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# Effect on Hepatic Parameters and Hepatocytes following administration of antihypertensive, hypolipidemic and hypoglycemic drugs in combination (Part II)

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**ABSTRACT**

Liver damage is the serious additive effect of drugs used for the treatment of patients suffers from diabetes, hypertension and hyperlipidemia. In continuation of our previous published research the present study is done to evaluate the toxic effects of combination of drugs prescribed for above stated diseases. Combinations of drugs were given to rabbits for the period of two months and their effects on liver function tests were observed. As compared to control rabbits, the combination Acarbose, Lisinopril and Atorvastatin showed increased Glutamic-Pyruvic Transaminase (GPT), Alkaline Phosphatase (ALP) and Gamma glutamyl transferase (γ-GT) ($p < 0.005$), while the animals received combination Metformin, Amlodipine and Atorvastatin showed significant decrease in Alkaline Phosphatase (ALP) and Gamma glutamyl transferase (γ-GT) ($p < 0.005$). The study proved increased in toxic effect of these drugs on hepatocytes when above combination are given, however, Glibenclamide, Losartan and Atorvastatin seems to be safer then others combinations given.

## INTRODUCTION

Blood pressure, serum cholesterol and glucose levels are the age associated risk factors are necessary to screened for controlling cardiovascular disorder. In complex diseases multiple mediators are involve in pathogenesis by similar or unusual mechanisms. Simultaneous blockade of these pathological targets can be achieved by using multiple medicines to yield better therapeutic approach (De Marchi *et al.*, 2011). The use of drugs in multiple disorders depends on the age, family history, life style, and other factors contributing such as diabetes, uncontrolled hypertension, and history of heart disease, total cholesterol and LDL. Several substances like phenobarbital, phenytoin, ethanol, grapefruit juice, and cigarette smoke induce hepatic enzymes and alter plasma levels of these drugs, resulting in extrahepatic adverse effects (Petri *et al.*, 2006 and Usui *et al.*, 2009). Enzyme enhancers play major role in increasing hepatotoxicity, as substrate competition occurs when ethanol is used in combination with acetaminophen.

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Ethanol reduces rate of metabolism of acetaminophen to its byproduct those are toxic and harmful. Interaction of ethanol (the enzyme inducer) with acetaminophen enhances liver damage (Clark *et al.*, 2002). And when ethanol is withdrawn the enzyme cytochrome P-450 which was slowed down in presence of Ethanol enhances the formation of toxic metabolites.

### Drug Induced Hepatoxicity

Thousand of chemical compound produce injury to the liver which can be interpreted by examination of liver enzymes and biochemical levels in blood (McDonald and Frieze, 2008). 5% of the hospitalizations and more than 50% acute liver failure results due to drug induced hepatotoxicities increasing mortality and morbidity rate. Many drugs have been withdrawn from Market or having limitation for use due to the possibility of drug induce liver injury e.g. troglitazone, bromfenac, pemoline, tolcapone, trovafloxacin and benoxaprofen (Bolesta and Roslund, 2008). Chance of hepatic injury further enhance due to the drugs affecting liver function if patient is already suffering due to chronic liver disease.

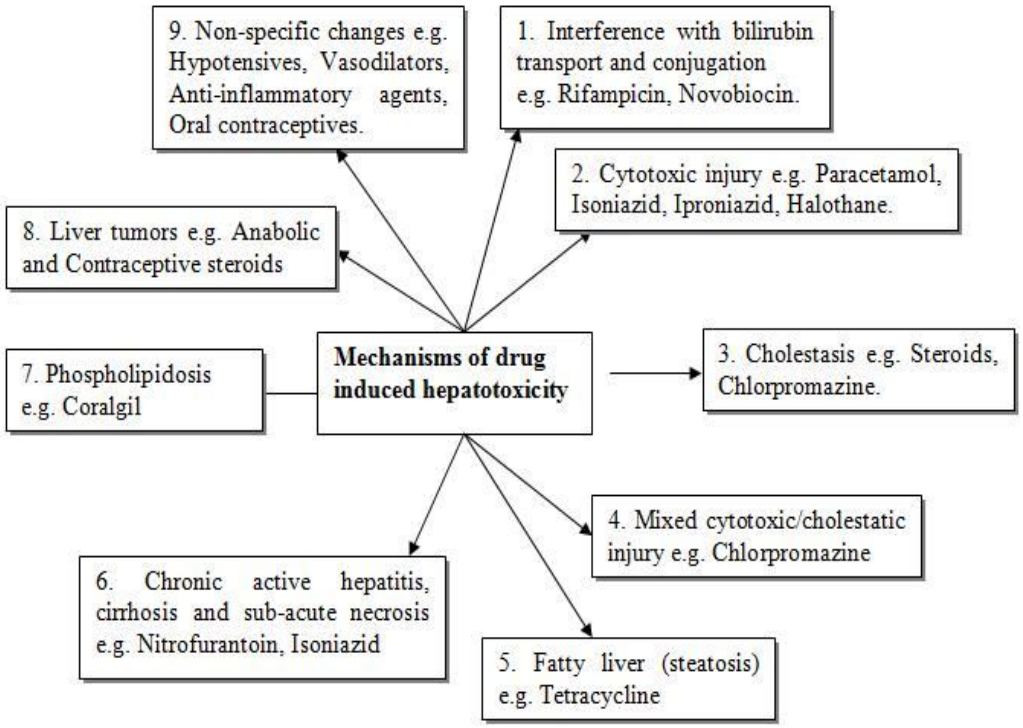


**Fig. 1:** Drugs Induced Liver Injuries (Cardona *et al.*, 2000- Wallace, 2004).

In liver cirrhosis patients rate Rates of drug may be reduced up to 50 percent. Therefore, all medicine inducing hepatoxicity should be taken with extreme caution (Conjeevaram *et al.*, 2007)**.**

Figure 2 explains various types of liver damage induced by some important drugs (Bolesta and Roslund, 2008)**.**

## BIOCHEMICAL TOXICITY TESTING

### Materials and Methods

#### *Sample Collection*

Blood samples collected in siliconised glass tube (Heyns *et al.*, 1981) and plasma was immediately separated out by centrifugation at 3000 rpm for 15 min to yield platelet poor plasma by Humax 14 K (Germany).

### Hepatic Parameters

**a)-** Bilirubin (Total and Direct – TBR and DBR), Bilirubin (Total and Direct) in the serum was estimated by photometric test (Jendrassik and Grof, 1938).

**b)-** Glutamic-Pyruvic Transaminase (GPT), GPT in the serum was estimated by kinetic method with reference to the International Federation of Clinical Chemistry (Bergmeyer and Horder, 1980).

**c)-** Gamma glutamyl transferase (γ -GT), γ -GT in the serum was estimated by colorimetric method (Szasz and Persijin, 1974 – Persijin and Van der silk, 1976).

**d)** Alkaline Phosphatase (ALP), Method use to the recommendation of international Federation of Clinical Chemistry (Tietz *et al.*, 1938).

### Statistical Analysis

All values were compared with control by taking mean and standard error to the mean using two-way analysis of variance (ANOVA) followed by post hoc. Data was reported as mean ± standard error to the mean with 95% confidence interval and p-values were observed.

## RESULT

### Hepatic parameters

Table 1 reveals the comparison of DBR, TBR, GPT, ALP, and γ-GT levels between control animals and animals kept on combination drugs for a period of 60 days. Animals received GlLAt combination revealed no significant changes in DBR, TBR, GPT, ALP and γ –GT levels at the completion of dosing. Animals received GLoAt combination revealed highly significant increase in GPT, ALP and γ–GT levels i.e. 119.24±0.25 µ/l, 63.08±1.70 µ/l and 21.47±2.10 µ/l as compared to control group 60.1± 0.31µ/l, 46±3.14 µ/l and 5.55± 0.46 µ/l respectively, while the other hepatic parameters were not altered significantly. Animals received MAAt combination showed highly significant decrease in ALP and γ–GT levels i.e. 27.42±2.25 µ/l and 2.72±0.25 µ/l with respect to control i.e. 46±3.14 µ/l and 5.55± 0.46 µ/l respectively, while the other hepatic parameters were not altered significantly.

**Table. 1:** Comparison of Hepatic Parameters Following 60 days Administration of Combinations.

| Parameters/ Groups | DBR (mg/dl) | TBR (mg/dl) | GPT (µ/l) | ALP (µ/l) | γ-GT (µ/l) |
|---|---|---|---|---|---|
| Control | 0.50 ±0.45 | 0.07 ± 0.34 | 60.10 ±0.31 | 46.00 ±3.14 | 5.55 ±0.46 |
| GlLAt | 0.45 ±1.21 | 0.17 ±0.25 | 61.47 ±0.43 | 42.48 ±0.25 | 4.81 ±0.12 |
| GLoAt | 0.43 ±0.51 | 0.13 ±0.57 | 119.24 ±0.25** | 63.08 ±1.70** | 21.47 ±2.10** |
| MAAt | 0.44 ±0. 04 | 0.10 ±0.43 | 65.70 ±0.57 | 27.42 ±2.25** | 2.72 ±0.25** |

Note: GLoAt = Glibenclamide, Losartan and Atorvastatin, GlLAt = Acarbose, Lisinopril and Atorvastatin and MAAt = Metformin, Amlodipine and Atorvastatin n=10, Mean ± S.E.M, *p < 0.05 significant with respect to control, **p <0.005 highly significant with respect to control.

## MICROSCOPIC TISSUE EXAMINATION

### Hepatic Tissue Examination

Gross examination of liver did not show any macroscopic alteration in any group. Microscopic examination of hepatic tissue in control animals and those received combinations GlLAt and MAAt did not reveal any microscopic changes in hepatic tissue (plate 1), however microscopic examination of hepatic tissue of animal group received Microscopic examination of hepatic tissue of two randomly selected animals received GLoAt combination revealed chronic inflammation with steatosis in one specimen of liver (plate 2). while the other showed marked congestion of blood vessels, hepatocytes are arranged in cords with dilated sinusoids (plate 5) as well as marked elevation in dimension of liver i.e. 7.5x6.5x3 cm. The section show normal architechure of liver and normal gall bladder.

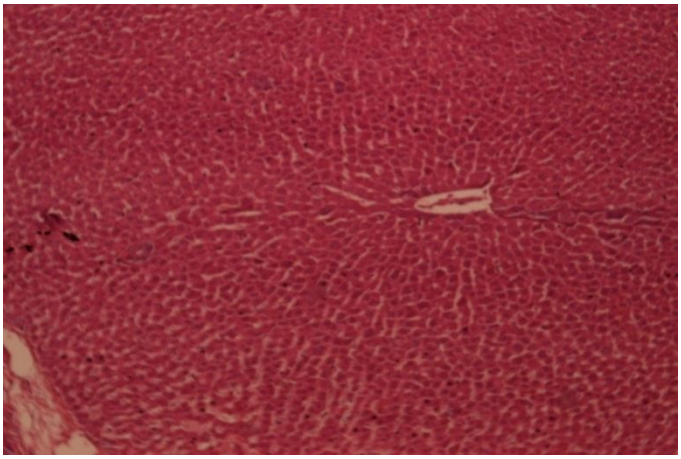

**Plate. 1:** Hepatic Tissue Showing No Change.

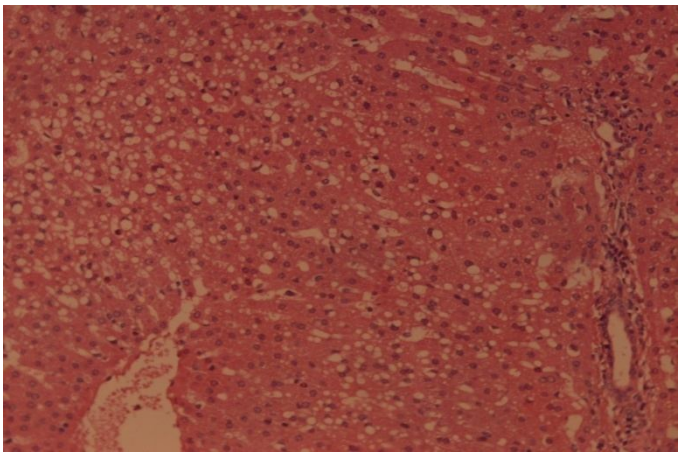

**Plate. 2:** Hepatic Tissue Showing Chronic Inflammation With Steatosis.

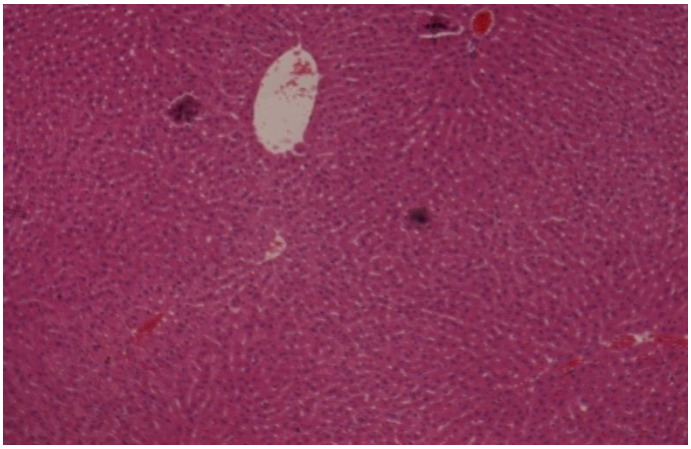

**Plate. 3:** Hepatic Tissue Showing Marked Congestion.

## DISCUSSION

A number of metabolic and physiological changes occur with normal aging process both in animals and humans. Hence elderly peoples are at greater risk of disorders like hypertension, diabetes, hyperlipidemia etc. The risk of hypertension usually increases with age since arteries become hardened and renal function decreases, which may be complicated due to associated hyperglycemia, as it may also cause changes in vascular function and structure (Agodoa *et al.*, 2001).

Similarly hyperlipidemia is another growing problem among the population. The main phenomena behind the prevalence of such disorders are reduced physical exercise, decreased oxygen consumption, relative increase in adipose over muscle mass, decreased insulin sensitivity, and increased blood pressure. All may contribute to the acceleration of atherosclerosis associated with the coronary heart disease such as angina, arrhythmias, myocardial infarction, heart failure and stroke (McGovern, 2005).

Drug interactions are of great concern because doctors and patients are unaware of the risks of toxicities due to simultaneous administration of drugs. It is therefore necessary to investigate such combinations that are less likely to interact with each other. Several studies have been done previously to determine the toxicities associated with the use of various combinations. But little work has done to evaluate toxicities associated with the simultaneous use of antihypertensive, antidiabetic and anti hyperlipidemic drugs. The present work has been therefore specially design to assess toxicity of not only individual drugs but various combinations that may be probably used in case of multiple disorders and to suggest a combination safer for the

patients (Agodoa *et al.*, 2001-Ji *et al.*, 2011). The animals received GlLAt combination did not show any significant change in liver function test similarly there were no changes in hepatic tissue although acarbose and atorvastatin both are contra indicated in liver diseases. However several studies support our finding that atorvastatin may help to attenuate liver steatosis (Ji *et al.*, 2011). More over combination contains lisinopril which is not associated with any liver damage. Thus GlLAt may be considered as safer in our results and finding.

Animals received combination GLoAt when compared with control showed highly significant increase in the level of GPT, ALP and γ-GT, while microscopic examination of the hepatic tissue of these animals also showed chronic inflammation with steatosis in one specimen and congestion in another specimen. Hence it may be concluded that this combination is not safer in comparison to other combinations (Tabak *et al.*, 2002).
Animals received MAAt combination showed highly significant change in ALP and γ-GT however, no changes were found in hepatocytes which is supported by the study that metformin protect liver damage due to inflammation (Ina Bergheim *et al.*, 2006- Sasaki *et al.*, 2010).

## CONCLUSION

It is concluded from the above study that the combinations GLoAt and MAAT used for the treatment of hypertension, diabetes and hyperlipidemia effecting the liver functions significantly, however, the combination GLoAt is safer hence no any change observed in hepatic function tests as well as hepatocytes. Multi drug therapy used for the management of multiple disorders among the younger has different strategy than in elderly patients as many drugs may be less effective and less suitable for elderly patients. Multiple drug administration increases the risk of toxicities hence treatment plans should be adjusted to minimize the chance of drug induced toxicities due to drug interaction.

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