, for one month in rats AST level was higher than control rats and there is a statistical high significant difference as shown by the P-value measures (p<0.01) (table 1 and figure 1).

While, NAC treated hepatic dysfunction rats in a dose of 200 mg/Kg for three consecutive days as well as, hepatic dysfunction rats treated with combination of NAC in the same dose and insulin in a dose of 0.5U/Kg, AST level had non-significant difference compared to control group (table 1 and figure 1).

Also, insulin treated hepatic dysfunction rats in a dose of 0.5U/Kg, AST level was higher than control rats and had a significant statistical difference as the P-value measures (p<0.05) (table 1 and figure 1).

B) ALT level in the five tested groups: After injection of CCl4 IP in a dose of 0.5 ml/kg, twice per week, for one month in rats, ALT level was higher than control rats and there was a significant statistical difference as shown by the P-value measures (p<0.01) (table 1 and figure 2).

While, NAC treated hepatic dysfunction rats in a dose of 200 mg/Kg for three consecutive days as well as, hepatic dysfunction rats treated with combination of NAC in the same dose and insulin in a dose of 0.5U/Kg, ALT level had non-significant difference compared to control group (table 1 and figure 2).

Also, insulin treated hepatic dysfunction rats in a dose of 0.5U/Kg, ALT level was higher than control rats and had a significant statistical difference as the P-value measures (p<0.05) (table 1 and figure 2).

C) ALP level in the five tested groups

Rats which had been treated with CCl4 in a dose of 0.5 ml/kg, twice per week, for one month ALP level was higher than control rats and had a high significant statistical difference as shown by the P-value measures (p<0.01) (table 1 and figure 3).

While, NAC treated hepatic dysfunction rats in a dose of 200 mg/Kg for three consecutive days, hepatic dysfunction rats treated with insulin in a dose of 0.5U/Kg hepatic dysfunction and hepatic dysfunction rats treated with combination therapy of NAC and insulin ALP level had non-significant difference compared to control group (table 1 and figure 3).

D) Total protein level in the five tested groups

We found that CCl4 treated hepatic dysfunction rats in a dose of 0.5 ml/kg, twice per week, for one month total
protein level was higher than control rats and there was a high significant statistical difference as shown by the P-value measures (p<0.01) (table 1 and figure 4).

While, NAC treated hepatic dysfunction rats in a dose of 200 mg/Kg for three consecutive days, hepatic dysfunction rats treated with insulin in a dose of 0.5U/Kg hepatic dysfunction and hepatic dysfunction rats treated with combination therapy of NAC and insulin total protein level had non-significant difference compared to control group (table 1 and figure 4).

Figure. (3): The results of ALP in the five groups (mean ± SE).
Data represents mean± SE of 5 observations.
*Statistically significant difference (p<0.05).
**Highly statistically significant difference (p<0.01) between control group and the other groups.

Figure. (4): The results of total protein in the five groups (mean ± SE).
Data represents mean± SE of 5 observations.
*Statistically significant difference (p<0.05).
**Highly statistically significant difference (p<0.01) between control group and the other groups.

2) Effect of CCl4 alone and in combination with NAC and insulin on glucose and insulin level

Table 2: The glucose and insulin levels among the studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CCl4</th>
<th>CCl4+NAC</th>
<th>CCl4+ insulin</th>
<th>CCl4 + insulin +NAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Glucose level</td>
<td>107±5.83</td>
<td>149.5±6.2**</td>
<td>112.1±7.6</td>
<td>78.5±4.31</td>
<td>77.7±6.49</td>
</tr>
<tr>
<td>Insulin level (μIU/mL)</td>
<td>10.4±0.71</td>
<td>19.6±3.1**</td>
<td>13.8±1.9*</td>
<td>18.2±1.8**</td>
<td>17.4 ±2.8 **</td>
</tr>
</tbody>
</table>

Data represents mean± SE of 10 observations in each group.
*Statistically significant difference (p<0.05).
**Highly statistically significant difference (p<0.01) between control group and the other groups.

A) Blood glucose level in the five tested groups
As shown in table 2 and figure 5, we found that blood glucose level in CCl4 treated rats in a dose of 0.5 ml/kg, twice per week, for one month was higher than control rats and there was a high significant statistical difference as shown in the P-value measures (p<0.01).

While, blood glucose level in NAC treated hepatic dysfunction rats in a dose of 200 mg/Kg for three consecutive days, hepatic dysfunction rats treated with insulin in a dose of 0.5U/Kg hepatic dysfunction and hepatic dysfunction rats treated with combination therapy of NAC and insulin had non-significant difference compared to control group (table 2 and figure 5).

B) Insulin level in the five tested groups: As shown in table 2 and figure 6, blood insulin level in CCl4 treated rats in a dose of 0.5 ml/kg, twice per week, for one month was higher than control rats and there was a high significant statistical difference as shown in the P-value measures (p<0.01).

Also, we found that blood insulin level in NAC treated hepatic dysfunction rats in a dose of 200 mg/Kg for three consecutive days was higher than control rats and had a significant statistical difference as the P-value measures (p<0.05) (table 2 and figure 6).

While, insulin treated hepatic dysfunction rats in a dose of 0.5U/Kg had blood insulin level higher than control rats and there was a high significant statistical difference as the P-value measures (p<0.01) (table 2 and figure 6). Moreover, blood insulin level in hepatic dysfunction rats treated with combination of NAC and insulin was higher than control rats and there was a high significant statistical difference as the P-value measures (p<0.01) (table 2 and figure 6).
Figure (5): The results of blood glucose level in the five groups.

Figure (6): The results of insulin level in the five groups. Data represents mean± SE of 5 observations.

* Statistically significant difference (p<0.05).

** Highly statistically significant difference (p<0.01) between control group and the other groups.

3) Effect of CCl4 alone and in combination with NAC and insulin on adiponectin level.

Table 3: Adiponectin levels among the studied groups

<table>
<thead>
<tr>
<th>Adiponectin level (pg/ml)</th>
<th>Control</th>
<th>CCl4</th>
<th>CCl4+NAC</th>
<th>CCl4+ insulin</th>
<th>CCl4 + insulin +NAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>186.77±19.71</td>
<td>413.49±29.85**</td>
<td>148.56±16.23</td>
<td>355.55±36.57**</td>
<td>184.56±10.42</td>
</tr>
</tbody>
</table>

Data represents mean± SE of 10 observations in each group.

*Statistically significant difference (p<0.05).

**Highly statistically significant difference (p<0.01) between control group and the other groups.

As shown in table 3 and figure 7, we found that adiponectin level in CCl4 treated rats in a dose of 0.5 ml/kg, twice per week, for one month was higher than control rats and had a high significant statistical difference as shown in the P-value measures (p<0.01). While, adiponectin level in both NAC treated hepatic dysfunction rats in a dose of 200 mg/Kg for three consecutive days and combination of treatment with NAC and insulin in hepatic dysfunction rats had nonsignificant difference compared to control group (table 3 and figure 7).

Also, adiponectin level in insulin treated hepatic dysfunction rats in a dose of 0.5U/Kg was higher than control rat and had a high significant statistical difference as the P-value measures (p<0.01) (table 3 and figure 7).

Figure (7): The results of adiponectin level in the five groups. Data represents mean± SE of 5 observations.

*Statistically significant difference (p<0.05).

**Highly statistically significant difference (p<0.01) between control group and the other groups.
**Histopathological results**

Table 4: Histopathological changes in the liver specimen in the five groups (mean± SE):

<table>
<thead>
<tr>
<th></th>
<th>Group1 (Control)</th>
<th>Group2 (CCL4)</th>
<th>Group3 (CCL4+NAC)</th>
<th>Group4 (CCL4+ insulin)</th>
<th>Group5 (CCL4+ insulin +NAC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td>Mean±SE</td>
</tr>
<tr>
<td><strong>Hydropic degeneration</strong></td>
<td>0±0 0%</td>
<td>2.6±0.16* 100%</td>
<td>0.8±0.2* 70%</td>
<td>1.2±0.25* 80%</td>
<td>0.4±0.16# 40%</td>
</tr>
<tr>
<td><strong>Sinusoidal dilatation</strong></td>
<td>0±0 0%</td>
<td>2±0.3* 100%</td>
<td>0.3±0.15* 30%</td>
<td>0.7±0.21* 70%</td>
<td>0.3±0.15* 30%</td>
</tr>
<tr>
<td><strong>Vascular congestion</strong></td>
<td>0±0 0%</td>
<td>1.9±0.28* 100%</td>
<td>0.5±0.17* 50%</td>
<td>0.7±0.26* 70%</td>
<td>0.2±0.13* 20%</td>
</tr>
<tr>
<td><strong>Lobular inflammation</strong></td>
<td>0±0 0%</td>
<td>2.7±0.15* 100%</td>
<td>0.5±0.17* 50%</td>
<td>1.7±0.4* 80%</td>
<td>0.2±0.13# 20%</td>
</tr>
<tr>
<td><strong>Portal inflammation</strong></td>
<td>0±0 0%</td>
<td>0.3±0.15 30%</td>
<td>0.3±0.15 30%</td>
<td>0.4±0.16* 40%</td>
<td>0±0 0%</td>
</tr>
<tr>
<td><strong>Necrosis</strong></td>
<td>0±0 0%</td>
<td>2.7±0.15* 100%</td>
<td>0.6±0.22* 50%</td>
<td>1.5±0.31* 80%</td>
<td>0.2±0.13# 20%</td>
</tr>
<tr>
<td><strong>Necroinflammatory score</strong></td>
<td>0±0 0%</td>
<td>5.7±0.33* 60%</td>
<td>1.4±0.5* 10%</td>
<td>3.8±0.77* 10%</td>
<td>0.4±0.16# 10%</td>
</tr>
<tr>
<td><strong>Steatosis</strong></td>
<td>0±0 0%</td>
<td>0.7±0.21* 100%</td>
<td>0.1±0.1# 70%</td>
<td>0.1±0.1# 80%</td>
<td>0.1±0.1# 40%</td>
</tr>
</tbody>
</table>

* Statistically significant difference between control group and the other groups, # statistically significant difference between group2 and the other groups.

Liver tissue of rats in the control group showed normal architecture of the hepatocytes without any pathological changes.

Rats in the untreated group (group II) showed significant liver damage when compared to the control group (table 4). The histological changes include severe centrilobular necrosis and inflammation, hydropic degeneration, sinusoidal dilatation, vascular congestion and steatosis (table 4).

However fibrosis was absent in all examined liver tissue (confirmed by massan trichrome stain).

Compared with the untreated group, treatment with NAC (group III) and (group V) treated with insulin and NAC showed significant reduction of all histopathological changes (table 6), while treatment with insulin (group IV) significantly reduced all the histopathological changes except lobular inflammation.

Compared with the control group, only rats treated with NAC and insulin (group V) showed hepatic recovery characterized by complete regeneration of the hepatocyte and hepatic tissue appeared more or less normal in most cases with insignificant difference between rats in the control group (table 4).

Although treatment with NAC (group III) and treatment with insulin (group IV) significantly reduced the degree of hepatic injury this improvement not yet reaches the normal hepatic morphology except for complete improvement of steatosis (table 4).
**DISCUSSION**

The liver is an important organ which plays a vital role in the metabolism, detoxification and excretion of various endogenous and exogenous substances such as xenobiotics. Liver damage can be produced by bacterial and viral infections, alcohol abuse, environmental pollutants, and several other factors.\(^{[12]}\) Despite different causes, liver injuries are frequently associated with excess oxidative stress.

The high prevalence of the hepatic damage and its relation to DM make the patient to use both antidiabetics and liver supports. Treatment of diabetes in the cirrhotic patient is complex because of the presence of liver damage. Moreover, the hepatotoxicity induced by some oral hypoglycemic drugs may complicate such treatment. Accordingly, the pharmacological therapy must be closely monitored.\(^{[9]}\) Accordingly, our study was designed to explore the possible drug interaction between classic antidiabetic agent “insulin” and hepatoprotective agent “N-acetyl cysteine” in rats.

N-acetyl cysteine exerts its antioxidant action by assisting glutathione biosynthesis and scavenging the reactive oxygen species (ROS) formed during oxidative stress.\(^{[12]}\) In this study, hepatic injury induced by CCl\(_4\), a classic experimental model has been extensively used for evaluation of hepatoprotective activity. The mechanism is involved in free radicals which are generated during CCl\(_4\) metabolism by hepatic cellular cytochrome P450, including trichloromethyl and oxygen-centered lipid radicals, lipid peroxidation, mitochondrial damage, DNA modification and even cell death in organisms.

As regarding liver function tests, we found that AST, ALT and ALP level in rats injected with CCl\(_4\) showed higher significant statistical difference than control rats and this was similar to Abdel-Moneim and his co-workers that showed that rats treated with CCl\(_4\) had significantly elevated levels of serum ALT, AST and ALP compared to normal control group.\(^{[13]}\) Kumar and his team also showed CCl\(_4\) treated rats significantly increased serum ALT and AST compared to controls.\(^{[14]}\)
This could be attributed to that CCl4 is activated by cytochrome CYP2E1, CYP2B1 or CYP2B2, and possibly CYP3A, to form the trichloromethyl radical, CCl3*. This radical can attach to cellular molecules (nucleic acid, protein, lipid), impairing crucial cellular processes such as lipid peroxidation, with the potential outcome of fatty degeneration (steatosis). This radical can also react with oxygen to form the trichloromethylperoxy radical CCl3OO*, a highly reactive species. CCl3OO* starts the chain reaction of lipid peroxidation, which attacks and destroys polyunsaturated fatty acids, in particular those accompanying with phospholipids. This affects the permeabilities of mitochondrial, endoplasmic reticulum, and plasma membranes, resulting in the loss of cellular calcium sequestration and homeostasis, which can lead heavily to subsequent cell damage. Among the degradation products of fatty acids are reactive aldehydes, especially 4-hydroxynonenal, which attach easily to functional groups of proteins and inhibit important enzyme activities.15

Administration of NAC along with CCl4 markedly restored levels of the hepatic enzymes and significantly improved the liver functions, this is in accordance with previous study that reported that NAC attenuates liver injury in rats induced by CCl4.15 Also, Sathish and his team showed similar results. NAC significantly attenuated the elevated serum levels of ALT and AST indicating that the proportion of damaged hepatocytes was reduced as a direct result of NAC administration.16

The explanation is that the NAC administration to control and intoxicated animals elevated low-molecular-weight thiols in the liver. The NAC administration under CCl4-induced intoxication prevented oxidative damage of liver cells, decreased membrane lipid peroxidation, protein carbonyls and mixed protein-glutathione disulphides formation. At the same time the NAC treatment of intoxicated animals produce decrease of the elevated levels of blood plasma ALT and AST activities and bilirubin. The in vitro exposure of human red blood cells to NAC increased the cellular low molecular weight thiol levels and retarded tertbutylhydroperoxide-induced cellular thiol reduction and membrane lipid peroxidation as well as efficiently inhibited hypochlorous acid-induced erythrocyte lysis. Thus, NAC can refill non-protein cellular thiols and protect membrane lipids and proteins due to its direct radical-scavenging properties.17

Administration of insulin along with CCl4 has little improvement effect on the liver enzymes. In accordance with, Shao and his team levels of the hepatic injury biomarkers ALT, AST and γGGT were significantly decreased in the group with received insulin therapy.18 Numerous studies have shown that insulin can decrease the production of reactive oxygen species, elevate antioxidant capacity and protect liver cells, β cells and the body from oxidative damage so improve the liver functions.19 Insulin therapy can also significantly enhance lipid metabolism and lower the incidence of chronic complications of diabetes.20

As regarding Insulin level, We found that insulin level in rats injected with CCl4 showed higher significant statistical difference than control rats and this was similar to Hemeida and his team that showed injection of CCl4 produced progressive increase in blood glucose level, insulin, insulin resistance and decreased glucose tolerance and utilization in male albino rats.21 Also, these results are in agreement with those of Bianchi and his co-workers who postulated that, glucose intolerance is often seen in patients with numerous liver diseases such as alcoholic cirrhosis, post necrotic cirrhosis, chronic active hepatitis, hemochromatosis and acute hepatitis.22

This could be attributed to that in patients with liver disease, peripheral blood concentrations of insulin are raised.23 Either decreased hepatic degeneration or hyper secretion of insulin by the pancreas, or both, are possible clarifications for the elevated peripheral blood insulin levels commonly observed in liver diseases. It is established that the elevated level of insulin in the peripheral blood of the patient with chronic liver disease is secondary to decreased hepatic degradation of insulin. There is no evidence for an increase in pancreatic secretion of insulin.24 In fact, in relation to peripheral blood glucose, subjects with chronic liver disease actually decrease in secretion of insulin as compared with healthy subjects.25

However, these results showed disagreement with Mion and his team work26 that showed that neither acute nor delayed cirrhotic rats were glucose intolerant. Basal plasma glucose insulin concentrations were identical in controls and treated rats (acute and delayed). These variations may be somewhat caused by the toxin used to induce cirrhosis.27

As regarding adiponectin level, We found that adiponectin level in rats injected with CCl4 showed higher significant statistical difference than control rats and this was similar to Yoda-Murakami and his team who made a study using the murine model of CCl4 induced liver injury also showed marked elevation of adiponectin mRNA expression within 18 h after CCl4 treatment, which suggests that adiponectin may act as an anti-inflammatory protein that helps in the repair process during tissue injury.28 Also, Uribe and his team that showed that adiponectin level was significantly higher in patients with NASH than in the normal liver group.29 This could be attributed to that adiponectin is not produced by the liver in normal conditions and CCl4-induced lesions cause adiponectin deposition in rat hepatocytes and in vitro stimulation of adiponectin expression.28
On the other side, it showed disagreement with Abdel-Moneim and his co-workers[13] that showed CCI4 caused a significant decline in serum concentrations of the anti-inflammatory adipocyte-derived adiponectin and this could be explained by expression of adiponectin in adipose tissue is lesser in subjects with insulin resistance than in normal subjects and is associated with higher degrees of insulin sensitivity.[30]

Adiponectin monitoring is essential in hepatic diseases as deficiency of adiponectin induces insulin resistance, whereas over-expression of adiponectin improves insulin sensitivity and glucose tolerance.[31]

The histopathological data of this study also support to the results of biochemical markers. There was congestion in the CCI4 group accompanied by hydropid degeneration, sinusoidal dilatation, lobular and Portal inflammation, parenchymal necrosis and steatosis. While CCI4+NAC group showed decrease in hepatic affection than CCI4 group, suggesting that NAC regressed the toxic damages. Our study also showed a decrease in necrosis hemorrhage, congestion, and hepatocyte hydropid degeneration in CCI4+insulin group compared to CCI4 alone but not decrease in lobular and portal inflammation. Also, in CCI4+ NAC+ insulin group showed much in improvement in hepatic affection compared to CCI4 group. In conclusion, NAC ameliorated hepatocellular damage and liver injury in this animal model of toxic liver injury associated with reduction of lipid peroxidation. This was similar to Sahin and Alatas that demonstrated that histopathological injury is less severe with co-administered NAC in liver injury induced by CCI4.[32]

CONCLUSION
Finally we concluded that a good control of carbohydrate metabolism including insulin level, insulin resistance and adiponectin level helped in improving the prognosis of liver dysfunction diseases. So, in treatment application of hepatoprotective agent in combination with anti diabetic agent would have better outcome than application of each drug alone as regarding laboratory and histopathological results.

Funding
The authors received funding from Research Funding Comittee Assiut, Faculty of Medicine, Assiut University.

REFERENCES


