Chelonian Conservation And Biology

Vol. 19No.1 (2024) | https://www.acgpublishing.com/ | ISSN - 1071-8443

DOI:doi.org/10.18011/2024.01(1).675-685

RUTA CHALEPENSIS LEAF EXTRACT'S PROTECTS METABOLIC INDICES ABNORMALITIES, TYPE 2 DIABETES, AND VARIOUS ORGANS TOXICITIES IN OBESE RAT

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Running title: RC, diabetes and liver-kidney functions in obese rat

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Abstract

Introduction: Plant phenolics have been used as food supplements due to their capacity to prevent several ailments. **Aim:**The effects of Ruta chalepensis extract (RCE) on α-amylase activity, type 2 diabetes, and lipid profile induced by obesity.**Methods***:* obesity was induced by a high-fat, high-fructose diet. Obese rats were given either RCEor acarbose daily by gastric gavage. **Results**:In a dosage-dependent manner, Ruta chalepensis extracts dramatically reduced pancreatic α-amylase activity in in vitro research.. RCE therapy in obese rats decreased blood glucose levels by 22% by decreasing the activities of intestinal, pancreatic, and blood α-amylase by 25, 22, and 27%, respectively and improved insulin sensitivity evidenced by oral glucose tolerance test (OGTT). Additionally, biochemical analysis and histological examination demonstrated that giving RCE to those with type 2 diabetes who had been exposed to HFFD corrected their lipid profiles and preserved their liver and kidney functions.**Conclusion**:This study shows that RCE is a beneficial functional food for diabetics due to its ability to control lipid profiles, blood glucose levels, and protect against liver and kidney toxicity.

Keywords: *Ruta chalepensis*, type 2 diabetes, lipid profile, α-amylase

Running title: Ruta chalepensis, diabetes and liver-kidney functions in obese rat

Introduction

Obesity is a metabolic illness characterized by abnormal protein metabolism, elevated blood glucose and cholesterol levels, and increased lipid levels. In 2016, the World Health Organization stated that more than 1.9 billion adults were overweight and that more than 650 million of these individuals were obese[1]. Obesity is a risk factor for metabolic syndrome and can cause cardiovascular disease (CVD), dyslipidemia, hypertension, and type 2 diabetes (T2DM)[2,3]. Currently, four weight-loss medicines (orlistat marketed as Xenical®, Roche Holding AG, Basel Switzerland or Alli®, GlaxoSmithKline, Brentford, UK; Contrave® from Nalpropion Pharmaceuticals, San Diego, CA, USA; Belviq® from Eisai, Tokyo, Japan; and Qsymia® from Vivus, Campbell, CA, USA) have received approval from the US Food and Drug Administration, controlling users' body weight by increased energy intake, appetite suppression, and inhibition of pancreatic lipase[4,5], which caused cause a number of harmful health effects, including intestinal pain, diarrhea, and gastrointestinal disorders.As an alternative to studies employing certain digestive enzyme inhibitors found in plants to treat diabetes and obesity while also preventing oxidative stress and other disruptions without having harmful effects on health.

One method of therapy for the prevention of obesity and type 2 diabetes is to postpone the absorption of glucose and fatty acids by suppressing α-amylase and lipase in the stomach [6]. In reality, type 2 diabetes poses one of the greatest risks to human health since it contributes to the emergence of several illnesses including liver and kidney toxicity, heart disease, and a host of others[9,10]. Alpha-amylase, a key enzyme, transforms large, soluble starch molecules into absorbable ones in saliva and pancreatic juice. Inhibitors of -amylase and -glucosidase slow the breakdown of carbohydrates in the small intestine and lessen the postprandial blood glucose spike.

Medicinal plants have been used extensively throughout history in a wide range of fields and contain a wide range of bioactive compounds with a wide range of therapeutic advantages. Previous studies have reported that*Ruta chalepensis* consummation protects from various perturbations and diseases such as type-1 diabetes, kidney and liver duysfunctions, and bacteria infection[11,12].

No previous research have looked at the effects of Ruta chalepensis extracts on type-2 diabetes. The goal of the current study is to ascertain how Ruta chalepensis extracts affect -amylase activity, OGTT, lipid profile, and liver-kidney functions..

Materiels and methods

Plant preparation

Fresh harvested plant material (*Ruta chalepensis*leaves) was collected from Monastir (Tunisia) in April 2020, and identified as *Ruta chalepensis*by a botanist (Professor Fathia Skhiri) at Monastir University. A voucher specimen (NO.Ea 22-37) has been deposited in the Herbarium of the

Laboratory of Bioressources: Biologie Integrative and Valorization, High Institute of Biotechnology of Monastir, University of Monastir, Tunisia.

*Ruta chalepensis*leaves were collected from Monastir (Tunisia) in April 2021 andwere dried at room temperature. The leaves of *RC* were dried at room temperature. *Ruta chalepensis* leaves powder (3kg) was extracted with ethyl acetate or methanol in a Soxhlet apparatus for 6–8 h in according of our previous study[11]. *Ruta chalepensis* leaves extracts were concentrated vacuum dried at 40 °C using a rotary vacuum evaporator.

α-amylase and lipase activities in vitro

The inhibitory activity of each extract against pancreatic lipase and α-amylase was measured by using p-nitrophenyl butyrate (NPB) and starch as substrate respectively, according to our recent publication[12].

Animals

Male Wistar rats weighed between179and 203 g were used in this study. The animals were fed on a pellet diet and water. The rats were preserved under controlled conditions. . The rat's experimentation was conducted by the international care and use Laboratory Animals (Code: 86/609/EEC), Monastir university.

Experimental Procedure

A total of 24 rats (18HFFD-rats and 6normal animals) weighed 165 g was used for this experimentation and was subdivided into four groups. Group1; referred to control rats fed a normalfood. Group 2: HFFD-rats: Rats fed high-fat fructose diet composed of 65% satndard diet, 15% sheep fats, 20% fructose and 0.1% cholic acid[4]. Group 3: Rats fed on HFFD and received 200 mg/kg bw of RCE daily by grastric gavage method named (HFFD + RCE). Group 4: Rats fed on HFFD and received 10 mg/kg bw of standard drug acarbose daily by gastric gavage method named (HFFD + Acar). Three months later,the blood was collected after decapitation, the blood is centrifuged 4000 rpm/15 mins and the serum were stored at -80°C until biochemical analysis. The small intestine was removed, and all samples were stored at –80°C for further use. The extract and the acarbose were administered daily to the rats one week before the experiment by gavage.

Analytical Methods

The α-amylase activity and serum glucose level were assayed by the determination of glucose level obtained from CNPG3 substrate (Kits Biomaghreb, Tunisia, ref 20033). The serum TC, LDL-C, and HDL-C rates were quantified by kits (Kits Biomaghreb, Tunisia, Ref 95516). GOT and GPT activities (Biolabo, ; and urea and creatinine levels were measured mixing 10 μL of sample and 1 mL of reagent and were quantified by commercial kits from Biomaghreb (Kits Biomaghreb, Tunisia, ref 11022 and 10025 for GOT and GPT; and ref

30023 and 25029 for urea and creatinine respectively).

For histological studies, hepatic, renal and heart pieces, were fixed in a formol buffer, and then embedded in paraffin.

Oral starch tolerance testing (OGTT) was performed at the 90 day animal experiments. The rats were fasted for 15 h and administrated starch (2 g/body weight) [13]together with RCE (200 mg/kg) or with acarbose at dose 10 mg/kg by gastric gavage method. Blood was also collected with a heparinized syringe from the tail vein of rats every 30 min. Blood glucose was determined every 30 minutes during 120 min using Roche ACCU-CHEK Active Blood Glucose Monitor.

Statistical Analysis

The values of each analysis were entered in StatView 5.0 for statistical analysis. Results are presented as means ± SD. The differences were determined using Fisher test.The significance between groups was accepted at $p \leq 0.05$.

Resultats

Effect of RCE on α-amylase and lipase activities in vitro

Table 1 showed that *Ruta Chalepensis* methanol, ethyl acetate and water extracts inhibited α-amylase activity in vitro with IC50 value 95, 275, and 120 µg/ml respectively(**Table 1**). Moreover, Table 1 showed the potential ability of methanol RC extract to control obesity and hypercholesterolemia by lipase activity. This study investigates that RCE inhibited lipase activity with IC_{50} value 137± 5.53 µg/ml. The inhibitory activity of EAWE against lipase activity was 2.56 and 4.16-fold higher than aqueus and ethyl acetate extracts respectively.

Effect of RCE administration in α-amylase activity and serum glucose level in HFFD-rats

Figure 1 showed that, a significant increases in the body weight of HFFD-rats by about 25% as compared to normal rats. This augmentation in body weight increases of intestinal, pancreatic and serum α-amylase activitiesby 80, 78 and 72% respectively as compared to normal rats. This increase of α-amylase activity caused a significant increase in blood glucose rate by 66%. However, the administration of RCE to HFFD-rats during 90 days causes a significant decrease in body weight by about 18%. This body weight decrease and/or accumulation of lipid in the liver, muscle... stimulates insulin activity and leads to reductions of 25, 22 and 27%, however, a significant decease in the intestinal, pancreatic and serum α-amylase activity of the RCE-treated rats was observed. In addition, administration of RC extrat to HFFD-rats decreased the blood glucose level by 11% as compared to obese untreated rats (**Figure 1**).

Effect of RCE administration on starch tolerance

Figure 2 showed that the administration of 2 g/kg of body weight of starch to HFFD- rats caused a fasting, a quick and considerable increase on blood glucose level by 92% 30 min after the starch load. The administration of RCE reduced the glycemic response by about 15% compared untreated HFFD-rats.

Effect of RCE administration on lipid profile of HFFD-rats

Table 2 indicates that HFF diet induced a significant risein serum TC and LDL-C by 98 and 142%, as compared to normal rats. It also shows that the HDL-C level in the serum of HFFD-rats underwent an decrease of 42%. In addition, the administration of RC extract to obese rats decreased the serum TC and LDL-C levels by 44 and 68%. Moreover, administration of RCE to HFFD rat increased the HDL-C level by 91% (**Table 2**).

Effect of RCE administration on liver function

Histological analysis of liver tissues showed that obesity induced three types of abnormalities; which are fatty infiltration (HFFD 1), lymphocytic infiltrate apparition in the portal spaces (HFFD 2) and Sinusoids were dilated (HFFD 3). In addition, enzymatic analysis showed that obesity induced a significant increase in GOT and GPT activities by 72 and 43% as compared to normal rats. In control rats, histopathology of the liver showed normal architecture. In HFFD-rats However, the administration of RCE at dose 200 mg/kg to HFFD-rats during 90 days partially protects liver function and tissues, evidenced by a significant decrease of serum GOT and GPT, reduction of fatty infiltration and inhibition of infiltration phenomena. Administration of RCE to HFFD-rats protects liver function and architecture evidenced by absence of fatty infiltration and lymphocytic infiltrate apparition in the portal spaces; and the reduction of hepatic sinusoidal infiltration**(Figure 3).**

Effect of RCE administration on kidney function

Histological analysis indicates that obesity inducedmany anaomalies in kidney tissues such as leukocyte infiltration, proximal and distal tubes expansion, and bowman capsule space increase. In RCE-treated HFFD rats Administration of RCE to HFFD-rats protects from kidney toxicity and alteration, showed by significant decrease of serum Creatinine and urea. Histological analysis showed that administration of RCE to obese improved kidney tissues, showed by decrease in proximal and distal tubes**(Figure 4).**

Effect of RCE administration on heart architecture in HFFD-induced type 2 diabetes

This study showed that obesity induced disorganized myocardial fibers. In addition the administration of Rc extract to obese rats protects from this thesis anomalies in heart tissues**(Figure 5).**

Discussion

In rats, this study showed that high-fat-fructose (HFF) diet consumption inducedlipid accumulation, evidenced by increase in body weight by 24% as compared to normal rats; and a significant persistent increase in α-amylase activity about 80%; and in blood glucose level by 66%. In fact, HFF-diet caused an increase in body weight and obesity probable through the accumulation of lipids in the body. The increased body fat might also be the cause of systemic insulin resistance and an increase in the α-amylase activity in the intestine, pancreas and serum of HFFD-rats, as evidenced by a significant increase in blood glucose level. In fact, Tijani*et al* have reported that high-fat diet-induced obesity and insulin resistance; and the reduction in body weight gain ameliorates basal glycemia, and insulin resistance [14]. Furthermore, HFF diet has been associated with impaired insulin-mediated metabolic effects in humans and animals [15,16].

Inaddition, this study showed that HFFD-induced hyperglycemic rats was confirmed here by increased food and water intakes during 90 days of HFF diet, by altered serum parameters such as hyperlipidemia (elevated serum LDLD-C and TC), elevated creatinine, urea, GOT, GPT and LDHactivities as indices of liver and kidney toxicities. Another studies have reported that high saturated fat, high calorie, processed carbohydrate diets increase the incidence of insulin resistance and impair carbohydrate metabolism, increase hepatic glucose production, and induce insulin resistance[17]. Locia-Morales et al have confirmed a positive association between tertiles of serum enzymatic activity of AMY2 and insulin resistance in children with obesity [18].

Herein, the results indicate for the first time that RCE induce potent antidiabetic activities. In fact, daily oral administration of RCE extract for 90 days decreased significantly α-amylase activity, ameliorated insulin sensitivity and/or activity (OGGT test) and reduced serum glucose concentration in HFFD-rats. In fact, the alpha-amylase are the calcium metalloenzymes and catalysis in the reaction which involves the hydrolysis of the alpha-1,4 glycosidic linkages of the starch, amylopectin, amylose and glycogen[19]; and the inhibition of this enzyme, inhibits the transformation of starch and glycogen into simple sugars and therefore an anti-hyperglycemic activity. This studydemonstrates that RC extract can prevent postprandial hyperglycemia; and this was confirmed by oral glucose tolerance test in diabetic rats which showed that RC extract potentially decreased the postprandial blood glucose level. This coincides with the work of Hamdiken *et al*, [20]who noticed, that treatment of diabetic rats with the extract of RC preserved islet cells, which is an improvement over in insulin secretion and/or activity [20]. Moreover, RC ingestion reverses gluconeogenesis and control protein loss [21]. The hypoglycemic effect of R. chalepensis, also, might be due to the presence of polyphenols subetances, and other such as acids, flavonoids, phytoestrogens, triterpenes. These compounds have been reported to be responsible for inhibition of digestives enzymes in the intestine suc as α-amylase.Oboh et al[22]have reported that rutin, one of the most abundant flavonoids in the RC extract, showed a strong inhibition of α-amylase and α-glucosidase. It has been well documented that RCE administration to streptozotocin-induced diabetes in rats stimulating pancreatic βregeneration

and enhancing glucose utilization in peripheral tissues.TC and LDL-C rates augmentation and decrease of HDL-C level are symptom of diabetes and as a consequence of insulinoresistance. The administration of RCE to HFFD-induced type 2 diabetes normalized serum LDL-C, TC and HDL-C. Similar antihyperlipidemic effects have been reported with ethanol extract of *Ruta chalepensis* leaves[11]. The effect was attributed to bioactive phytocompounds in RCE such as vanillic acid, Coumarin, Caffeic acid, gallic acid and others.

In thisstudy, obesity induced hypatocytes damages showed by an increase in GOT and GPT activities. The administration, of RC extract to HFFD-rats with RCE significantly ameliorated the GOT and GPT levels compared to diabetic untreated rats. In addition, administration of RCE to HFFD-rats improved liver histology compared to untreated diabetic rats. This result in accordance with previous study has reported that bioactive drugs exist in *Ruta chalepensis* leaves as vanillic acid, Coumarin, Caffeic acid, gallic acid protect from liver toxicity and histological damaged.

Our data show that urea and creatinine concentrations increased in HFFD- rats but were blunted by RCE treatment. The observation highlights a possible nephroprotective potential of the plant. Previous studies reported that the major polyphenol including vanillic acid and gallic acid existed in RCE possessing anti-toxicity activity and suggest benefits in kidney toxicity and dysfunction.

Results of this study showed that HFF-diet induced myocardial fiber disorganization in rats. However, administration of RCE is highly effective at preventing heart tissue damage by reducing alteration of heart tissues. These results are consistent with previous reports that herbal flavonoids and polyphenols supplementation to diabetes-induced heart damage, improved and minimised cardiac injury.

In conclusion, this study indicated that RC ameliorate type 2 diabetes by the inhibition of α-amylase activity and the prevention of obesity.

Conflict of Interest

The author declares that there is no conflict of interest

Funding: The authors extend their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work throught Large Research Project Under grant number RGP2/101/45.

Acknowledgement

The authors extend their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work throught Large Research Project Under grant number RGP2/101/45.

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	IC50 *(µg/mL)*	
Ruta chalepensis	**α-amylase**	**Lipase**
Water extract	120 ± 3.37	351± 6.73
Methanol extract	95 ± 10	137± 5.53
Ethyl acetate extract	271 ± 0.21	571± 9.12
Standard acarbose	67 ± 0.07	

Table 1: α-amylase and lipase inhibition activity of Ruta chalepensis extracts *in vitro*(IC50, mg/mL) (n = 3).**Note:** Values are Means of triplicates; and results were presented mean mean±SD

Table 2: Blood TC, LDL-C, and HDL-C, GOT, GPT, creatinine and urea in obese-rats induced type 2 diabetes treated by methanol Ruta chalepensis extract. *p<0.05 as compared to controls rats; #p<0.05 as compared to untreated HFFD-rats; @p<0.05 as compared to HFFD-rats + RCE.

	Control rats	***HFFD-rats***	***HFFD-rats + RCE***	***HFFD-rats + Acar***
Average food intake(g/rat)	21.5 ± 1.3	29.1 ± 2.3*	25.1 ± 1.3*#	23.3 ± 0.7*#@
Average water intake(ml/rat)	19.3 ± 1.7	24.5 ± 2.3*	22.1 ± 1.1#	21.1 ± 0.8#@
Lipid profile				
TC (g/L)	0.79 ± 0.08	1.57 ± 0.30*	0.87± 0.05#	0.95 ± 0.12#
HDL-C (g/L)	0.61± 0.01	0.35± 0.05*	0.67± 0.1#	0.49± 0.03*#@
LDL-C (g/L)	0.49± 0.01	1.19± 0.13*	0.37± 0.08#	0.41 ± 0.03*#@
Liver toxicity indices				
GOT(UI/l)	33,12 ± 4,3	57,11±7,7*	37,17± 3,7#	44,87±6,4*#@
GPT (UI/l)	13,80 ± 1,3	30,88±5,1*	17,16±3,8#	19,12±5,5*#@
Kidney toxicity indices				
Creatinine (mg/dl)	0.61 ± 0.05	1.31±0.07*	0.81± 0.03*#	0.93 ± 0.07*#@
Urea (mg/dl)	30.3 ± 1.1	53.7±2.1*	40± 1.3*#	45.3 ± 3.1*#@

Figure 1

Effect of the administration of methanol Ruta chalepensis extract. on body weight **(A)** intestine**(B)**, pancreas **(C)** and serum **(D)** α-amylase activity; in HFFD-rats. Values are given as mean mean±SDfor groups of 8 animals each. The values are statistically presented as follows: *p < 0.05 vs controls at day; #p < 0.05 vs. HFFD-untreated rats.;@p < 0.05 vs methanol Ruta chalepensis extract-HFFD treated rats.

Figure 2

Serum glucose level **(A)** and oral glucose tolerance test **(B)** on HFF-diet induces type 2 diabetes in rats. Values are given as mean mean±SDfor groups of 8 animals each. The values are statistically presented as follows: *$p < 0.05$ vs controls at day; #$p < 0.05$ vs. HFFD-untreated rats.;@$p < 0.05$ vs methanol Ruta chalepensis extract-HFFD treated rats.

Figure 3

Effect of methanol Ruta chalepensis extract ingestion on HFF-diet induced liver toxicity. Liver of normal rats (Con) showed normal architecture. Liver of obese rats showed fatty infiltration (**FI**) and hepatic leukocyte sinusoidal infiltration (**LSI**) and portal spaces lymphocytic infiltrate apparition (**PSLI**) (HFFD 2). Administration of methanol Ruta chalepensis extract to HFFD-rats protects tissues, showed by absence of fatty infiltration and lymphocytic infiltrate (H&E 100X).

Figure 4

Effect of methanol Ruta chalepensis extract ingestion on HFF-diet induced kidney tissues alteration. Histological analysis of kidney of obese rat showed leukocyte infiltration (**LI**) and alteration collecting system (**ACS**). Administration of methanol Ruta chalepensis extract-treated to obese rats rats impooved kidney architecture (H&E 100X)..

Figure 5

Effect of methanol Ruta chalepensis extract ingestion on HFF-diet induced heart tissues alteration. Histolofical analysis observed that obesity induced anarchized myocardial fibers (AMF) and lipid lobule accumulation (LLA). The administration of methanol Ruta chalepensis extract to obese rats at dose 200 g/kg daily during three months protects from these anomalies (H&E 100X)..

Figure 1

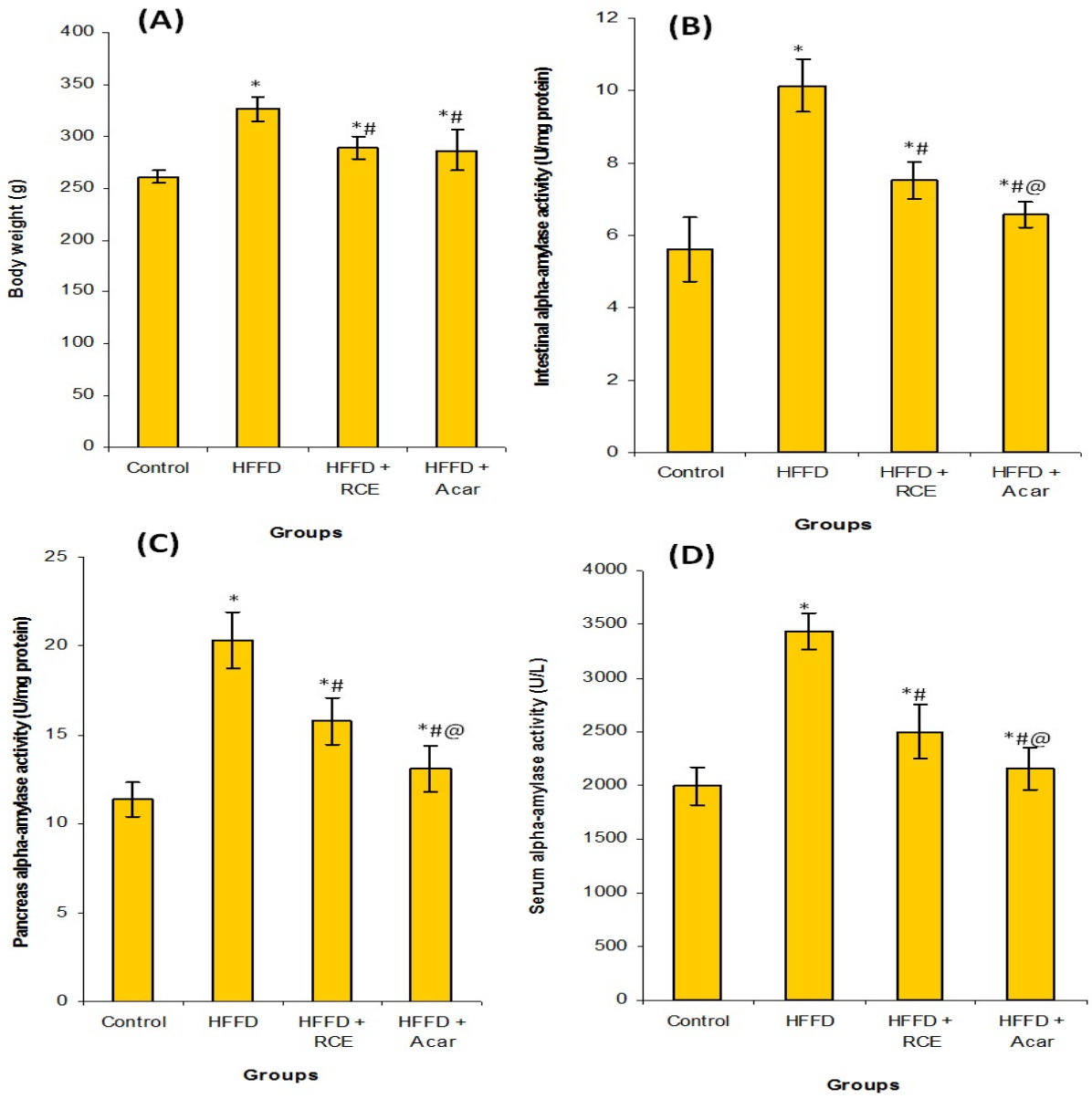
(A)
Body weight (g)
400
350
300
250
200
150
100
50
0
*
*#
*#
Control
HFFD
HFFD + RCE
HFFD + Acar
Groups
(B)
Intestinal alpha-amylase activity (U/mg protein)
12
10
8
6
4
2
0
*
*#
*#@
Control
HFFD
HFFD + RCE
HFFD + Acar
Groups
(C)
Pancreas alpha-amylase activity (U/mg protein)
25
20
15
10
5
0
*
*#
*#@
Control
HFFD
HFFD + RCE
HFFD + Acar
Groups
(D)
Serum alpha-amylase activity (U/L)
4000
3500
3000
2500
2000
1500
1000
500
0
*
*#
*#@
Control
HFFD
HFFD + RCE
HFFD + Acar
Groups

Figure 2

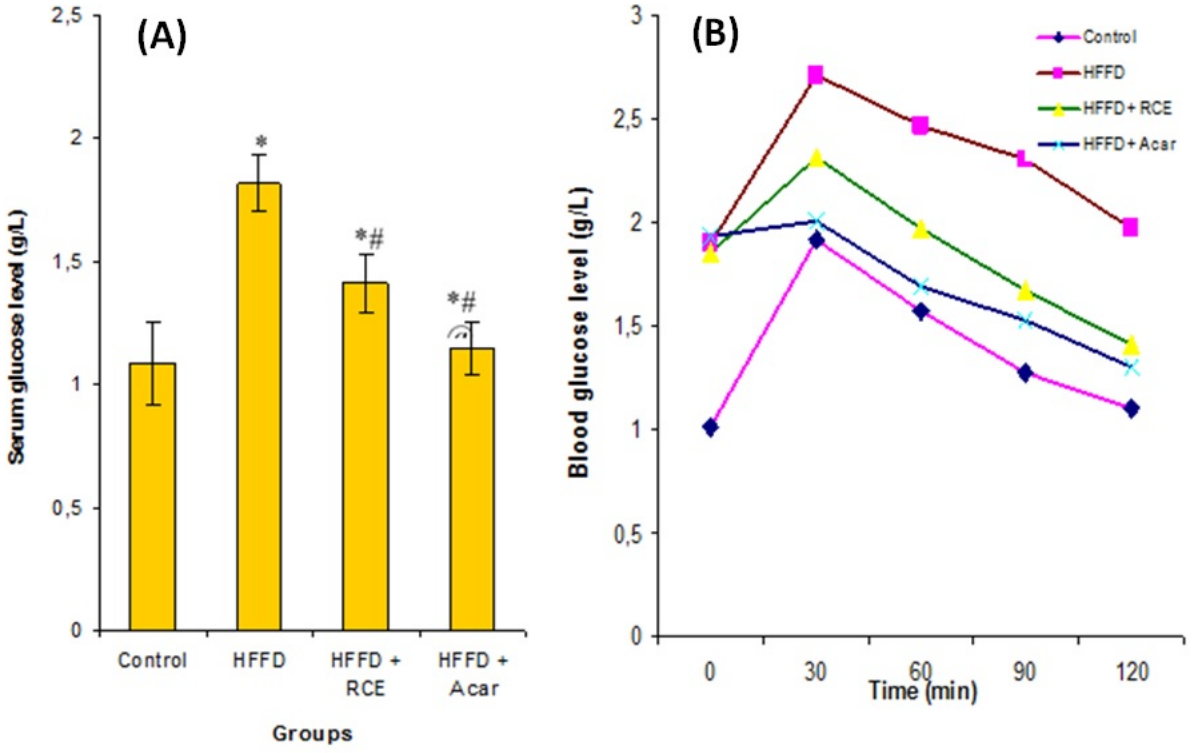

Figure 3

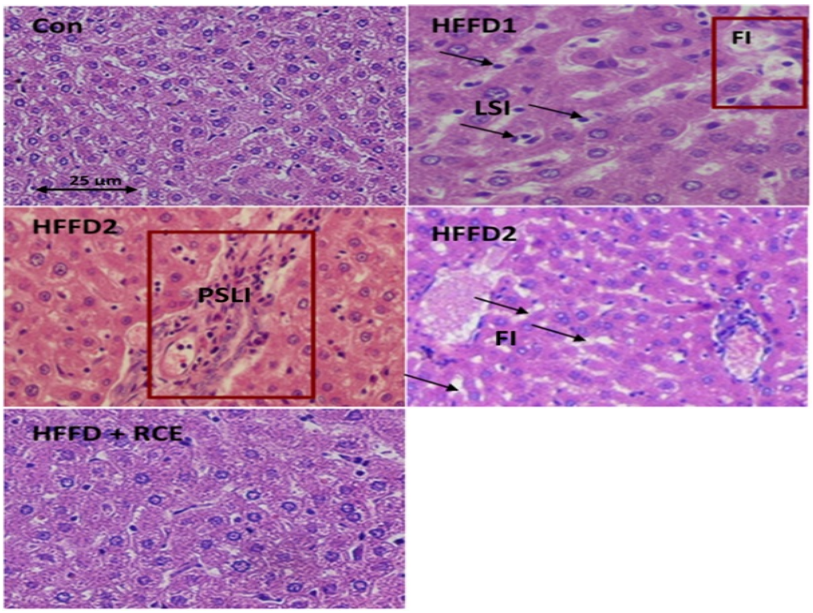

Figure 4

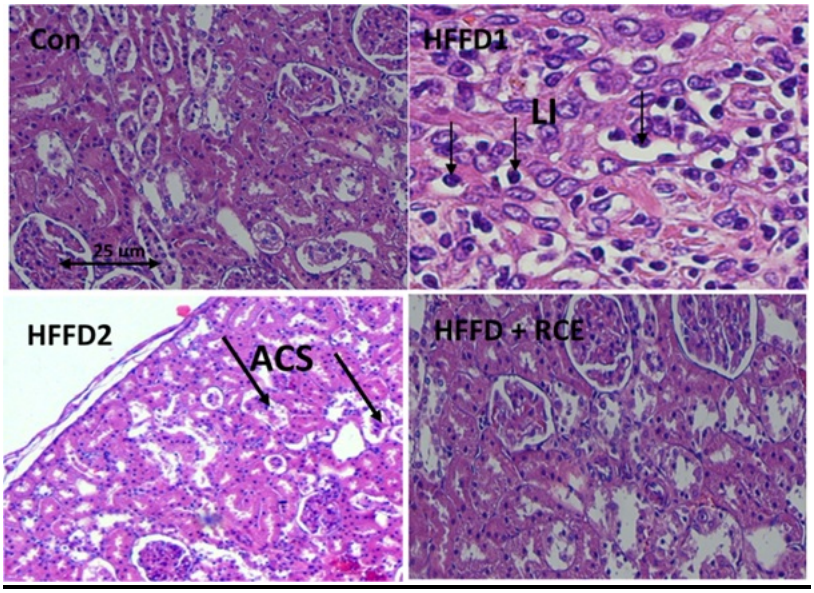

Figure 5