



## Role of Liver in Progression of Insulin Resistance in Relation to IGF-I and Insulin Levels in Rats with Acute Hepatotoxicity

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**SUMMARY.** The aim of the present study was to investigate the role of liver in progression of insulin resistance in relation to IGF-I levels in rats with acute hepatotoxicity, induced by carbon tetrachloride (CCl<sub>4</sub>) and acetaminophen. Wistar rats were divided into four equal groups of six rats each. Group-I (served as Control1) received olive oil, group-II received CCl<sub>4</sub>, group-III (served as control 2) received gum acacia and group-IV received acetaminophen. After 48 h of treatment, fasting blood samples were collected to determine biochemical parameters, and liver and pancreas in all groups were collected for histological evaluations. The levels of serum fasting glucose, AST, ALT, ALP, total bilirubin and insulin resistance were significantly more in group II & IV when compared with their respective control groups. Fasting insulin and IGF-I levels in toxicant treated groups were shown significantly lower than in control groups. The liver sections of toxicant treated rats showed hydropic degeneration (ballooning) in centrilobular hepatocytes with single cell necrosis surrounded by neutrophils. The serum IGF-I level could be a useful marker for identifying subjects at risk of developing type-II diabetes mellitus and possible cardiovascular complications.

### INTRODUCTION

Hepatotoxicity is often associated with glucose intolerance and insulin resistance <sup>1,2</sup>. Recent studies in patients with liver cirrhosis have shown that the failure of insulin to stimulate glucose uptake can be attributed to defects in nonoxidative glucose metabolism <sup>3</sup>, but the underlying mechanisms remain unknown. Lang *et al.* <sup>4</sup> suggested that the normal liver produces a “factor” that facilitates glucose uptake in extra hepatic tissues and lack of this factor may be the cause of the insulin resistance in chronic liver failure. The mysterious factor could be Insulin-like growth factor I (IGF-I), because it is produced a large extent in the liver and has been reported to be reduced in patients of liver cirrhosis <sup>5</sup>. It was reported the hypoglycemic activity of IGF-I by stimulating the glucose uptake similar to the effects of insulin in normal <sup>6</sup>, diabetic rats <sup>7</sup> and in humans <sup>8</sup>. The chemical structure of IGF-I is similar to that of insulin, and the

biological action of IGF-I, although mediated predominantly via its own receptor, is to a lesser extent exerted through the insulin receptor <sup>9</sup>. Most of the circulating IGF-I is bound to specific carrier proteins, and some of these IGF-I-binding proteins may modulate the biological effects of the hormones <sup>10</sup>. In the present study, we investigated the role of liver in progression of insulin resistance in relation to IGF-I and insulin levels in rats with carbon tetrachloride (CCl<sub>4</sub>) & acetaminophen induced acute liver toxicity.

### MATERIALS & METHODS

#### *Animals*

Male Wistar albino rats (Mahaveer Enterprises, Hyderabad, India) of 12 weeks old (250–300 grams) were selected and housed in polypropylene cages in a room where the congenial temperature was 27 °C ± 1 °C and 12 h light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7

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days and supplied with a standard pellet diet and water *ad libitum*. All procedures using animals were reviewed and approved by the Institutional Animal Care and Use Committee.

### **Induction of acute hepatotoxicity in rats**

In this study, acute hepatotoxicity in rats was induced by two known toxicants i.e. carbon tetrachloride (CCl<sub>4</sub>) and acetaminophen. Carbon tetrachloride (1:1 v/v mixture of CCl<sub>4</sub> and olive oil, 2 ml/kg/i.p.)<sup>11</sup> and Acetaminophen (2.5 g/kg/oral)<sup>12</sup> were administered to 12 h fasted rats to induce acute hepatotoxicity.

### **Study Design**

Animals were divided into four groups of six rats in each. Group-I: (served as control 1) received olive oil, group-II received CCl<sub>4</sub> mixed with olive oil, group-III (served as control 2) received gum acacia and group-IV received acetaminophen suspension in gum acacia. After 2 days, the blood samples were collected from the carotid artery and serum was separated. All the rats were sacrificed; the livers and pancreas were removed immediately, washed with ice-cold saline and stored in formalin for histopathological examination.

### **Biochemical Evaluations**

The activities of serum hepatic marker enzymes namely aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum fasting glucose levels were estimated using standard kits from Labtrol (Span) Merck Laboratories by using Selectra Junior Autoanalyzer (Vital Scientific, Spankeren, Netherlands). Fasting serum insulin concentrations were determined by enzyme linked immuno sorbent assay (ELISA) kit (Mercodia AB., Sweden) and fasting serum IGF-I concentrations were determined by immuno enzymometric assay IEMA by using OCTEIA Rat/Mouse IGF-I kit (Immunodiagnostic System Ltd., UK). Insulin resistance was, assessed by using the previously validated homeostasis model, calculated from the fasting insulin and glucose concentrations according to the formula: HOMA-IR = (insulin X glucose)/405<sup>13</sup>. Similarly, insulin sensitivity was, assessed by using the previously validated homeostasis model, calculated from the fasting insulin and glucose concentrations according to the formula: HOMA-S = 1/ HOMAIR<sup>13</sup>.

### **Histopathological Evaluations**

For the histopathological observations, fresh

liver and pancreas tissues previously trimmed to approximately 2 mm thickness, were placed in plastic cassettes and immersed in neutral buffered formalin for 24 h. The fixed tissues were processed routinely and then embedded in paraffin blocks, sectioned with microtome (0.7 μ thickness) deparaffinized and rehydrated using standard techniques. The extent of toxicant-induced necrosis was evaluated by assessing morphological changes in liver sections stained with hematoxylin and eosin using standard techniques and photographed.

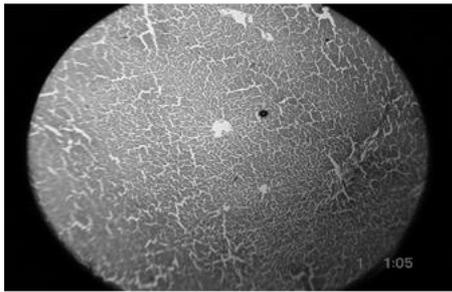
### **Statistical Analysis**

All variables were expressed as means ± SD. Data were processed using Excel and analyzed using SAS version 9.1.3 (SAS Institute Inc., Cary, NC, USA). The four animal groups in this parallel study were labeled as: CCl<sub>4</sub>, GACG, OOCG and PIHG. Statistical analysis between all groups was carried out using one-way analysis of variance (ANOVA) and Dunnett test on ten relevant biochemical parameters (BW, Glucose, AST, ALT, ALP, T-Bilirubin, IGF-I, Insulin, HOMA-IR and HOMA-S) with group as a factor. Within each ANOVA group means were compared with a t-test and 95% confidence intervals (CI) for difference in group means were computed. P-values and CI were unadjusted for multiple comparisons. A *p*-value < 0.05 was considered statistically significant.

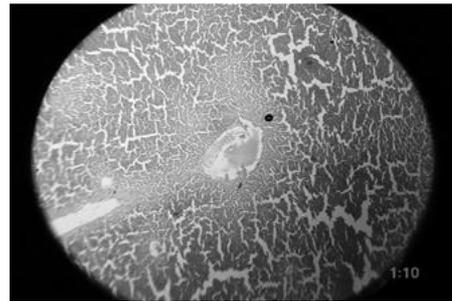
## **RESULTS**

### **Biochemical Evaluations**

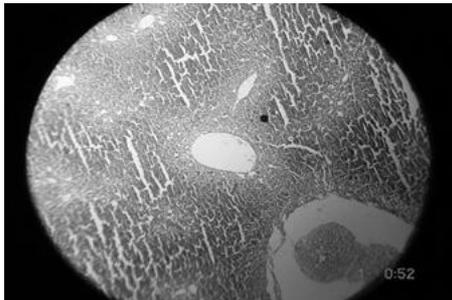
The serum glucose, AST, ALT, ALP, T-Bilirubin, IGF-I, Insulin, HOMA-IR and HOMA-S levels in control and toxicant treated groups were shown in Table 1. The elevated levels of AST, ALT, ALP and total bilirubin concentrations in both toxicants (CCl<sub>4</sub> and acetaminophen) treated groups indicates the successful induction of acute hepatotoxicity in rats<sup>11,12</sup>. The glucose, AST, ALT, ALP, total bilirubin and insulin resistance (HOMA-IR) were significantly elevated (*P* < 0.0001, *P* < 0.0001, *P* < 0.0001, *P* < 0.0001, *P* = 0.0005 and *P* = 0.0009, respectively) in the toxicant treated groups when compared with control groups (Table 1). Insulin sensitivity (HOMA-S) was, however not significantly altered in these groups when compared with control treated rats. On the other hand fasting levels of insulin and serum IGF-I were significantly reduced (*P* < 0.0001, *P* < 0.05, respectively) in toxicant treated groups when compared with control groups.



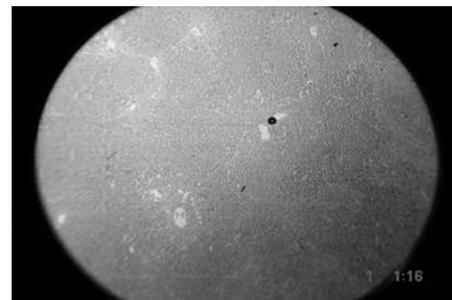
**Figure 1.** Liver tissue of rats treated with olive oil showing normal histology.



**Figure 3.** Liver tissue of rats treated with gum acacia showing normal histology.



**Figure 2.** Liver tissue of rats treated with CCl<sub>4</sub> showing hydropic ballooning.



**Figure 4.** Liver tissue of rats treated with acetaminophen showing regeneration of hepatocytes and obliteration of hepatic sinusoids.

	<b>Group-1 (Olive oil)</b>	<b>Group-2 (CCl<sub>4</sub>)</b>	<b>Group-3 (Gum Acacia)</b>	<b>Group-4 (Acetaminophen)</b>	<b>P-Value</b>
Body Weight (g)	262 ± 12.55	266.67 ± 46.55	266.00 ± 14.75	265.00 ± 22.36	0.994
Glucose (mg/dL)	86.2 ± 18.34	166.83 ± 31.83	94.00 ± 7.07	142.00 ± 22.54	<0.0001
ALT (IU/l)	91.16 ± 10.36	321.02 ± 52.62	74.46 ± 6.51	779.14 ± 49.65	<0.0001
AST (IU/l)	181.38 ± 24.07	778.17 ± 49.84	183.54 ± 18.25	449.80 ± 39.74	<0.0001
ALP (IU/l)	236.08 ± 172.15	646.22 ± 171.82	238.70 ± 75.94	929.88 ± 30.50	<0.0001
T-Bilirubin(IU/l)	0.024 ± 0.01	0.26 ± 0.1	0.05 ± 0.03	0.22 ± 0.14	0.0005
IGF-I (ng/ml)	500.06 ± 248.99	291.77 ± 131.14	498.41 ± 105.02	250.09 ± 141.80	0.0427
Insulin (µU/l)	0.30 ± 0.08	0.21 ± 0.10	0.90 ± 0.31	0.27 ± 0.11	<0.0001
HOMA-IR	0.066 ± 0.02	0.08 ± 0.04	0.21 ± 0.09	0.09 ± 0.03	0.0009
HOMA-S	17.42 ± 8.29	15.17 ± 8.01	5.45 ± 2.49	12.20 ± 5.62	0.0532

**Table 1.** Anthropometric and biochemical characteristics of the study. Data are means ± SD (n=6).

**Histopathological Examination**

In control groups of rats, liver sections showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and central vein (Figs. 1 and 3). In CCl<sub>4</sub> treated animals liver sections showed hydropic degeneration (ballooning) in centrilobular hepatocytes with single cell necrosis surrounded by neutrophils (Fig. 2). The liver sections of rats treated with acetaminophen showed the presence of ballooning, inflammatory cells around

the central vein (Fig. 4). No changes were observed in the sections of toxicant group pancreas when compared with control animals (not shown).

**DISCUSSION**

In the present study we made an attempt to investigate the role of liver in progression of insulin resistance in relation to IGF-I and insulin levels by inducing hepatotoxicity using two known toxicants, CCl<sub>4</sub> and acetaminophen.

CCl<sub>4</sub> treatment has been reported to induce “human-like” liver cirrhosis in rats, in which the hepatotoxicity is irreversible and recognized as the micronodular cirrhosis that closely resembles the human disease<sup>14,15</sup>. Although acetaminophen was considered to be safe at therapeutic doses, it produces a centrilobular hepatic necrosis at higher doses<sup>16</sup>. Whereas the initial biochemical and metabolic events that occur in the early stages of toxicity have been well described, but the precise mechanisms of hepatocyte death are poorly understood. Necrosis is recognized as the mode of cell death in this toxicant induced hepatotoxicity and apoptosis has been ruled out<sup>17,18</sup>. Acute hepatotoxicity in rats was confirmed by the elevation of serum AST, ALT, ALP, total bilirubin levels and histological changes in both toxicants treated groups when compared with respective control groups. The pathogenesis of insulin resistance in liver cirrhosis is still unknown, and several factors may be involved, including lower plasma concentrations of IGF-I, increased plasma concentrations of free fatty acids, growth hormone, glucagon, catecholamines, and possibly altered membrane lipid composition<sup>19</sup>. The present study demonstrates that the rat model of liver cirrhosis induced by CCl<sub>4</sub> treatment is associated with marked insulin resistance and significant lowering of plasma IGF-I levels. The growth hormone-IGF-I axis is abnormal in both insulin-dependent and non-insulin dependent diabetes mellitus. Many patients with insulin-dependent diabetes have some hepatic resistance to growth hormone, with elevated serum growth hormone concentrations and decreased serum IGF-I concentrations. This is probably as a result of insufficient insulin action on the liver<sup>20</sup>. The present study results demonstrated that liver cirrhosis produced a significant reduction of serum IGF-I levels in rats, probably due to abnormal growth hormone-IGF-I axis. In this study, a significant

hepatotoxicity was noticed but the  $\beta$ -cells of the pancreas were not affected. Significant increase in blood glucose levels associated with low levels of insulin and IGF-I in this model with intact  $\beta$ -cells is very difficult to explain. The most probable explanation is involvement of GH-IGF-I-insulin axis. Most of the circulating IGF-I is, derived from the liver and is regulated by the growth hormone (GH), insulin, and nutritional intake. It has profound effects on the regulation of proliferation and differentiation of many cell types, as well as metabolism<sup>21-23</sup>. It has been hypothesized that IGF-I, working through its own receptor, enhances the insulin sensitivity which accounts for part of its glucose lowering activity. IGF-I seems to be a substrate for the synthesis of preproinsulin, proinsulin and insulin<sup>24</sup>. Since the molecular structures of preproinsulin and IGF-I are similar it may be explained that the low levels of insulin are secondary to decreased levels of IGF-I.

In conclusion, the present study indicates that the acute hepatotoxicity produced by CCl<sub>4</sub> and acetaminophen, results in lowering of serum IGF-I levels which in turn down regulates pancreas to produce insulin secondary to liver pathology and abnormal pattern in GH-IGF-I axis. The serum IGF-I levels therefore could be a useful marker for identifying subjects at risk of developing type-II diabetes mellitus. Further studies preferably are required to definitively prove this hypothesis.

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