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RESVERATROL EFFECT ON FRUCTOSE-INDUCED NASH: A MECHANISTIC APPROACH

***Enas A. Abd El-Haleim, Ashraf K. Bahgat and Samira Saleh**

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt

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ABSTRACT

Background: High-fructose beverages have been extensively used in recent years leading to the development of NAFLD and NASH. The present study evaluates the effect of resveratrol on fructose-induced metabolic abnormalities in rats. A number of genes known to be critically involved in lipid metabolism were investigated.
Methods: Fructose-enriched diet (FED) was given to rats for 12 weeks. Resveratrol (RES) (70 mg/kg) was administered orally from the 9th week till the end of experimental period. Body weight, oral glucose tolerance test (OGTT), liver index, insulin resistance (HOMA), serum and liver triglycerides (TGs), oxidative stress (liver MDA, GSH and SOD), serum AST/ALT ratio and TNF-α were measured. Additionally, hepatic gene expression of suppressor of cytokine signaling -3 (SOCS-3), sterol regulatory element binding protein-1c (SREBP-1c), fatty acid synthase (FAS), malonyl CoA decarboxylase (MCD), transforming growth factor-β1 (TGF-β1) and adipose tissue gene expression of leptin and adiponectin was investigated.
Results: Resveratrol treatment ameliorated the fructose-induced damaged liver, glucose tolerance impairment, insulin resistance, oxidative stress & dyslipidemia. There was an improvement in genes responsible for NASH development.
Conclusions: Resveratrol improved the NASH-related fructose-induced disturbances due to its antioxidant and anti-inflammatory capacity beside its attenuating effects on hepatic genes expression and correcting the balance between adiponectin and leptin.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are now the number one cause of liver disease in Western countries, in the Middle East, Far East, Africa, the Caribbean and Latin America (LaBrecque *et al*., 2014). The Pathogenesis of these conditions involves a multi-hit process in which increased accumulation of triglycerides in face of insulin resistance results in increased susceptibility to inflammatory damage mediated by increased expression of inflammatory cytokines and adipokines, oxidative stress. The most reproducible risk factors for NAFLD/NASH are central obesity, insulin resistance, fasting hyperglycemia and hypertriglyceridemia (Farrell and Larter, 2006). Interplay of multiple metabolic genetic expressions plays an important role in NASH development. Suppressor of cytokine signaling (SOCS) proteins appear to play a key role in the induction of insulin resistance in obese rodents (Emanuelli *et al*., 2001). Besides, increased expression of SOCS-3 was supplementary to enhanced expression of sterol-regulatory element-binding protein (SREBP)-1c, a transcriptional activator of all lipogenic enzymes (Ueki *et al*., 2005). These changes would eventually end up by an increased rate of fatty acid synthesis and would lead to classic fatty liver and increased lipogenesis (Foretz *et al*., 1999). Moreover, adipose tissue plays a key role in energy homeostasis, since its metabolic products, adipokines (leptin and adiponectin), exert local, peripheral and central effects. Leptin is thought to participate in NASH development while adiponectin is considered as an anti-inflammatory adipokine (Tsochatzis *et al*., 2006). Recent studies ascertained that obesity is a systemic, low-grade inflammation (Greenberg and Obin, 2006) and that enhanced expression of TNF-α is one of the earliest events after liver injury and that it represents a major trigger of the cytokine response (Tilg and Diehl, 2000). Currently, the role of oxidative stress in disease progression from steatosis to steatohepatitis and potentially cirrhosis cannot be ignored (Garcia-Ruiz *et al*., 1995).

On the other hand, it is well known that fructose is a highly lipogenic sugar molecule, which triggers the accumulation of TGs and FFAs into the hepatic tissues as well as in circulating blood, and leads to insulin resistance (Basciano *et al*., 2005). Fructose feeding has therefore been historically utilized as a model for studying various aspects of hepatic dyslipidemia and insulin resistance. Therapeutic options for these conditions

****Corresponding author: Enas A. Abd El-Haleim,***
Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

are limited. Alternative treatment options are therefore intensively sought after. An interesting candidate is the natural polyphenol resveratrol. Several antioxidants were found to be effective in NAFLD treatment (Lam and Younossi, 2009). One of these is resveratrol, a polyphenol with known antioxidant and antinflammatory effects. Although the antioxidant effects of resveratrol have been proposed to contribute to its beneficial effects, the underlying molecular mechanism is not completely understood. Resveratrol treatment in mice fed a high calorie diet consistently improved various health parameters including glucose homeostasis and survival (Baur *et al*., 2006), and has therefore been suggested to act as a calorie restriction mimetic. Moreover, resveratrol decreased NAFLD severity in rats (Bujanda *et al*., 2008). In the present study, we investigated the effect of resveratrol treatment on fructose-induced NASH in rats and explored the potential molecular mechanisms through which the protective effects of resveratrol may work. Because the interaction between adipose tissue and liver is critical for the regulation of glucose and lipid metabolism, a number of genes known to be critically involved in lipid metabolism were investigated in both tissues.

MATERIALS AND METHODS

Animals and diet

Adult male albino rats, weighing 150-200 g, were used in the present study. They were purchased from the animal house of the National Cancer Institute (Cairo, Egypt). Animals were kept under the appropriate conditions of controlled humidity, temperature and light. All animals except the normal group were given 10% fructose in drinking water for 12 weeks in order to induce NASH (Elliott *et al*., 2002). All experiments on laboratory animals were performed in accordance with the protocol approved by Faculty of Pharmacy, Cairo University Research Ethics Committee, Cairo, Egypt. PT number (600). Resveratrol (70 mg/kg p.o) (Zhu *et al*., 2008**)** (Finest Nutrition, Walgreen) was suspended in 0.5% carboxymethyl cellulose (CMC).

Experimental design

After a period of adaptation, animals were randomly assigned into a normal control group and a fructose enriched diet group (FED). The FED group was distributed into 2 groups, a control FED group and resveratrol treated group, each composed of 8-12 rats. The normal control group was fed a normal chow diet consisting of 66% carbohydrates, 22% protein, 6% fats, 3% fiber and 3% mineral and vitamin mixture and purchased from Alfa Media Trade (Giza, Egypt). The high fructose fed groups received the same diet plus fructose (10%) in drinking water for a period of 12 weeks. Treatment was carried out for 4 weeks from the 9^{th} week until the end of the 12 weeks experimental period. Group I: normal control, II: control FED, III: resveratrol 70mg/kg (RES). Body weight was determined during the experimental period (12 weeks) at two weeks intervals.

Blood sampling and serum preparation

At the end of the treatment period, blood samples were taken from retro-orbital sinus of rats under ether anesthesia, after being fasted for 18 h, to minimize feeding-induced variations in lipid pattern. Blood samples were allowed to clot at room temperature then serum was separated by centrifugation of blood at 3000 rpm for 15 minutes using a centrifuge (Hettich universal 32A, Germany). Each sample was divided into several aliquots, one for each estimated biochemical parameter, and stored at -20ºC until analysis is performed.

Tissue sampling

Animals were then sacrificed by cervical dislocation. Livers and epididemal fat were carefully and rapidly excised. The removed livers were washed with cold normal saline and dried on filter papers then weighed for the determination of liver index. Liver index percent was determined (= liver weight / body weight x 100). Samples of the liver, taken 5 mm away from the edge of the largest hepatic lobe were frozen at -80ºC for the determination of hepatic genes expression. The other lobes of the liver were homogenized in ice-cold saline, using a homogenizer (Heidolph Diax 900, Germany), to prepare 20% homogenate. The prepared homogenate was divided into several aliquots that were stored at -20 ºC until assayed later for estimation of the chosen biochemical parameters. The remaining part of the large hepatic lobe was fixed with 10% formaldehyde for histopathological examination. Besides, the epididdemal fat located above the epididymus was dissected and frozen at -80ºC for estimation of adipose tissue gene expression. The dead bodies were frozen till incineration.

Extraction of liver lipids

Liver lipids were extracted according to the method of Bligh and Dyer (Bligh and Dyer, 1959) for the determination of liver triglycerides.

Histopathological examination

The liver fixed with 10% formaldehyde was dehydrated and embedded in paraffin wax, cut into sections of 7-10 µm thickness, stained with hematoxyl in and eosin (H&E) and then examined under light microscope. Images were acquired with a Leica ICC50 HD digital camera attached to a Leica motorized light microscope system. Steatosis area was determined using leica application suite (LAS) image analysis program which automatically detects, measures and evaluates multiple image features.

Estimation of Biochemical Parameters

Blood glucose and Oral Glucose Tolerance Test (OGTT)

Two days before the end of the treatment period, rats were fasted for 18 h, with free access to water, to minimize feeding-induced variations in glucose pattern. Glucose level was determined in a blood sample obtained from the tail vein. The OGTT was carried out using glucometer test strips *(Fine test, Korea)*. Rats were given oral glucose (20% solution in a dose 2 g/kg of body weight), and droplets of blood from the tail vein were withdrawn at 0 (prior to glucose administration), 15, 30, 60 and 120 min after glucose load, to evaluate the resulting blood glucose concentrations.

Serum insulin and Insulin resistance

Insulin was determined using *DRG® Insulin (Rat) ELISA (EIA-2048) version 8.0, 2013*. Insulin resistance was calculated using homeostasis model of assessment

(HOMA) = blood glucose (mmol/L) X serum insulin (pmol/L) / 155

Serum and liver TGs

Serum and liver TGs were determined according to the method of Bucolo and David (Bucolo and David, 1973).

Oxidative stress parameters

Liver homogenate was used for determination of thiobarbituric acid reactive substances (TBARs), measured as MDA (Mihara and Uchiyama, 1978), reduced glutathione (GSH) (Beutler *et al*., 1963) and superoxide dismutase (SOD) (Marklund and Marklund, 1974).

Liver function tests

Serum alanine transaminase (ALT) and aspartate aminotransferase (AST) were determined according to the method of Reitman and Frankel (Reitman and Frankel, 1957).

Serum TNF-α

TNF-α was determined using *Quantikine® ELISA Rat TNF-α Immunoassay kit. c2012 R&D Systems, Inc.*

Hepatic and adipose tissue genes expression

Hepatic genes expression of SOCS-3, SREBP-1c, fatty acid synthase (FAS), malonyl CoA decarboxylase (MCD), TGF-β1, and adipose tissue gene expression of leptin and adiponectin were measured using real time-polymerase chain reaction (RT-PCR).

RNA isolation and RT-PCR

Total RNA was isolated from the liver samples stored for determination of hepatic genes expression using *Thermo Scientific GeneJET RNA Purification Kit* following the manufacturer's protocol. Total RNA was purified using the supplied purification column, after which it was reverse transcribed using *Thermo Scientific RevertAid First Strand cDNA Synthesis Kit*. RT-PCR analysis was performed using the SYBR Green procedure and *Stratagene Mx3000P* instrument. Expression of mRNA values was normalized relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal standard. The following primer pairs were employed *(Primer-Blast* program was used to assist in the design of primers). At the end of a RT-PCR running with SYBR Green chemistry, the relative quantification was determined according to the method of Pfaffl (Pfaffl, 2001). The RT-PCR results were analyzed with applied biosystem software.

Statistical analysis

Data are expressed as means ± standard error (S.E.M). Comparisons between means were carried out using one way analysis of variance (ANOVA) test followed by Tukey Kramer multiple comparison's test. For all statistical tests, the level of significance was fixed at $p< 0.05$. GraphPad Prism® software package, version 6 (GraphPad Software, Inc., USA) was used to carry out all statistical tests.

RESULTS

Body weight and liver index percent

As presented in Fig.1a, there was a gradual gain in body weight in all groups although the extent was variable. Comparing body weights on week 12, fructose-fed rats reached a body weight of 330.0 g compared to 289.8 g in control rats, indicating a 10% more weight gain in the FED group. This rise in body weight was virtually normalized by RES treatment.

Table 1. Primer pairs sequences

Primer name	Forward primer	Reverse primer
GAPDH	5'-ACAAGATGGTGAAGGTCGGTGTGA-3'	5'-TTGAACTTGCCGTGGGTAGAGTCA-3'
SOCS-3	5'-CTGGCCGCCGCCTCGTCTCGG-3'	5-ACGGCACTCCAGTAGAATCCG-3'
SREBP1c	5'-GGAGCCATGGATTGCACATT-3'	5'-GCTTCCAGAGAGGAGCCCAG-3'
FAS	5'-AGAGGCTGTTCTCAAGGAAGG-3'	5-AGGGTACATCCCAGAGGAAGT-3
MCD	5'-CGGCACCTTCCTCATAAAGC-3'	5'-GGGTATAGGTGACAGGCTGGA-3'
Leptin	5'-TTCAAGCTGTGCCTATCCACAAAG-3'	5'-TGAAGCCCGGGAATGAAGTC-3'
Adiponectin	5'-GGAAACTTGTGCAGGTTGGATG -3'	5'-GGGTCACCCTTAGGACCAAGAA-3'
TGF-β1	5'-TGAGTGGCTGTCTTTTGACG-3'	5'-ACTTCCAACCCAGGTCCTTC-3'

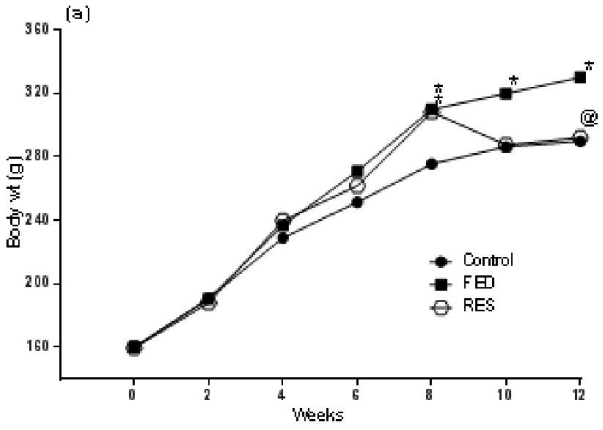


Fig. 1. Effect of resveratrol (70 mg/kg) on the body weight (a) and percentage of liver index (b) in fructose-induced NASH in rats

FED= fructose enriched diet, RES= resveratrol, liver index= liver weight/body weight. Values are means ± S.E.M. (S.E.M was omitted in Fig.1 (a) body weight for clarification). n = 8 – 12 rats. The significance of the difference between means was tested by ANOVA followed by Tukey Kramer multiple comparisons test.

* Significantly different from control, @ Significantly different from FED at $p< 0.05$.

Fig.1b shows that there was an increase in liver index in the FED group compared to control and that treatment with RES normalized it.

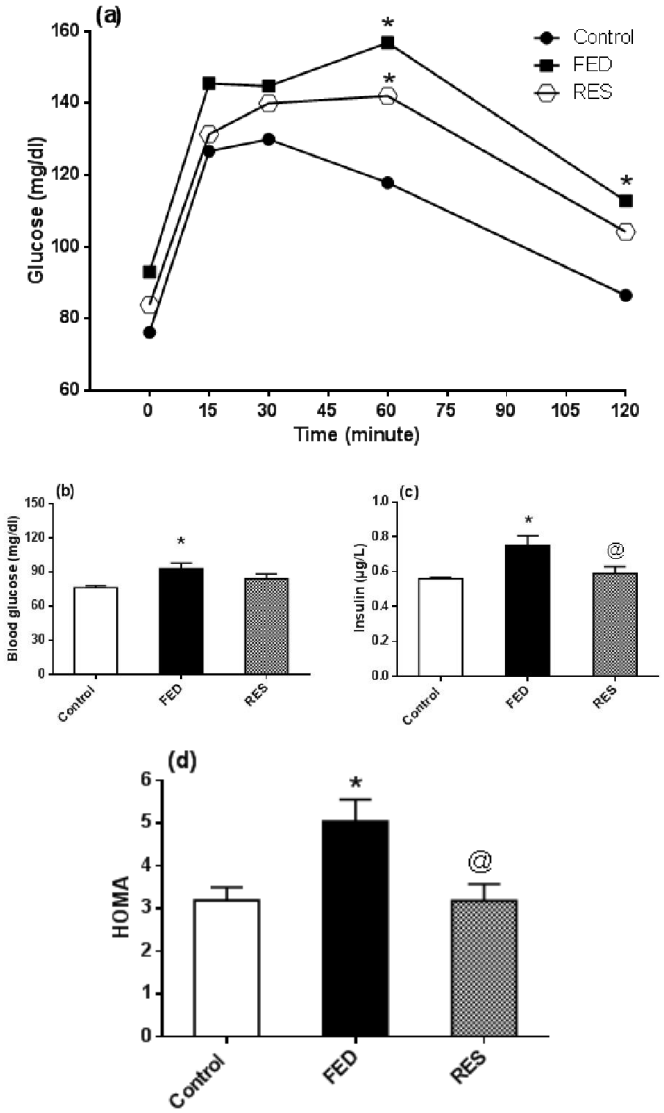


Fig. 2.Effect of resveratrol (70 mg/kg) on OGTT (a), blood glucose (b), insulin (c) and HOMA (d) in fructose-induced NASH in rats

FED= fructose enriched diet, RES= resveratrol, OGTT=oral glucose tolerance test, HOMA= homeostasis model of assessment. Values are means ± S.E.M. (S.E.M was omitted in Fig.2 (a) OGTT for clarification). n = 8 – 12 rats. The significance of the difference between means was tested by ANOVA followed by Tukey Kramer multiple comparisons test.

* Significantly different from control, @ Significantly different from FED $p < 0.05$.

OGTT, blood glucose, insulin and HOMA

Fig. 2a depicts the impairment of glucose tolerance in the fructose-fed group as compared to the control. Thus, in the control group ingestion of glucose caused a marked steady rise in blood sugar level that reached a peak of 126.7 mg/dl at 15 min; it was maintained at 30 min (130.0 mg/dl) and became nearly normal 2 hours after glucose loading. In FED rats, blood glucose peaked at 15 minutes, there was another peak at 60 minutes, and was still 26% higher than normal after 2 hours. Moreover, the peak blood glucose level in the FED group was higher (156.9 mg/dl) and delayed. Glucose tolerance was improved but not normalized by RES treatment. Beside the alterations in OGTT, feeding rats with fructose caused an increase in blood glucose level to 93.00 mg/dl compared to control (76.25 mg/dl), an increase in serum insulin level to 0.753 µg/L compared to control (0.560 µg/L) leading to a 58% rise in HOMA index. Treatment did not affect blood sugar level but it caused a recovery of both insulin level and HOMA index. Fig.2b, 2c & 2d illustrate the changes in blood glucose, insulin and HOMA respectively.

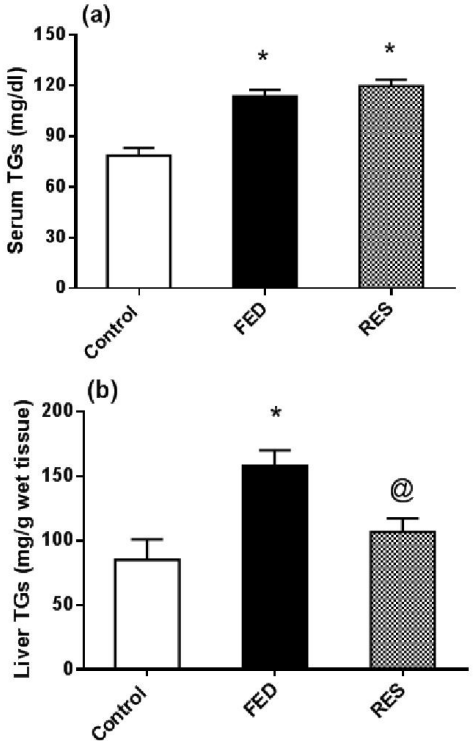


Fig. 3. Effect of resveratrol (70 mg/kg) on liver TGs (a) and serum TGs (b) in fructose-induced NASH in rats

FED= fructose enriched diet, RES= resveratrol, TGs= triglycerides.
Values are means ± S.E.M. n = 8 – 12 rats.
The significance of the difference between means was tested by ANOVA followed by Tukey Kramer multiple comparisons test.

* Significantly different from control, @ Significantly different from FED at $p < 0.05$.

Serum and liver TGs

The changes in serum and liver triglycerides are illustrated in Fig.3a and 3b respectively. Fructose fed rats showed a rise of 44.5% in serum TGs and 86.3% in hepatic triglycerides content as compared to the control group. RES was effective in preventing the accumulation of liver TGs, but failed to resume serum TGs to the control levels.

Hepatic MDA, GSH contents and SOD activity

Fig.4 shows the changes in the redox balance in the liver. Fructose feeding significantly elevated liver MDA by 59% and

decreased SOD activity by 17% without affecting liver GSH content. Being an antioxidant free radical scavenger, RES prevented the upsurge of MDA and normalized hepatic SOD activity.

Serum AST, ALT, AST/ALT ratio and TNF-α

As shown in Fig.5 (a, b &c), feeding fructose slightly increased the activity of AST without affecting ALT; there was a 2 fold increase in the AST/ALT ratio.

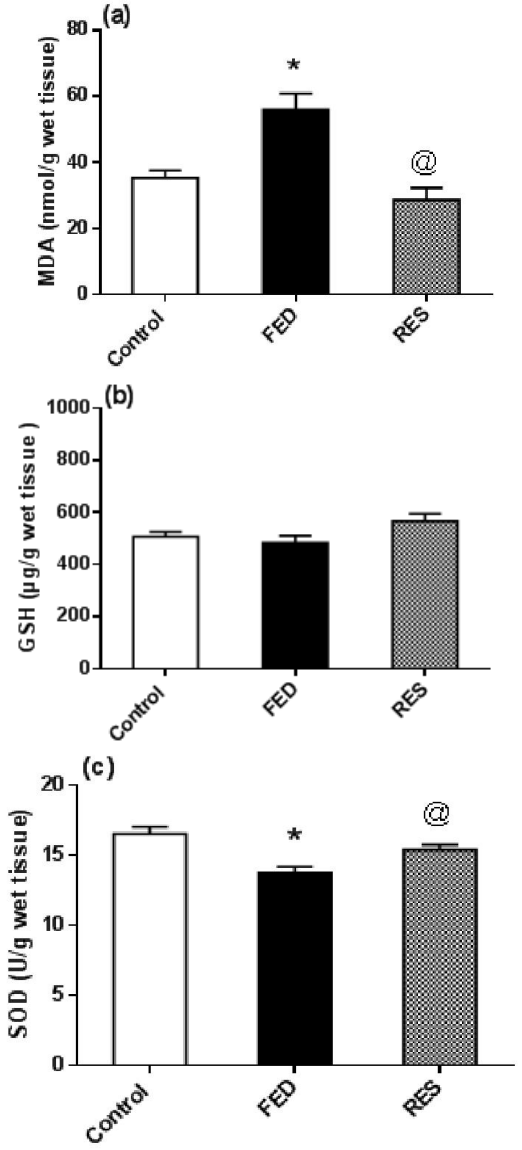


Fig. 4. Effect of resveratrol (70 mg/kg) on liver MDA (a), GSH (b) and SOD (c) in fructose-induced NASH in rats

FED= fructose enriched diet, RES= resveratrol, MDA= malonaldehyde, GSH = glutathione, SOD= superoxide dismutase. Values are means ± S.E.M. n = 8 – 12 rats. The significance of the difference between means was tested by ANOVA followed by Tukey Kramer multiple comparisons test.

* Significantly different from control, @ Significantly different from FED at p< 0.05.

Additionally, Serum TNF-α (Fig.5d) was augmented (1.6 folds) compared to control. RES treatment opposed the injurious effect of FED and normalized both AST/ALT ratio and serum TNF-α level.

Hepatic expression of SOCS-3, SREBP-1c, FAS, MCD and TGF-β1

The expressions of SOCS-3, SREBP-1c, FAS, MCD and TGF-β1 in the control group were 1.287, 0.177, 1.04, 1.21 and 0.0084 respectively. Fructose feeding displayed profound changes in these signaling pathways.

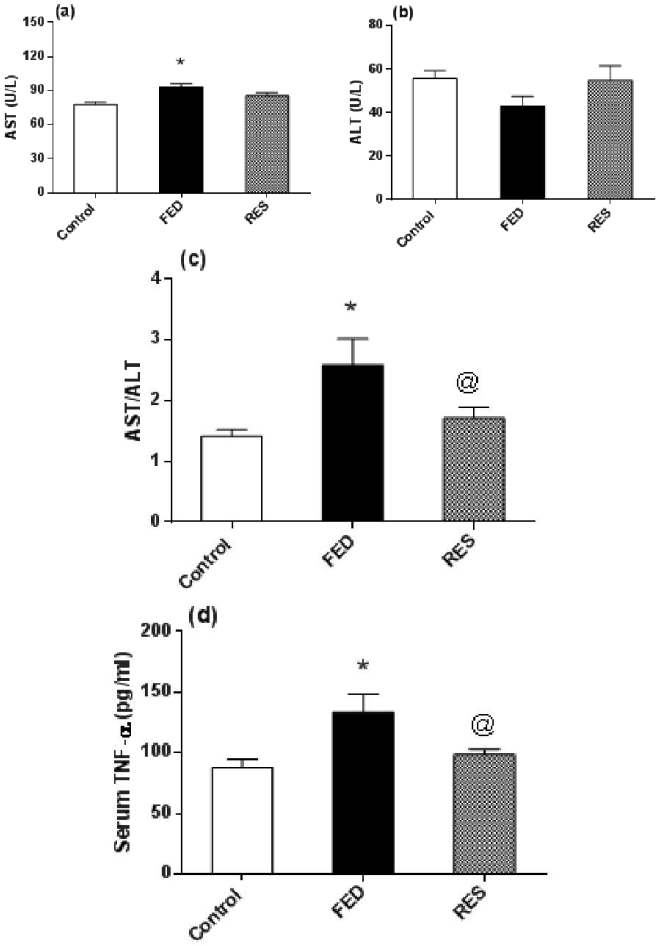


Fig. 5. Effect of resveratrol (70 mg/kg) on serum AST (a), ALT (b), AST/ALT ratio (c) and serum TNF-α (d) in fructose-induced NASH in rats

FED= fructose enriched diet, RES= resveratrol, AST= aspartate aminotransferase, ALT= alanine aminotransferase, TNF-α= tumor necrosis factor-α. Values are means ± S.E.M. n = 8 – 12 rats. The significance of the difference between means was tested by ANOVA followed by Tukey Kramer multiple comparisons test.

* Significantly different from control, @ Significantly different from FED at p< 0.05.

Thus, there was about 7 folds increase in SOCS-3 gene expression, 2 folds increase in SREBP-1c, 8 folds increase in FAS, whereas MCD gene expression revealed a 4 folds decline compared to control group. Treatment of the rats given FED with RES trimmed down SOCS-3 gene expression to 3.933 compared to FED (9.500) (Fig.6a) and caused

moderation of SREBP-1c by 68.7%. The hepatic expression of the FAS gene, responsible for *de novo* lipogenesis, was reduced to 5.14 compared to FED (8.60). Furthermore, MCD gene expression in liver was increased by 3.0 folds compared to FED group (Fig.6b, 6c&6d). TGF-β gene expression was upregulated in fructose fed rats (1.4 folds) as compared to the control counterpart and was normalized by treatment with RES as shown in Fig.6e.

Adipose tissue genes expression of leptin and adiponectin

Fig.7 represents the alterations in the expression of leptin and adiponectin genes in adipose tissue.

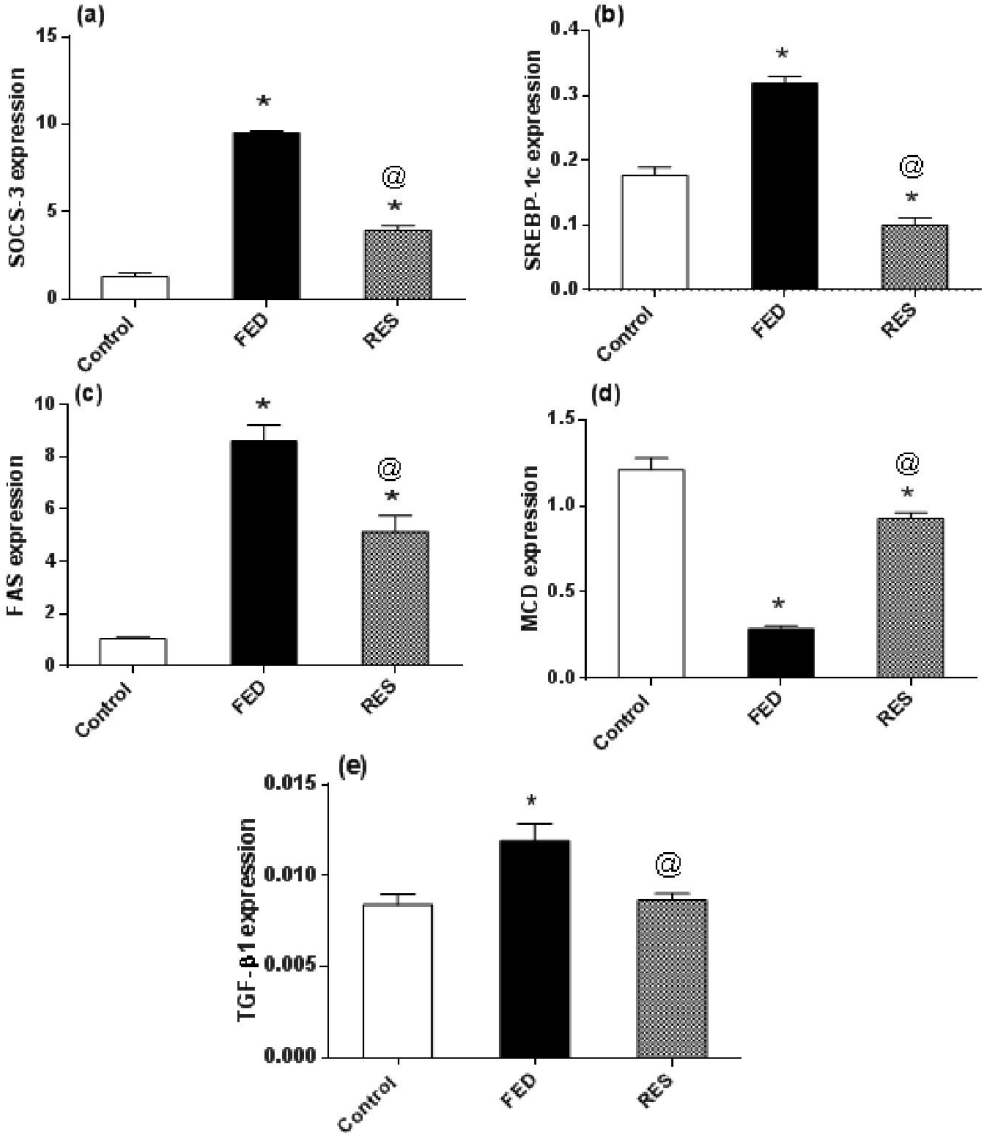
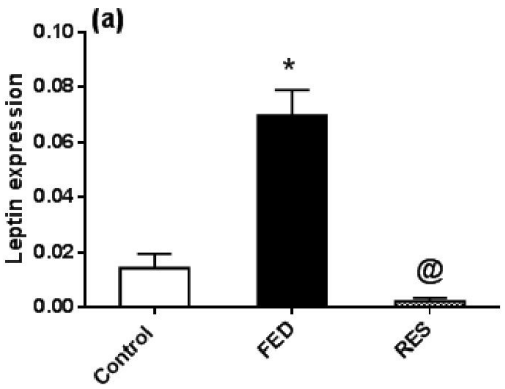


Fig. 6. Effect of f resveratrol (70 mg/kg) on hepatic genes expression: SOCS-3 (a), SREBP-1c (b), FAS (c), MCD (d) and TGF-β1 (e) in fructose-induced NASH in rats

FED= fructose enriched diet, RES= resveratrol, SOCS-3= suppressor of cytokine signalling-3, SREBP-1c= sterol regulatory element binding protein-1c, FAS= fatty acid synthase, MCD= malonyl Co-A decarboxylase. Gene expression (level of mRNA) was expressed in arbitrary units based on calculation expression level relative to the internal standard. Values are means ± S.E.M. n = 8 – 12 rats. The significance of the difference between means was tested by ANOVA followed by Tukey Kramer multiple comparisons test.

* Significantly different from control, @ Significantly different from FED at $p < 0.05$.



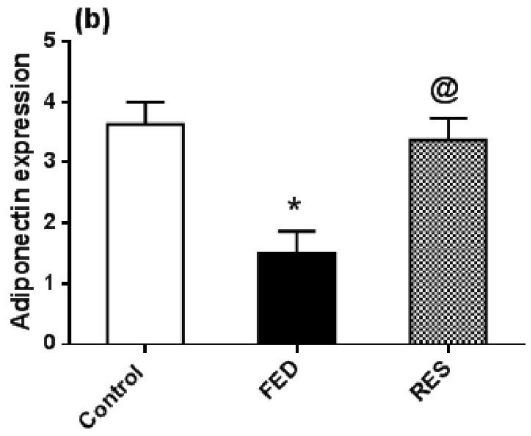


Fig. 7. Effect of resveratrol (70 mg/kg) on adipose leptin (a) And adiponectin (b) genes expression in fructose-induced NASH in rats

FED= fructose enriched diet, RES= resveratrol. Gene expression (level of mRNA) was expressed in arbitrary units based on calculation expression level relative to the internal standard. Values are means ± S.E.M. n = 8 – 12 rats. The significance of the difference between means was tested by ANOVA followed by Tukey Kramer multiple comparisons test.

* Significantly different from control, @ Significantly different from FED at p< 0.05.

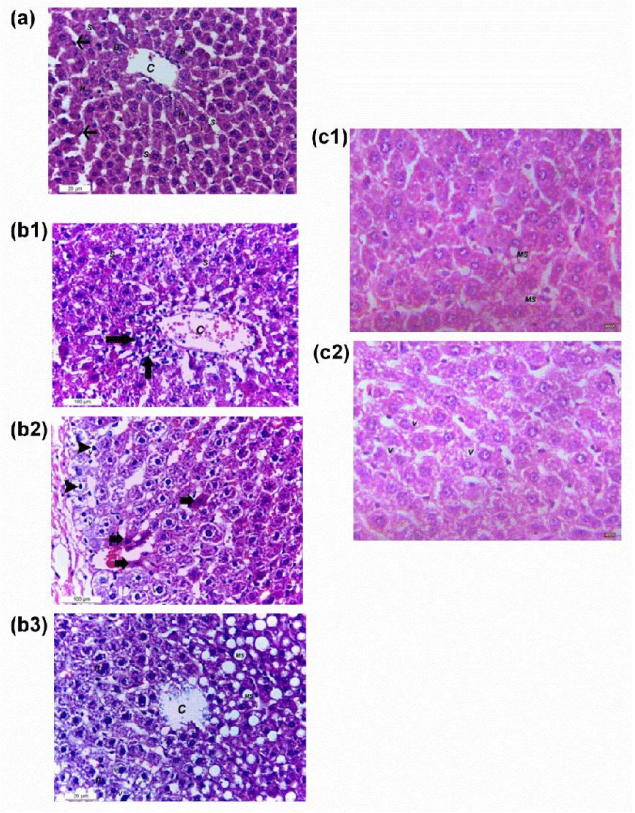


Fig. 8. Representative photomicrographs of liver sections of control (a), FED (bs) and resveratrol (70 mg/kg) (cs) in fructose-induced NASH in rats. (H&E x 400)

a: Control rat liver showing a central vein (C), hepatocytes (H) arranged in the form of plates separated from each other by irregular blood sinusoids (S).

b1: Fructose-fed rat liver showing cellular infiltration (arrows) near the central vein (C).

b2: Fructose-fed rat liver showing pyknotic hepatic nuclei (arrowheads), cytoplasmic vacuolations (V) and apoptotic hepatic cells (arrows).

b3: Fructose-fed rat liver showing central vein (C) with macrovesicular steatosis (MS) compressing hepatic nuclei to the right side of the vein , and cytoplasmic vacuolations (V) , pyknotic nuclei (P) and binucleated hepatic cells to the left side of the vein.

c1: Resveratrol-treated rat liver showing macrovesicular steatosis (MS).

c2: Resveratrol-treated rat liver showing vacuolations (V) of the cytoplasm of the hepatic cells.

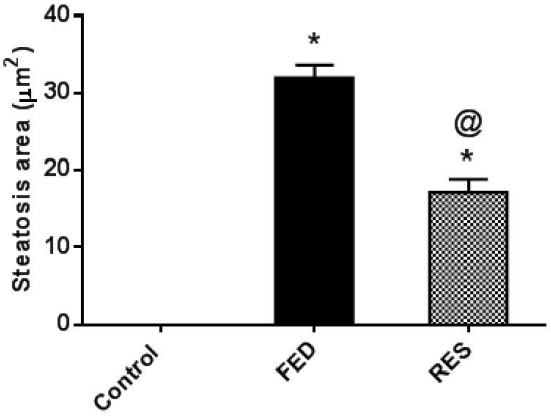


Fig. 9. Effect of resveratrol (70 mg/kg) on steatosis area in fructose-induced NASH in rats

FED= fructose enriched diet, RES= resveratrol. Values are means ± S.E.M. n = 400 images.

The significance of the difference between means was tested by ANOVA followed by Tukey Kramer multiple comparisons test.

* Significantly different from control, @ Significantly different from FED at p< 0.05.

It is obvious that feeding rats with fructose produced an almost 5 folds augmentation in leptin gene expression and 2 folds suppression in adiponectin expression. Cytoplasmic vacuolations, increased Kupffer cells' number, pyknosis and apoptosis of hepatic nuclei. Notably, RES treatment caused a marked improvement in the general structure of the liver tissue and markedly decreased the average steatosis area to 46% and prevented most of the histopathological abnormalities observed in the FED group.

DISCUSSION

Several studies emphasized that consumption of high fructose diets led to obesity, fatty liver, hypertriglyceridemia and insulin resistance (Stanhope *et al*., 2009; Tappy and Le, 2010). In the liver, fructose increases *de novo* synthesis of fatty acids (Mayes, 1993) and increases inflammation (Nomura and Yamanouchi, 2012), both contribute to increased hepatic lipogenesis. Resveratrol is a natural dietary polyphenol which has been shown to combine antioxidant, anti-inflammatory and antihyperlipidemic effects (Bujanda *et al*., 2008; Zhu *et al*., 2008). In the present study, when resveratrol was given to

rats fed high fructose diet it prevented the gain in body weight and modulated the severity of liver damage seen microscopically and relieved the development of NASH. It ameliorated insulin resistance via reducing hepatic lipid deposition, prevented lipid peroxidation and reactive oxygen species (ROS) formation and inhibited inflammation by suppressing the production of TNF-α. In the present study, the first observation with resveratrol was a desirable decrease in body weight that could also be attributed to the enhanced insulin sensitivity and decreased accumulation of body-fat mediated in part by adipokines changes as resveratrol reduced leptin and increased adiponectin mRNA levels. This is in harmony with a number of previous studies, reporting that resveratrol supplementation decreases body weight (Lagouge *et al*., 2006), has an anti-obesity potential (Chen *et al*., 2012) and affects leptin adiponectin balance (Eseberri *et al*., 2013; Kim *et al*., 2011). In contrast, other studies denied the beneficial effects of resveratrol on body weight and obesity (Baur *et al*., 2006; Tauriainen *et al*., 2011).

The discrepancy could be due to the duration of study, dosage of resveratrol, and age of animals.In the histopathological examination, the only abnormality seen was macrovesicular steatosis together with hepatic cell vacuolations, both of which are less than observed in the FED group. Several investigators have demonstrated a similar hepatoprotective effect of resveratrol against steatosis in mice (Tauriainen *et al*., 2011) or rats (Shang *et al*., 2008 a) fed high fat diet. The histological improvement of resveratrol was extended to involve an improvement in insulin sensitivity. This lends credit to previous studies showing improved insulin sensitivity in rats fed high fat diet (Shang *et al*., 2008 b) and in obese humans (Timmers *et al*., 2011). The enhanced insulin sensitivity was reflected herein by decreased hepatic TGs deposition. This was in accordance with other investigators who demonstrated a reduction in hepatic fat deposition in experimental NAFLD models (Bujanda *et al*., 2008; Shang *et al*., 2008 b).

Another important property of resveratrol is having anti-inflammatory activity. This was shown in the present study by preventing the rise in serum TNF-α. Anti-inflammatory activity was also indicated by inhibition of inflammation in mice fed high fat diet (Kim *et al*., 2011), in NAFLD rats (Bujanda *et al*., 2008) and decreasing IL-6 and TNF-α mRNA and reduced the Kupffer cells' number induced in the injured liver of mice after bile duct ligation (Chan *et al*., 2011). In obese humans, resveratrol decreased expression levels of genes of inflammatory pathways, and plasma levels of several inflammatory markers (Timmers *et al*., 2011). Being an effective antioxidant, resveratrol could also inhibit the progression of steatosis or steatohepatitis. In the fructose fed rats it decreased hepatic MDA content and increased SOD activity. Resveratrol has been shown to prevent free radicals and inflammatory cytokines-induced hepatic damage by scavenging ROS and decreasing lipid peroxidation (Leonard *et al*., 2003). Resveratrol administration reduces oxidative stress in obese rats (Gomez-Zorita *et al*., 2012; Franco *et al*., 2013) and in hyperlipidemic rats (Zhu *et al*., 2008). Similar findings were reported clinically in normal, healthy subjects in the postprandial state where resveratrol suppressed oxidative and inflammatory stress responses to in high fat high carbohydrate meal (Ghanim *et al*., 2011).

The observed resveratrol-induced improvement in NASH related parameters and histopathology was associated with a significant hepatoprotective effect revealed by the reduction in AST/ALT ratio. Other study showed that, resveratrol significantly reduced both ALT, AST serum levels in cholestatic liver injury (Chan *et al*., 2011). On the molecular basis, resveratrol decreased hepatic SOCS-3 gene expression, therefore preventing insulin resistance as the enhanced expression of SOCS-3would result in decreased tyrosine phosphorylation of insulin receptor substrate proteins and consequently attenuation of insulin action. Another contributing role of SOCS-3 arises from its ability to increase fatty acid synthesis by up-regulation of SREBP-1c expression, presumably through persistent hyperinsulinemia (Ueki *et al*., 2005). Additional findings of resveratrol treatment are down-regulation of lipogenic genes expression of SREBP-1c and FAS that reported in this study and in an earlier study (Rayalam *et al*., 2008).

Resveratrol may improve fructose-induced metabolic abnormalities by activating adenosine monophosphate kinase (AMPK) pathway (Shang *et al*., 2008 b). Because resveratrol activates AMPK, one would expect that, AMPK activation in the liver shuts down anabolic processes like cholesterol and TG biosynthesis by reducing the activities of SREBP-1c and FAS. AMPK activation also promotes catabolic processes. This would lead to a reduction in lipid synthesis and higher fatty acid oxidation rates and finally, prevention of liver steatosis. Furthermore, resveratrol positively activates sirtulin-1 (SIRT-1) through activation of AMPK (Canto *et al*., 2010). In turn, SIRT-1 activation gives protection against insulin resistance (Rutanen *et al*., 2010) and results in SREBP-1c inhibition. The expression of hepatic MCD gene, which is responsible for fatty acid oxidation, was upregulated by resveratrol leading to suppression of lipogenesis and enhanced β-oxidation. As for adipose tissue, resveratrol attenuated the expression of leptin gene, indicating an enhancement of leptin sensitivity and at the same time it increased adiponectin expression. Both of these actions would, in turn, lead to activation of AMPK (Minokoshi *et al*., 2002; Yamauchi *et al*., 2002).

The reduction in adipose leptin gene expression was associated with decreased hepatic SOCS-3 expression with consequent enhancement of leptin sensitivity (Howard *et al*., 2004). The reduction observed in oxidative stress, serum TNF-α and leptin expression in addition to stimulation of adiponectin expression was reflected in reduced TGF-β expression in liver by resveratrol treatment. As an anti-inflammatory, resveratrol decreased TGF-β1 mRNA expression. It has been reported that resveratrol decreases NF-kB which controls transcription of TGF-β induced by CCl4 (Chavez *et al*., 2008). There is an interplay between obesity, leptin, adiponectin, TNF-α and TGF-β. In conclusion, the current work justifies the use of resveratrol to reverse NASH and its associated insulin resistance and protect the liver from fructose induced hepatic steatosis. This can be attributed to its antioxidant and anti-inflammatory effects in addition to anti-hyperlipidemic effect which is mediated by modulation of hepatic and adipose tissue genes expression. Clinical trials in this area are scarce and have primarily been performed with a focus on overweight/obese participants without a focus on

NAFLD/NASH and histological liver changes. Future clinical studies with appropriate design are needed to clarify the true impact of RES treatment in NAFLD/NASH patients.

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