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Original Article

Effect of Irbesartan and Rutin in Alcohol Plus High Fructose Corn Syrup Induced Insulin Resistance Syndrome in Rats

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ABSTRACT

The objective of the study was to invistigate the effect of Irbesartan and rutin in High Fructose Corn Syrup (HFCS) plus alcohol induced Insulin Resistance Syndrome in rats. Our study demonstrated the beneficial effects of Irbesartan and rutin on both abnormal metabolic characters and vascular dysfunction in insulin resistant rats. Initially we have validated the predaibetic Insulin Resistance Syndrome rat model by feeding High Fructose Corn Syrup to the male Sprague Dawley rats. Development of Insulin Resistance Syndrome and glucose intolerance was assessed by performing the plasma biochemical analysis such as plasma glucose, triglyceride, insulin and total cholesterol levels. Glucose intolerance was assessed by performing an intraperitoneal glucose tolerance test. And Histopathological studies of Liver were conducted. In the present study, we examined the effect Irbesartan and rutin and the relationship between the plasma adiponectin concentration and adiposity, body fat distribution, lipid profile, renal profile, liver enzymes, Cardio vascular parameters, histopathology of Liver, in High Fructose Corn Syrup fed Sprague-dawley rats. It was concluded that Irbesartan and rutin are effective in treating Insulin Resistance Syndrome and alcohol induced liver toxicity Further studies are needed to explore the underlying mechanisms.

Keywords: High fructose corn syrup, Irbesartan, Rutin, Alcohol.

INTRODUCTION

Insulin Resistance Syndrome is a set of risk factors that includes: abdominal obesity, a decreased ability to process glucose (increased blood glucose and/or insulin resistance), dyslipdemia, and hypertension. Insulin Resistance Syndrome is thought to be caused by adipose tissue dysfunction and insulin resistance. Dysfunctional adipose tissue also plays an important role in the pathogenesis of obesity-related insulin resistance[1]. Both adipose cell enlargement and infiltration of macrophages into adipose tissue result in the release of pro-inflammatory cytokines and promote insulin resistance. Insulin resistance appears to be the primary mediator of Insulin Resistance Syndrome. Insulin promotes glucose uptake in muscle, fat, and liver cells, and can influence lipolysis and production of glucose by hepatocytes[2]. Additional contributors to insulin resistance include abnormalities in insulin secretion and insulin receptor signaling, impaired glucose disposal, and pro-inflammatory cytokines. These abnormalities, in turn, may result from obesity with related increases in free fatty acid levels and changes in insulin distribution (insulin accumulates in fat). To mimic this nongenetic prediabetic insulin resistance condition in rats, we are feeding the rats with a high calorie food in the form of High Fructose Corn Syrup (HFCS)[3]. Development of vascular dysfunction was reported in HFCS fed insulin resistant animals, further PPAR- γ agonists such as Irbesartan had shown promising improvement in the metabolic abnormalities associated with insulin resistant. In addition to its effect on metabolic parameters the vascular protective nature has been demonstrated in several experiments, these agents performing the vascular protection through anti oxidative, anti-inflammatory and antiproliferative mechanisms. But the conventional TZDs are well known for their side effects such as weight gain and heart failure; hence these compounds are not advisable to obese patients who are already at great risk for CVDs. Therefore there is a tremendous need to develop a drug to treat metabolic syndrome and associated CVDs and it should be free of above mentioned side effects[4]. On the other side, RAS has identified as a key player in the development of CVDs associated with diabetes, most of the deleterious effects of RAS are mediated through AT_1 receptor stimulation by Ang II. Blockade of RAS is found to be beneficial in these situations, but multidimensional therapeutic interventions are required to combat coexisting diabetes and CVDs, till now there is no single drug available to treat the above condition, so there is a need of combination regimen to treat metabolic and cardiovascular disorders effectively[5]. Fortunately the serendipitous discovery of some of the ARBs for their additional PPAR-γ agonistic property has given the idea for the development of new and evaluation of existing drugs in metabolic syndrome. This discovery raised the hope for the development of new dual pharmacophores in future.

Irbesartan is reported to be a dual pharmacophore (ARB/ PPAR- γ)[6]. Some studies have demonstrated the beneficial effects of Irbesartan on metabolic profile in insulin resistant and diabetic conditions, but there are no systematic studies carried out to evaluate the beneficial role of these dual pharmacophore on vascular protection in Insulin Resistance Syndrome. In this present work we studied the effect of Irbesartan on both vascular and metabolic parameters. Administration of alcohol at this condition further increases the risk. Combination of HFCS and alcohol may increase reactive oxygen species this will damage the liver[7]. Some studies shows that rutin a Flavonoid

glycoside having powerful anti oxidant as well as powerful anti inflammatory property in the present work we studied the effect of rutin on vascular and metabolic parameters[8].

MATERIALS AND METHODS

Animals

Male Sprague Dawley (SD) rats of 160-180 g body weight were procured from the national institute of nutrition, Hyderabad India. The animals were maintained under controlled room Temperature (22±2 °C) and humidity (55 ± 5%) with a 12-h light / dark cycle. Animals were housed in three animals per cage and allowed to food and water ad libitum[9]. Rats were grouped and fed with either standard chow diet (NPD) or High Fructose Corn Syrup (HFCS). All experiments and protocols described in present study were approved by the Institutional Animal Ethical Committee (IAEC) of Andhra University College of Pharmaceutical Sciences, Visakhapatnam. (Regd. No.516/01/A/CPCSEA).

Chemicals

Irbesartan and Rutin were procured from sigma chemicals, St Louis, USA. Alcohol was purchased from neon labs, Mumbai, INDIA. All other chemicals and reagents were used of analytical grade.

Dose Selection

In the present study treatment control groups 10 % High Fructose Corn Syrup (HFCS), alcohol at a dose of (20% v/v) and the treatment groups Irbesartan dose (5mg/kg), rutin (50mg/kg) were administered by per oral route in morning throughout the study period.

Experimental Design

High Fructose Corn Syrup (HFCS) Induced Insulin Resistance Syndrome: Experimental study was carried out using adult Male Sprague Dawley rats weighing between 150-170g. The animals were housed in polypropylene cages of dimension 16”×9”×7”. The cages were maintained under clean and hygienic conditions[10]. Animals were acclimatized to light and temperature with a 12h-12h dark-light cycle, The rats were fed with commercial pelleted rat feed and water ad libitum.

Total 60 adult male rats were selected for the study and divided in to 10 groups each containing 6 rats. Group 1 is the Control (fed with normal pellets chows). Group 2 rats have received HFCS (10% v/v.p.o.) with normal pellets). Group 3 animals have received Alcohol (20% v/v p.o) with normol pellets. Group 4 animals were fed on HFCS (10% v/v) + Alcohol (20% v/v) with normol pellets. Group 5 animals received HFCS + Irbesartan (5mg/kg) p.o with normol pellets. Group 6 animals received Alcohol + Irbesartan (5mg/kg) p.o with normol pellets. Group 7 animals received HFCS + alcohol + Irbesartan (5mg/kg) p.o with normol pellets. Group 8 animals received HFCS + rutin (50mg/kg) p.o with normol pellets. Group 9 animals received Alcohol + rutin (50mg/kg) p.o with normol pellets. Group 9 animals received HFCS + alcohol + rutin (50mg/kg) p.o with normol pellets.

Composition of The Atherogenic Diet: 1% Cholesterol (Sd fine-chem limited), 0.5% Cholic acid (LOBA CHEMIE), 5% Lard oil (Open Market). These diets were provided in addition to normal pellet chow[11].

Treatment Protocol: Induction of Insulin Resistance Syndrome in the experimental animals was carried out by feeding the animals with the High Fructose Corn Syrup .Induction of liver toxicity in the experimental animals was carried out by feeding the animals with the alcohol. Irbesartan was administered orally in a dose of 5mg/kg, p.o. daily for the entire study

period. Rutin was administered orally in a dose of 50mg/kg, p.o. daily for the entire study period. 24hrs before the sacrifice of the study animals, they were kept on fast but they had access to water. The blood samples were collected in eppendroffs tubes by puncturing the retro orbital plexus for biochemical estimation. Then experimental animals were sacrificed and the liver were collected and were kept in 15% V/V formalin solution for histopathological examination.

Statistical Analysis

All results are expressed as mean ± SEM. Statistical analysis was performed using the Graph pad prism 5, Graph pad software. One-way ANOVA followed by Dunnett's test was performed.

Histopathological examination

The liver was fixed in 10% formalin and embedded in paraffin. Five-micron thick sections were prepared and stained with hematoxylin and eosin (H&E)[12]. The tissue sections were evaluated under light microscopy by a blinded pathologist.

RESULTS AND DISCUSSION

Initially we have validated the predaibetic Insulin Resistance Syndrome rat model by feeding high fructose corn syrup to the male Sprague Dawley rats. The Insulin Resistance Syndrome rats showed a significant increase in the activity of serum LDL VLDL levels. And Treated rats showed a significant results. It was observed from the tables 1-3. The Insulin Resistance Syndrome rats showed a significant increase in the activity of serum LDL VLDL levels. The increased blood levels of total cholesterol, LDL, VLDL as well as lowered levels of HDL in high fructose corn syrup rat have been identified in the development of hypercholestremia, which is one of the risk factors for CAD. Administration of Irbesartan and rutin produces a significant decrease in the activity of LDL VLDL Our findings showed that obese rats treated with the Irbesartan and rutin exhibited significant decreases in LDL, VLDL activity from table3. The Irbesartan and rutin could prevent the development of atherosclerosis through regulating vascular inflammatory processes in rats fed with a high fructose corn syrup.

The current data showed a significant increase in the activity of enzymes AST and ALT in the Insulin Resistance Syndrome rats compared with control rats from table 3. Liver is bombarded by the free fatty acids (FFA) that pour out of the adipose tissue into the portal blood. This can directly cause inflammation within the liver cells, which then release further pro-inflammatory cytokines, leading to more hepatocyte injury and affecting the integrity of liver cells. The present results demonstrate that the Irbesartan and rutin showed a significant decrease in the activity of both AST and ALT from table 2 and showing a hepatic protective action.

The results obtained showed significant correlation between adiponectemia and adiposity, body fat distribution, in atherogenic diet fed obese animals. The preliminary investigation of effects of Irbesartan and rutin showed to improve the overall picture of metabolism especially the fatty acid metabolism. The plasma adiponectin levels were found to be significantly decreased in HFCS group ($p<0.001$), and a significant increase was observed in HFCS + IRBESAR-TAN (5mg/kg) groups ($p<0.001$), ALCOHOL + IRBESARTAN (5mg/kg) (obese + treated) group ($p<0.01$), HFCS + RUTIN (50mg/kg) group ($p<0.01$), ALCOHOL + RUTIN (50mg/kg) group ($p<0.01$), when compared to control group.

Histopathology Report

Histopathological studies of Liver were conducted. The results found are highly encouraging. The regenerative changes were observed in the group of animals treated with the Irbesartan and rutin the tissue elements lost due to the induction of disease condition namely Insulin Resistance syndrome and alcohol induced liver toxicity, the cardiovascular co-morbidity were regenerated and restored in the treated animals. High Fructose Corn Syrup Section studied shows liver parenchyma with partially effaced architecture. Most of the hepatocytes show apoptotic changes (Short-arrow, Fig.1), while some show cytoplasmic vacuolations (Long-arrow, Fig.2). Most of the central veins (Long-arrow, Fig.1) and sinusoids are dilated and congested (Short-arrow, Fig.2). Alcohol+Irbesartan [5 mg/kg]: Section studied shows liver parenchyma with intact architecture. Some of the hepatocytes show regenerative changes (Arrow, Fig.1), while few show regenerative changes (Arrow, Fig.1). Most of the central veins are dilated and congested (Arrow, Fig.2). There are seen scattered mononuclear inflammatory infiltrations within parenchyma. Alcohol + Irbesartan [5mg/kg]: Section studied shows liver parenchyma with intact architecture. Some of the hepatocytes show regenerative changes (Arrow, Fig.1), while few show regenerative changes (Arrow, Fig.1). Most of the central veins are dilated and congested (Arrow, Fig.2). There are seen scattered mononuclear inflammatory infiltrations within parenchyma. Alcohol+Rutin [50mg/kg]. Section studied shows liver parenchyma with intact architecture. Few of the central veins (Short-Arrow, Fig.1) and sinusoids show congestion (Long-Arrow, Fig.1). Some of the hepatocytes show regenerative changes (Arrow, Fig.2). There are seen few mononuclear inflammatory infiltration within parenchyma. Control Section studied shows liver parenchyma with intact architecture. Most of the perivenular hepatocytes and periportal hepatocytes appear normal. Within the hepatic parenchyma are seen few scattered mononuclear inflammatory cells [Fig.2, arrow].

The study showed that Irbesartan and rutin significantly counters the High Fructose Corn Syrup and alcohol induced cardiovascular properties and improves the lipid profile in the experimental animals. Adiponectin is a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism. In the present study, we examined the effect Irbesartan and rutin and the relationship between the plasma adiponectin concentration and adiposity, body fat distribution, lipid profile, renal profile, liver enzymes, CVS parameters, histopathology of Liver, in High Fructose Corn Syrup fed Sprague-dowley rats.

CONCLUSION

The results obtained in our study of weight gain/loss, weight of liver, fat distribution, lipid profile, liver enzymes, glucose, histopathology & the plasma adiponectin concentration, in the treated and untreated experimental animals indicate that properties of Irbesartan and rutin improve the overall health. However, further extensive investigation is needed to understand the overall mechanisms of improving overall metabolic changes. Thus Irbesartan and rutin are effective in treating Insulin Resistance Syndrome and alcohol induced liver toxicity Further studies are needed to explore the underlying mechanisms.

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Table 1. Effect of irbesartan and rutin on body weight and glucose levels in hfcs plus alcohol induced insulin resistance syndrome in rats

Groups	Body weight (Mean ± sem)	Glucose levels (Mean ±sem)
Control	181.0 ± 0.91	91.16 ± 1.35
HFCS	228.2 ± 3.32^{a***}	157.66 ± 1.47^{a***}
Alcohol (20%v/v)	216.6 ± 0.91^{a***}	138.16 ± 2.58^{a***}
HFCS + alcohol (20%v/v)	244.0 ± 1.82^{a***}	168.00 ± 0.96^{a***}
HFCS+ irbesartan (5mg/kg)	209.15 ± 0.85^{b***}	112.83 ± 2.08^{b***}
Alcohol (20%v/v) + irbesartan (5mg/kg)	213.0 ± 1.29^{b***}	114.0 ± 2.43^{c***}
HFCS+alcohol (20%v/v) + irbesartan (5mg/kg)	215.5 ± 0.64^{b***}	131.5 ± 2.17^{d***}
Hfcs + rutin (50mg/kg)	207.0 ± 1.47^{b***}	117.50 ± 2.86^{b***}
Alcohol (20%v/v) + rutin (50mg/kg)	206.25 ± 1.54^{b***}	104.66 ± 1.54^{c***}
HFCS+ alcohol (20%v/v) +rutin (50mg/kg)	212.5 ± 1.04^{b***}	115.2 ± 1.96^{d***}

Results are expressed as mean ± SEM. (n=6).Data was analyzed by One way ANOVA followed by Dunnett's multiple comparison tests.*P<0.05, **P<0.01, ***P<0.001, ns=non significant.

Table 2. Effect of irbesartan and rutin on total cholesterol, HDL and LDL levels in HFCS plus alcohol induced insulin resistance syndrome in rats

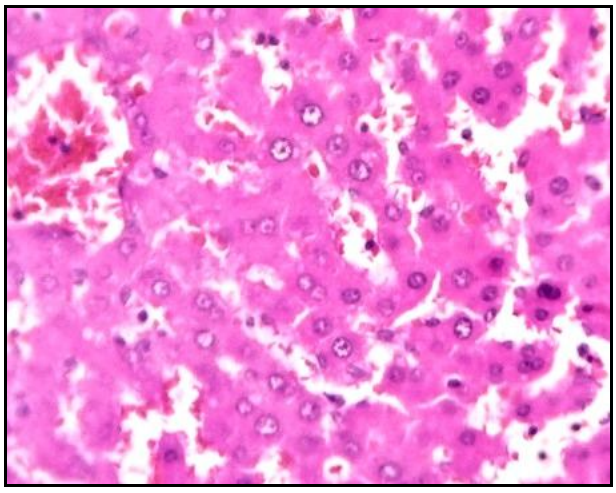
Groups	Total cholesterol Levels (Mean ±sem)	HDL levels (Mean ± sem)	LDL levels (Mean ± sem)
Control	50.16± 1.66	30.33± 0.76	25.66 ± 1.17
HFCS	76.83 ± 1.60^{a***}	23.50 ± 0.88^{a***}	32.66 ± 1.85^{a***}
Alcohol (20%V/V)	82.00 ± 1.23^{a***}	21.35 ± 0.88^{a***}	28.00 ± 0.57^{a***}
HFCS + Alcohol (20%V/V)	90.83 ± 1.24^{a***}	18.66 ± 0.76^{a***}	37.50 ± 0.76^{a***}
HFCS + irbesartan (5mg/kg)	43.00 ± 0.89^{b***}	36.16± 1.10^{b***}	18.83 ± 0.74^{b***}
Alcohol (20%v/v) + IRBESARTAN (5mg/kg)	47.83 ± 1.42^{c***}	27.33 ± 0.86^{c***}	18.66 ± 0.71^{c***}
HFCS+ Alcohol (20%v/v) + IRBESARTAN (5mg/kg)	45.33 ± 1.66^{d***}	27.83 ± 0.86^{d***}	19.50 ± 0.84^{d***}
HFCS + Rutin (50mg/kg)	57.50 ±1.54^{b***}	29.00 ± 0.89^{b***}	19.16 ± 0.60^{b***}
Alcohol (20%v/v) +Rutin (50mg/kg)	44.00 ± 1.29^{c***}	33.66 ± 1.26^{c***}	19.00 ± 0.51^{c***}
HFCS + Alcohol (20%v/v) + Rutin (50mg/kg)	56.66 ± 1.25^{d***}	32.83 ± 0.79^{d***}	18.16 ± 0.70^{d***}

Results are expressed as mean ± SEM. (n=6).Data was analyzed by One way ANOVA followed by Dunnett's multiple comparison tests.*P<0.05, **P<0.01, ***P<0.001, ns=non significant.

Table 3. Effect of irbesartan and rutin on liver weight, SGPT and SGOT levels in HFCS plus alcohol induced insulin resistance syndrome in rats

Groups	Liver weight (Mean ± sem)	ALT (SGOT) Leves (Mean ± sem)	AST (SGPT) Levels (Mean ± sem)
Control	2.57 ± 0.02	36.68 ± 0.97	25.29 ± 0.19
HFCS	3.28 ± 0.12 ^{a***}	85.36 ± 0.45 ^{a***}	82.56 ± 1.06 ^{a***}
Alcohol (20%v/v)	3.18 ± 0.26 ^{a***}	90.55 ± 0.76 ^{a***}	87.20 ± 0.60 ^{a***}
HFCS + alcohol (20%v/v)	3.92 ± 0.17 ^{a***}	110.25 ± 1.25 ^{a***}	98.20 ± 0.67 ^{a***}
HFCS+irbesartan (5mg/kg)	2.90 ± 0.08 ^{b***}	67.32 ± 1.53 ^{b***}	52.86 ± 2.16 ^{b***}
Alcohol (20%v/v) +irbesartan (5mg/kg)	2.92 ± 0.09 ^{c**}	55. 12 ± 0.89 ^{c***}	42.26 ± 0.54 ^{c***}
HFCS+alcohol (20%v/v) + Irbesartan (5mg/kg)	2.79 ± 0.10 ^{d***}	60.26 ± 0.77 ^{d***}	47.13 ± 0.70 ^{d***}
HFCS + rutin (50mg/kg)	2.83 ± 0.06 ^{b**}	56.12 ± 0.52 ^{b***}	46.48 ± 0.58 ^{b***}
Alcohol (20%v/v) + rutin (50mg/kg)	2.87 ± 0.02 ^{c***}	47.86 ± 0.26 ^{c***}	35.56 ± 0.07 ^{c***}
HFCS + alcohol (20%v/v) + rutin (50mg/kg)	2.80 ± 0.06 ^{d***}	52.16 ± 1.58 ^{d***}	40.06 ± 0.74 ^{d***}

Results are expressed as mean ± SEM. (n=6).Data was analyzed by One way ANOVA followed by Dunnett's multiple comparison tests.*P<0.05, **P<0.01, ***P<0.001, ns=non significant.



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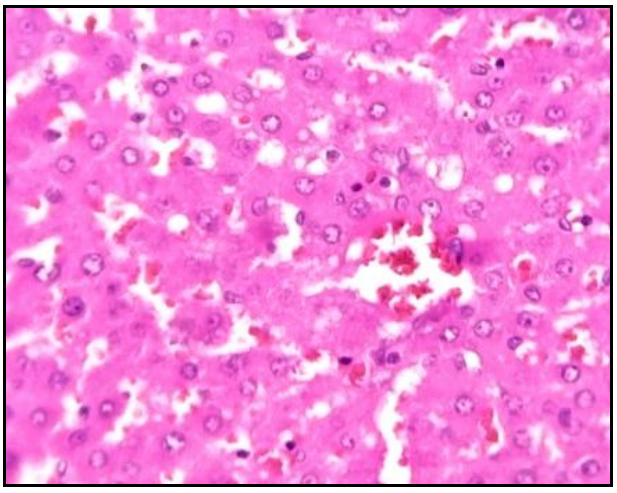
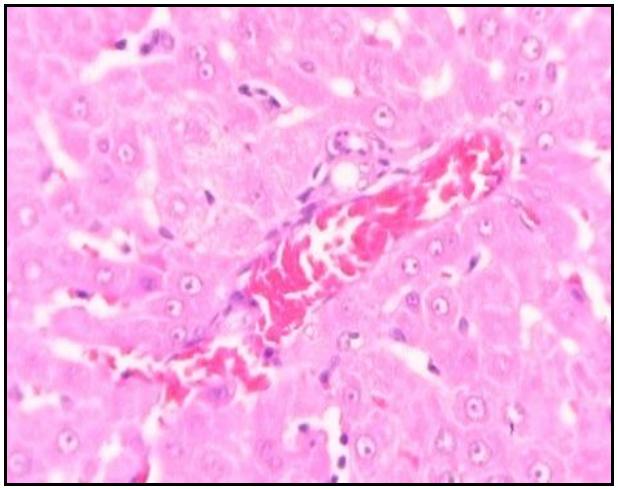


(Fig.1b, H&E, x400)

Figure 1. High Fructose Corn Syrup Section shows liver parenchyma with partially effaced architecture



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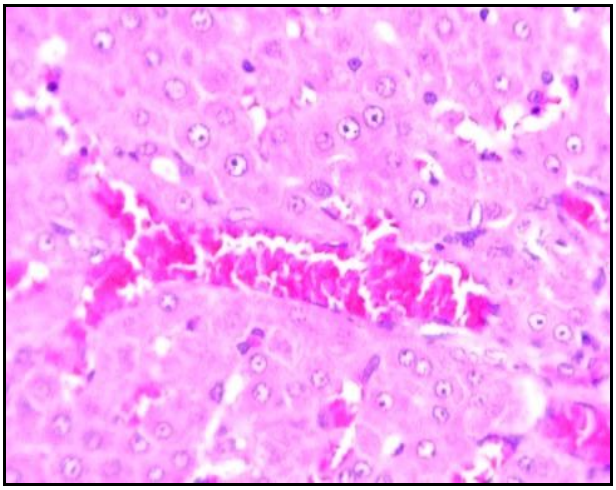

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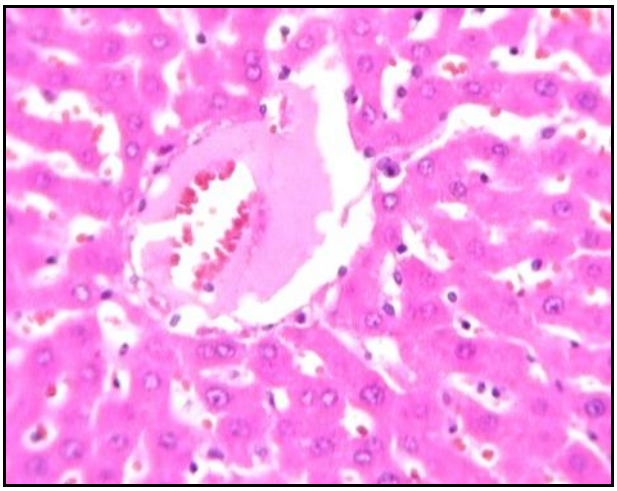
Figure 2. Alcohol + Irbesartan [5 mg/kg]: Section studied shows liver parenchyma with intact architecture



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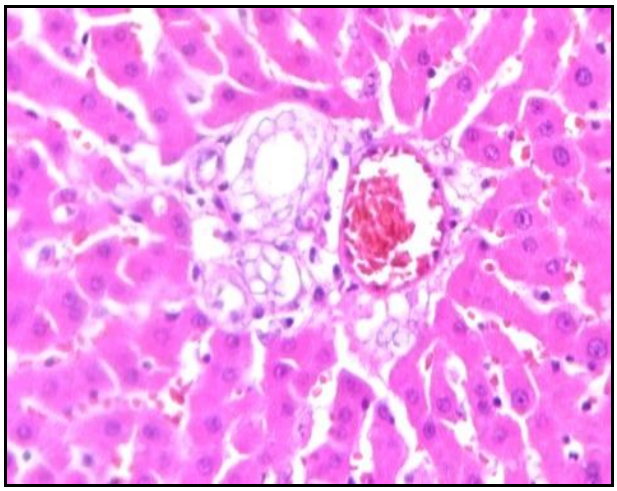

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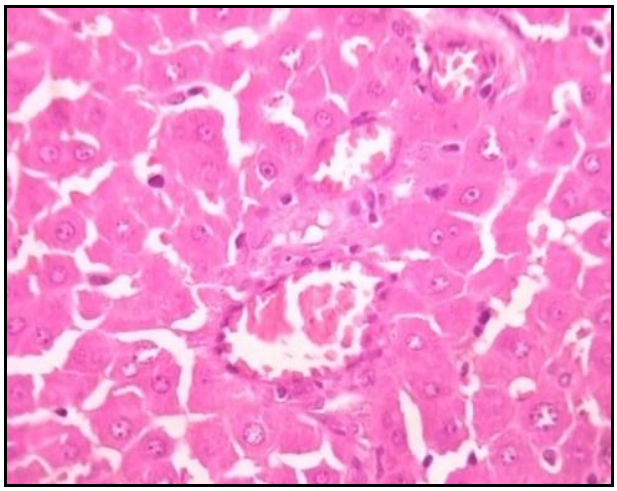
Figure 3. Alcohol + Rutin [50mg/kg].Section studied shows liver parenchyma with intact architecture



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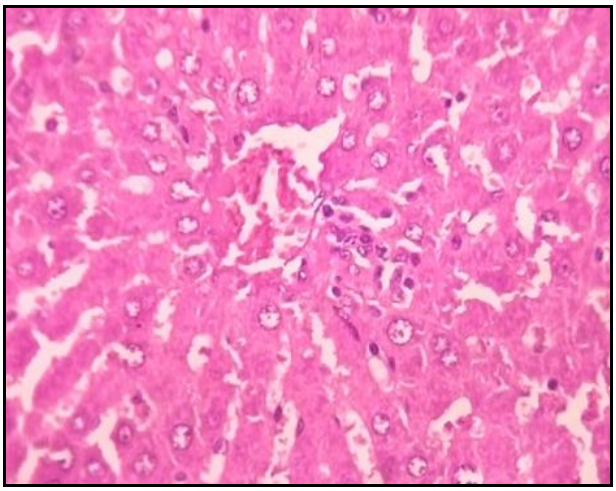

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Figure 4. Control: Section studied shows liver parenchyma with intact architecture