

Effect of CB1-Antagonist (AM251) on Metabolic Syndrome (Prophylactic and Therapeutic)

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Abstract

The present study was designed to investigate the possible prophylactic and therapeutic effect of cannabinoid receptors type1 (CB1) antagonist (AM251) on fructose-induced metabolic syndrome in rats, through administration of high fructose diet for 12 weeks. Prophylactic treatment by CB1-antagonist (AM251) significantly returned triglyceride, inflammatory, oxidative stress parameters and liver enzymes to the normal values while it significantly improved blood pressure, glucose, insulin, and IR and lipid profile but not significantly returned to the normal values. Interestingly, it significantly decreased body weight to values less than corresponding normal group. Histopathological findings confirmed that CB1-antagonist (AM251) in both prophylactic and therapeutic group improved histopathological changes of the liver and aorta induced by high fructose (60%) diet, with no drug had the upper hand of improvement of these histopathological changes.

Keywords: CB1-antagonist; AM251; Metabolic syndrome; Prophylactic treatment; Therapeutic treatment; Drugs; Histopathology

Introduction

The metabolic syndrome (MetS), defined as the clustering of multiple metabolic abnormalities, including abdominal obesity, dyslipidemia (elevated serum triglyceride and depressed serum high-density lipoprotein cholesterol), dysglycemia and hypertension [1]. Metabolic syndrome (MetS) is associated with glucose intolerance, obesity, ageing, elevated blood pressure and dyslipidemia, all of which are risk factors of highly cardiovascular morbidity and mortality [2]. The prevalence of MetS is increasing continuously to provide challenges for medical research beyond its clinical and public health importance. The pathophysiology of MetS seems to be largely attributable to insulin resistance with the implication of excessive flux of fatty acids [3] but also to a pro-inflammatory state resulting from the production of cytokines from adipocytes and macrophages [4]. Thus, increased inflammatory factors and reactive oxygen species (ROS) are associated with detrimental cardiovascular alterations linked to MetS [5].

Marijuana and its derivatives have been used in medicine for centuries; however, it was not until the isolation of the psychoactive component of Cannabis sativa, 9-tetrahydrocannabinol; (THC) and the subsequent discovery of the endogenous cannabinoids signaling system [6]. Endocannabinoids are lipid mediators of the same cannabinoid (CB) receptors that mediate the effects of marijuana. The endocannabinoid system (ECS) consists of CB receptors, endocannabinoids, and the enzymes involved in their biosynthesis and degradation, and it is present in both brain and peripheral tissues, including the liver [7].

The cannabinoids exert their pharmacologic action through the interaction with the specific receptors CB1 and CB2, which were described in the late 1980s and later were cloned [8]. The CB1 receptors are primarily distributed to the brain and adipose tissue, but are also found in the myocardium, vascular endothelium, and sympathetic nerve terminals. The CB2 receptors are primarily located in the lymphoid tissue and peripheral macrophages. Both receptors function as trans membrane G-proteins [9,10].

Existence of CB3 receptors has been postulated, but the receptor itself has not yet been cloned [11]. Cannabinoid receptors have affinity for at least two endogenous ligands which are small lipid molecules arachidonylethanolamide (anandamide) and 2-arachidonoylglycerol (2-AG). Under normal conditions, the endocannabinoid system is not tonically active; rather endocannabinoids are produced on demand, act locally, and are rapidly inactivated via cellular uptake and enzymatic hydrolysis. Cannabinoid antagonists were developed; of which rimonabant has been the most extensively studied. It has a high affinity for the central CB1 receptors [12]. There is increasing evidence showing that the endocannabinoid system plays a central role in regulating metabolism and body composition by enhancing the central orexigenic drive and increasing peripheral lipogenesis [13]. Control of food intake and body composition is the result of a series of complex interactions between the adipocytes, the mesolimbic system, the hypothalamus, and the gastrointestinal tract [14]. Leptin, an endogenous hormone, can reduce the food intake. Serum concentration of leptin is directly proportional to the degree of adiposity, but obese individuals have lower sensitivity to leptin. An adipose tissue-specific protein, adiponectin, stimulates fatty acid oxidation and a decrease in body weight. Its levels are reduced in obesity [15].

EC system over-activity may result from increased EC synthesis, CB (mainly CB1) receptor overexpression, and/or decreased EC degradation. Conversely, pharmacological modulation to correct over-activity of the EC system may theoretically involve reduction of EC production, blockade of cannabinoid (CB) receptors, and/

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or enhancement of EC degradation [16]. The most advanced pharmacological approach targets CB1 receptors [17]. There are different possible mechanisms by which CB1 receptor antagonists produce their effects on the CB1 receptor. The ligands can be pure competitive antagonists of CB1 receptor activation by endogenously released ECs (neutral antagonists), or they can act as inverse agonists modulating constitutive CB1 receptor activity by shifting it from an active 'on' to an inactive 'off' state [18].

Material and Methods

Materials

Drugs:

AM251 is a CB₁ receptor antagonist: C₂₂H₂₁Cl₂IN₄O Mw 555.24. It was received from TOCRIS, in the form of ten vials, each vial contain 10 mg white solid powder, each vial was dissolved in absolute ethanol to form stock solution, the stock solution was diluted immediately before administration, the dilution was as follow, 18:1:1 normal saline, tween-80 and absolute ethanol respectively. It was given by a dose of (1 mg/kg/day) intraperitoneally [19].

Normal saline: Produced by El-Nasr pharmaceutical company.

Heparin: (MW=12000-15000 g/mol) in the form of ampoule (Amoun pharmaceutical company, Egypt).

Urethan: White crystals dissolved in normal saline (Sigma-Aldrich, USA).

Chemicals:

- Ethanol, clear colorless liquid (Guangdong, China)
- Tween-80, aqueous solution of polyethylene sorbitol ester
- Fructose, C₆H₁₂O₆, Molecular weight: 180.16, white crystallized powder (Sigma-Aldrich, USA)

Kits:

1. Kits for measurement of lipid profile (Diamond Diagnostic Company, Egypt).
2. Kits for measurement of serum glucose (biodiagnostic, Giza, Egypt).
3. Kits for measurement of serum insulin, rat insulin ELISA kit (biodiagnostic, Giza, Egypt)
4. Kits for measurement of serum tumor necrosis factor alpha (TNF- α), ELISA kits derived from (Immunotech, France).
5. Kits for measurement of serum nitric oxide (NO), (biodiagnostic, Giza, Egypt)
6. Kits for measurement of serum malondialdehyde (MDA) (biodiagnostic, Giza, Egypt).
7. Kits for measurement of serum glutathione, (biodiagnostic, Giza, Egypt).
8. Kits for measurement of serum catalase, (biodiagnostic, Giza, Egypt).
9. Kits for measurement of serum super oxide dismutase (SOD) (biodiagnostic, Giza, Egypt).
10. Kits for measurement of liver enzymes (ALT&AST), (Diamond Diagnostic Company, Egypt).

Apparatus:

- Spectrophotometer (Spectro UV-V is RS Model UV-2500, USA)
- ELISA analyzer (Bioscience Company, USA)
- Oscillograph (D-Channel Modular Universal Oscillograph, Curvilinear, 115 VAC, 60 Hz, A Harvard Bioscience Company, USA)
- A Pressure transducer (PX81-5V, Omega Engineering International)
- Light microscope (XSZ-107BN, China)
- Nikon digital camera (DXM 1200, Japan)
- Digital scale (China weighing scale)

Animals: 130 Male albino rats initially weighing 150-170 g were used for the experiments. Rats were bred in the animal house of pharmacology department of AL-Azhar University; the animals were handled according to the guide lines of local ethical committee which comply with the international laws for use and care of laboratory animals. Each 5 rats were housed in a separate cage, at room temperature, exposed to natural daily light-dark cycles and had access to tap water ad libitum throughout the experiments. The standard chow diet was composed of 50% starch, 21% protein, 4% fat, 4.5% cellulose, standard vitamins and mineral mix. The high fructose diet (for induction of metabolic syndrome) was composed of 60% fructose, 21% protein, 5% fat, 8% cellulose, and standard vitamins and mineral mix.

Method

Experimental protocol: After resting and observation period of 1 week, rats were divided into three main groups.

Control (C) group: This group received a standard rat chow diet and the vehicle of the tested drugs for 12 weeks, (n=30).

Prophylactic (P) groups: Consists of 10 rats, received a high fructose diet and tested drugs or its vehicle for 8 weeks, rats were fed a high fructose diet and received CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for 8 weeks.

Therapeutic (T) groups: Consists of 50 rats, received a high fructose diet for 12 weeks and received the tested drugs or its vehicle for 4 weeks started at the end of 8th week till the end of 12th week, this group was divided into 5 subgroups, each subgroup consists of 10 rats were fed a high fructose diet for 12th weeks and CB1 antagonist (AM251) (1 mg/kg/day) from the end of 8th week till the end of 12th week of fructose administration.

The first day of fructose feeding was recognized as day 1.

Body weight: It was measured at the start, at the end of 8th week and at the end of 12th week of the study for all studied groups.

Collection of blood samples: Collection of blood samples were done at the start (for 10 rats of normal control group), at the end of 8th week (for 10 rats of normal control group & prophylactic groups) and at the end of 12th week (for 10 rats of normal control group & therapeutic groups). Blood samples were collected from the retro-orbital venous plexus of rat eye by using heparinized capillary tubes. The collected blood samples were then centrifuged (Cooling centrifuge, sigma 2 k 15) at 300 round/minute for 30 minutes. Then the serum was transferred into clean vials and stored at -18°C for biochemical

parameters determination [20]. All biochemical measurements were done in department of biochemistry, faculty of medicine, Cairo University.

Biochemical analysis:

Measurement of serum glucose, triglyceride (TG), HDL-cholesterol, LDL-cholesterol and total cholesterol (TC): Kits for measurement of lipid profile, (Diamond Diagnostic Company, Egypt).

Measurement of insulin and insulin resistance (HOMA-IR): Kits for measurement of serum insulin, rat insulin ELISA kit (biodiagnostic, Giza, Egypt).

Measurement of serum total glutathione (GSH): Kits for measurement of serum glutathione, (biodiagnostic, Giza, Egypt).

Measurement of plasma malondialdehyde (MDA): Kits for measurement of serum malondialdehyde (MDA) (biodiagnostic, Giza, Egypt).

Measurement of plasma nitric oxide (NO): Kits for measurement of serum nitric oxide (NO) (biodiagnostic, Giza, Egypt).

Measurement of serum tumor necrosis factor alpha (TNF-α): Kits for measurement of serum tumor necrosis factor alpha (TNF-α), ELISA kits derived from (Immunotech, France).

Determination of serum catalase: Kits for measurement of serum catalase, (Biodiagnostic, Giza, Egypt).

Determination of serum superoxide dismutase (SOD): Kits for measurement of serum super oxide dismutase (SOD) (biodiagnostic, Giza, Egypt).

Determination of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST): Kits for measurement of liver enzymes (ALT&AST), (Diamond Diagnostic) Company, Egypt).

Measurement of systolic and diastolic blood pressure (Vascular histological analysis): After collection of blood samples, rats were sacrificed and the Aorta specimens were taken, fixation of vessel (Aorta) samples obtained from control and other groups were made in 10% formaldehyde. After that, paraffin sections at 4 micron thickness obtained from routine pathological processes were stained with hematoxylin-eosin and examined by light microscopy for lymphatic infiltration in place, hypertrophy of smooth muscles and sub endothelial edema [21].

Histological examination of the liver: After collection of blood samples, rats were sacrificed and liver specimens were taken, fixed in 10% formalin immediately after removal, then processed and embedded in paraffin, fragments of liver tissue were cut, being kept for 24 hours in a solution of 10% buffered formaldehyde. Shortly thereafter, this solution was replaced with 70% alcohol. After this step, the livers were immersed in paraffin and then cut with a thickness of 4 mm and stained with hematoxylin-eosin, in order to semi-quantitatively assess hepatic steatosis, which was classified into hydropic change, microvascular, macrovascular and mixed micro-macro vascular steatosis, according to Yerian L [22].

Statistical analysis: All values are expressed as Mean ± SEM. Data were statistically analyzed using independent samples student (t) test for comparison between two groups. Significance was set at p ≤ 0.05. Data were computed for statistical analysis by using statistical package for social science (SPSS), version 16 (SPSS Inc, USA), running on IBM compatible computer.

Results

Effect of prophylactic treatment with CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for 8 weeks on body weight, serum glucose, insulin, IR, blood pressure, lipid profile, inflammatory cytokines, oxidative stress and liver enzymes of fructose enriched diet group

Administration of CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally with fructose enriched diet for 8 weeks produced the following effects-

Effect of fructose (60%) enriched diet plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for 8 weeks on serum glucose, insulin and insulin resistance: Administration of CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally with fructose enriched diet for 8 weeks, the mean serum glucose, insulin and insulin resistance were 6.93 ± 0.13 mmol/L, 11.77 ± 0.30 μU/L and 3.63 ± 0.13 respectively.

There was significant (P<0.05) decrease in serum glucose, insulin and insulin resistance in fructose plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally group in comparison to high fructose diet group at the end of 8th week. The mean serum glucose, insulin and insulin resistance values did not return to the normal values in high fructose diet plus ALA group, at the end of 8th week as shown in Table 1 and Figure 1.

Effect of fructose (60%) enriched diet plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for 8 weeks on body weight and blood pressure: Administration of CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally with fructose enriched diet for 8 weeks; the mean body weight was 201.50 ± 1.79 g at the end of 8th week. There was significant decrease in body weight between high fructose diet plus CB1 antagonist (AM251) group when compared to either normal or high fructose diet groups at the end of 8th week, as shown in Table 2 and Figure 2.

Administration of CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally with fructose enriched diet for 8 weeks, the mean SBP & DBP were 137.50 ± 1.11 mmHg & 86.50 ± 1.24 mmHg, respectively

	Normal at 8 weeks	Fructose plus CB1-antagonist at 8 weeks	Fructose 8 weeks
	Mean ± S.E	Mean ± S.E	Mean ± S.E
Glucose (mmol/L)	5.92 ± 0.08	6.93 ± 0.13**	8.05 ± 0.15
Insulin (IU/L)	7.99 ± 0.09	11.77 ± 0.30**	14.27 ± 0.36
IR	2.10 ± 0.05	3.63 ± 0.13**	5.12 ± 0.21
Cholesterol (mg/dl)	129.77 ± 1.40	159.01 ± 3.67*	166.86 ± 5.92
TG (mg/dl)	73.82 ± 0.76	78.62 ± 1.74#	145.01 ± 4.34
HDL (mg/dl)	47.05 ± 1.37	41.31 ± 1.11**	33.43 ± 1.56
LDL (mg/dl)	68.17 ± 1.69	102.44 ± 2.59*	104.81 ± 8.65
ALT (U/L)	22.80 ± 0.67	26.6 ± 1.95#	49.20 ± 1.48
AST (U/L)	12.30 ± 0.63	16.00 ± 1.84#	23.90 ± 0.69

Table 1: Effect of fructose (60%) diet plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for 8 weeks, on serum glucose, insulin, IR, lipid profile and liver enzymes at the end of 8th week.

Each value represents the Mean ± S.E of 10 readings (P<0.05; n=10)
 *=significant fructose plus CB1 antagonist for 8 week when compared to normal at 8 weeks.
 #=significant fructose plus CB1 antagonist for 8 weeks when compared to fructose at 8 weeks.

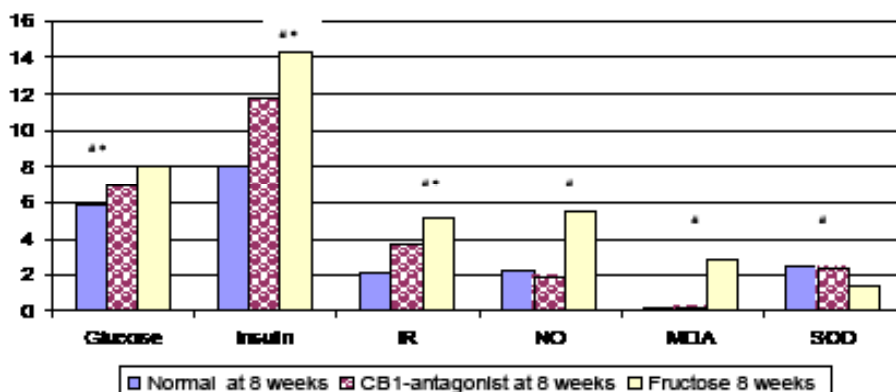


Figure 1: Effect of fructose (60%) diet plus CB1 antagonist (1 mg/kg/day) intraperitoneally for 8 weeks, on serum NO (nmol/L), MDA (nmol/L), SOD (U/ml), glucose (mmol/L), insulin (μ U/L) and IR (index).

Each value represents the Mean \pm S.E of 10 readings. ($P < 0.05$; $n = 10$)

*=significant fructose plus CB1 antagonist for 8 week when compared to normal at 8 weeks.

#=significant fructose plus CB1 antagonist for 8 weeks when compared to fructose at 8 weeks.

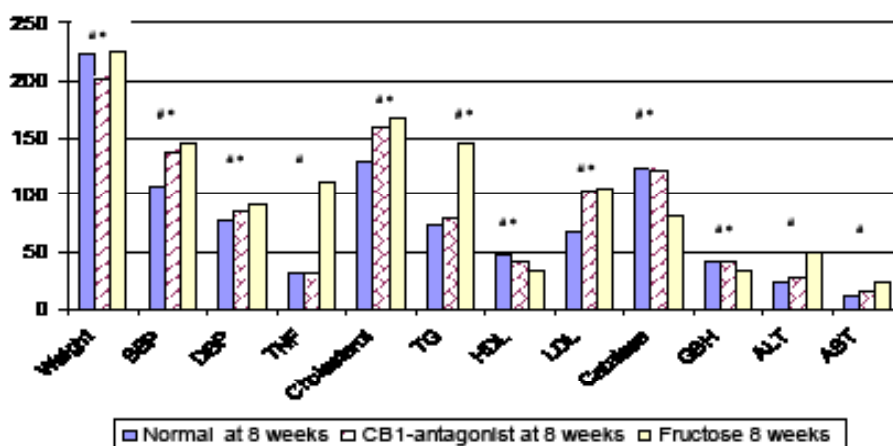


Figure 2: Effect of fructose (60%) diet plus CB1 antagonist (1 mg/kg/day) intraperitoneally for 8 weeks, on body weight (gm), blood pressure (mmHg), TNF- α (pg/mg), Catalase (μ U/L), GSH (nmol/gPtn), SOD (U/ml) lipid profile (mg/dl) and liver enzymes (U/ml).

Each value represents the Mean \pm S.E of 10 readings. ($P < 0.05$; $n = 10$)

*=significant fructose plus CB1 antagonist for 8 week when compared to normal at 8 weeks

#=significant fructose plus CB1 antagonist for 8 weeks when compared to fructose at 8 weeks

at the end of 8th week. There was significant decrease in both SBP & DBP in high fructose diet plus CB1 antagonist (AM251) group in comparison to high fructose diet group at the end of 8th week. The mean SBP & DBP values were not returned to the normal values in high fructose diet plus CB1 antagonist (AM251), at the end of 8th week as shown in Table 2 and Figure 2.

Effect of fructose (60%) enriched diet plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for 8 weeks on lipid profile: Administration of CB1 antagonist (AM251) (1 mg/kg /day) with fructose enriched diet for 8 weeks, the mean total cholesterol, triglyceride, HDL and LDL were 159.01 ± 3.67 mg/dl, 78.62 ± 1.74 mg/dl, 41.31 ± 1.11 mg/dl and 102.44 ± 2.59 mg/dl respectively as shown in Table 1 (Figures 1 and 2).

There was significant ($p < 0.05$) decrease in serum, triglyceride and significant increase in serum HDL cholesterol, while there was no significant ($p < 0.05$) difference of total cholesterol & LDL cholesterol in fructose enriched diet plus CB1 antagonist (AM251) (1 mg/kg /day) in

comparison to high fructose diet group at the end of 8th week. The mean serum triglyceride and HDL cholesterol did not return to the normal in high fructose diet CB1 antagonist (AM251), at the end of 8th week as shown in Table 1 and Figure 2.

Effect of fructose enriched diet plus CB1-antagonist (1 mg/kg/day) intraperitoneally for 8 weeks on inflammatory factors (TNF- α & NO): Administration of CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally with fructose enriched diet for 8 weeks, the mean TNF & NO were 31.19 ± 1.18 pg/mg & 1.91 ± 0.15 nmol/L respectively at the end of 8th week.

There was significant decrease in serum TNF&NO in fructose enriched diet plus CB1 antagonist (AM251) group in comparison to high fructose diet group at the end of 8th week. The mean serum TNF & NO were returned to the normal values in high fructose diet plus CB1 antagonist (AM251) group, at the end of 8th week as shown in Table 2 (Figures 1 and 2).

	Normal at 8 weeks	Fructose plus CB1-antagonist at 8 weeks	Fructose 8 weeks
	Mean ± S.E	Mean ± S.E	Mean ± S.E
Weight (g)	223.00 ± 2.03	201.50 ± 1.79**	225.50 ± 1.89
SBP (mmHg)	107.00 ± 1.49	137.50 ± 1.11**	145.00 ± 1.49
DBP (mmHg)	77.50 ± 1.30	86.00 ± 1.24**	90.50 ± 1.74
TNF (pg/mg)	31.81 ± 1.35	31.19 ± 1.18#	110.29 ± 8.18
NO (nmol/L)	2.23 ± 0.19	1.91 ± 0.15#	5.46 ± 0.22
MDA (nmol/L)	0.21 ± 0.02	0.21 ± 0.01#	2.87 ± 0.44
Catalase (μU/L)	123.83 ± 2.12	122.101.89#	81.49 ± 5.78
GSH (nmol/gPtn)	41.91 ± 0.95	40.96 ± 0.82#	32.16 ± 1.68
SOD (U/ml)	2.45 ± 0.09	2.34 ± 0.06#	1.36 ± 0.13

Each value represents the Mean ± S.E of 10 readings (P<0.05; n=10)

*=significant fructose plus CB1 antagonist for 8 week when compared to normal at 8 weeks.

#=significant fructose plus CB1 antagonist for 8 weeks when compared to fructose at 8 weeks.

Table 2: Effect of fructose (60%) diet plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for 8 weeks, on body weight, blood pressure, TNF, NO and oxidative stress parameters at the end of 8th week.

Effect of fructose enriched diet plus CB1 antagonist (AM251) (1 mg/kg/kg) intraperitoneally for 8 weeks on oxidative stress parameters (MDA, Catalase, GSH and SOD): Administration of CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally with fructose enriched diet for 8 weeks, the mean serum MDA, Catalase, GSH & SOD were 0.21 ± 0.01.nmol/L, 122.10 ± 1.89 μU/L, 40.96 ± 0.82 nmol/gPtn & 2.34 ± 0.06 U/ml respectively at the end of 8th week.

There was significant (p<0.05) decrease in serum MDA and significant increase (p<0.05) in catalase, GSH & SOD in fructose enriched diet plus Telmisartan group in comparison to High fructose diet group at the end of 8th week, as shown in Table 2, Figures 1 and 2. The mean serum MDA, Catalase, GSH & SOD were returned to the normal values in high fructose diet plus CB1 antagonist (AM251) group at the end of 8th week.

Effect of fructose enriched diet plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for 8 weeks on liver enzymes (ALT & AST): Administration of CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally with fructose enriched diet for 8 weeks, the mean serum ALT & AST were 26.60 ± 1.95 U/L & 16.00 ± 1.84 U/L respectively at the end of 8th week.

There was significant (p<0.05) decrease in ALT & AST in fructose enriched diet plus CB1 antagonist (AM251) group in comparison to high fructose diet group at the end of 8th week. The mean serum ALT & AST were returned to the normal values in high fructose diet plus CB1 antagonist (AM251) group, at the end of 8th week as shown in Table 1 and Figure 2.

Histopathological results at the end of 8th week after fructose (60%) diet administration for 8 weeks

Effect of fructose enriched diet for 8 weeks on liver structure: In the liver of normal rats, there was normal hepatic lobules, normal central hepatic vein with radiating normal hepatocytes with no evidence of hydropic or fatty changes as shown in Figures 3 and 4, after administration of high fructose diet for 8 weeks, there was pathological changes of liver structure in the form of marked congestion of central hepatic vein with marked hydropic changes and prominent micro vascular fatty changes of liver hepatocytes as shown in Figure 5.

Effect of fructose (60%) enriched diet for 8 weeks on structure of aorta: In the aorta of normal rats, the intima were intact, normal vascular smooth muscle fiber, with no evidence of hypertrophy or inflammation, as shown in Figure 6 after administration of high fructose diet for 8 weeks, there was hypertrophy of vascular smooth muscle fiber with marked subendothelial edema, as shown in Figure 7.

Effect of fructose enriched diet plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for 8 weeks on liver structure: Administration of high fructose diet plus CB1 antagonist (1 mg/kg/day) intraperitoneally for 8 weeks, the normal hepatic architecture was preserved with minimal hydropic changes as shown in Figures 8 and 9.

Effect of fructose enriched diet plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for 8 weeks on structure of aorta: Administration of high fructose plus CB1 antagonist (1 mg/kg/day) intrapretonially for 8 weeks, there was preservation of normal structure of aorta, as shown in Figure 10.

Histopathological results at the end of 8th week

Histopathological results at the end of 8th week after fructose (60%) diet administration for 8 weeks (control and normal).

	Normal at 12 weeks	CB1 antagonist at 12 weeks	Fructose at 12 weeks
	Mean ± S.E	Mean ± S.E	Mean ± S.E
Weight (g)	247.00 ± 1.63	214.00 ± 3.02#	250.00 ± 2.80
SBP (mmHg)	115.00 ± 1.49	141.50 ± 2.24**	162.50 ± 2.38
DBP (mmHg)	76.50 ± 1.67	87.00 ± 1.63**	99.50 ± 1.74
TNF (pg/mg)	29.29 ± 1.36	88.85 ± 5.56**	140.71 ± 5.72
NO (nmol/L)	2.11 ± 0.16	5.69 ± 0.44**	8.95 ± 0.44
MDA (nmol/L)	0.27 ± 0.01	1.95 ± 0.42**	3.63 ± 0.43
Catalase (μU/L)	128.99 ± 0.91	94.55 ± 2.64#	49.95 ± 2.68
GSH (nmol/gPtn)	39.52 ± 0.56	32.02 ± 1.20#	24.00 ± 1.22
SOD (U/ml)	2.30 ± 0.06	1.32 ± 0.06**	0.77 ± 0.04

Table 3: Effect of fructose (60%) plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally, for the last 4 weeks, of fructose 12 weeks administration on body weight, blood pressure, TNF-α, NO & oxidative stress.

Each value represents the Mean ± S.E of 10 readings (p<0.05; n=10)

*=significant CB1 antagonist when compared to normal at the end of 12th week.

#=significant CB1 antagonist when compared to fructose 12 weeks at the end of 12th week.

	Normal at 12 weeks	CB1-antagonist at 12 weeks	Fructose at 12 weeks
	Mean ± S.E	Mean ± S.E	Mean ± S.E
Glucose (mmol/L)	6.07 ± 0.12	9.51 ± 0.14**	10.45 ± 0.14
Insulin (IU/L)	7.14 ± 0.13	13.57 ± 0.28**	15.67 ± 0.23
IR	1.93 ± 0.07	5.74 ± 0.18**	7.28 ± 0.19
Cholesterol (mg/dl)	128.28 ± 1.45	186.75 ± 2.59*	188.55 ± 2.51
TG (mg/dl)	68.27 ± 0.49	146.95 ± 6.88**	172.19 ± 6.29
HDL (mg/dl)	44.82 ± 0.86	33.69 ± 0.60**	28.49 ± 0.52
LDL (mg/dl)	67.71 ± 1.64	123.67 ± 5.59*	125.6 ± 5.28
ALT (U/L)	23.50 ± 0.95	31.02 ± 4.35**	63.70 ± 3.69
AST (U/L)	14.10 ± 0.73	22.50 ± 0.83**	30.40 ± 0.88

Table 4: Effect of fructose (60%) plus CB1 antagonist (1 mg/kg/day) intraperitoneally, for the last 4 weeks, of fructose 12 weeks administration on serum glucose, insulin, IR, lipid profile and liver enzymes.

Each value represents the Mean ± S.E of 10 readings (p<0.05; n=10)

*=significant CB1 antagonist when compared to normal at the end of 12th week.

#=significant CB1 antagonist when compared to fructose 12 weeks at the end of 12th week.

Effect of fructose enriched diet for 8 weeks on liver structure:

In the liver of normal rats, there was normal hepatic lobules, normal central hepatic vein with radiating normal hepatocytes with no evidence of hydropic or fatty changes as shown in Figures 3 and 4 after administration of high fructose diet for 8 weeks, there was pathological changes of liver structure in the form of marked congestion of central hepatic vein with marked hydropic changes and prominent micro vascular fatty changes of liver hepatocytes as shown in Figure 5.

Effect of fructose (60%) enriched diet for 8 weeks on structure of aorta: In the aorta of normal rats, the intima were intact, normal vascular smooth muscle fiber, with no evidence of hypertrophy or inflammation, as shown in Figure 6, after administration of high fructose diet for 8 weeks, there was hypertrophy of vascular smooth muscle fiber with marked subendothelial edema, as shown in Figure 7.

Effect of fructose enriched diet plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for 8 weeks on liver structure: Administration of high fructose diet plus CB1 antagonist (1 mg/kg/day) intraperitoneally for 8 weeks, the normal hepatic architecture was preserved with minimal hydropic changes as shown in Figures 8 and 9.

Effect of fructose enriched diet plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for 8 weeks on structure of aorta: Administration of high fructose plus CB1 antagonist (1 mg/kg/day) intraperitoneally for 8 weeks, there was preservation of normal structure of aorta, as shown in Figure 10 and 11.

Histopathological results at the end of 12th week after administration of high fructose diet (Therapeutic)

Effect of fructose enriched diet for 12 weeks on liver structure (Normal and Control): In the liver of normal rats, there was normal hepatic lobules, normal central hepatic vein with radiating normal hepatocytes with no evidence of hydropic or fatty changes as shown in Figures 12 and 13 after administration of high fructose diet for 12 weeks, there was changes in liver structure in the form of, marked congestion of central hepatic vein with marked hydropic, micro and macrovascular fatty changes of liver hepatocytes as shown in Figure 14.

Effect of fructose enriched diet for 12 weeks on structure of aorta (Normal and Control): In the aorta of normal rats, the intima were intact, normal vascular smooth muscle fiber, with no evidence of hypertrophy or inflammation, as shown in Figures 15 and 16, after administration of high fructose diet for 12 weeks, there was hypertrophy of vascular smooth muscle fiber with marked sub endothelial edema, and lymphocytic infiltration of vascular smooth muscle as shown in Figures 17 and 18.

Effect of CB1 antagonist (1 mg/kg/day) intraperitoneally, for the last 4 weeks, of fructose 12 weeks administrations on liver structure: Administration of high fructose diet for 12 weeks plus CB1 antagonist (1 mg/kg/day) intraperitoneally, for 4 weeks, started at the end of 8th week till the end of 12th week, produced preservation of the normal hepatic architectures with minimal hydropic changes of liver hepatocytes as shown in Figures 19 and 20.

Effect of CB1 antagonist (1 mg/kg/day) intraperitoneally, for the last 4 weeks, of fructose 12 weeks administrations on structure of aorta: Administration of high fructose diet for 12 weeks plus CB1 antagonist (1 mg/kg/day) intraperitoneally for 4 weeks, started at the end of 8th week till the end of 12th week, produced preservation of

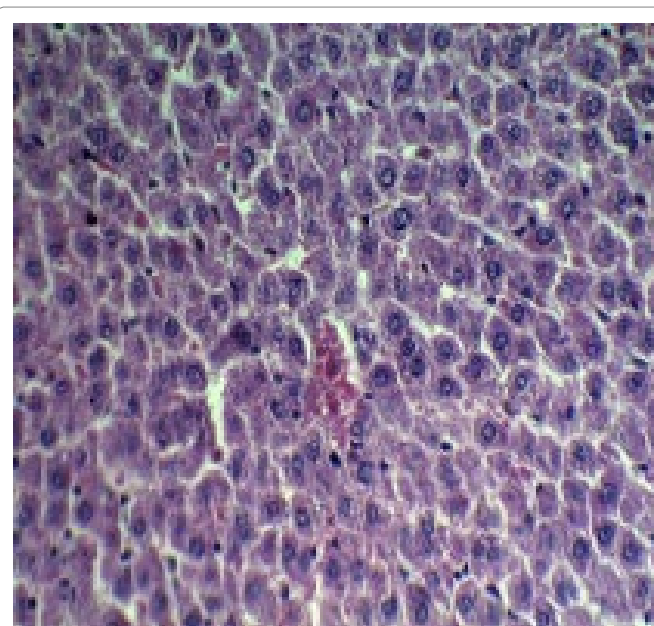


Figure 3: Section of the liver of normal rat showing normal liver architecture, with normal hepatocytes, no hydropic or fatty changes (H/E X200).

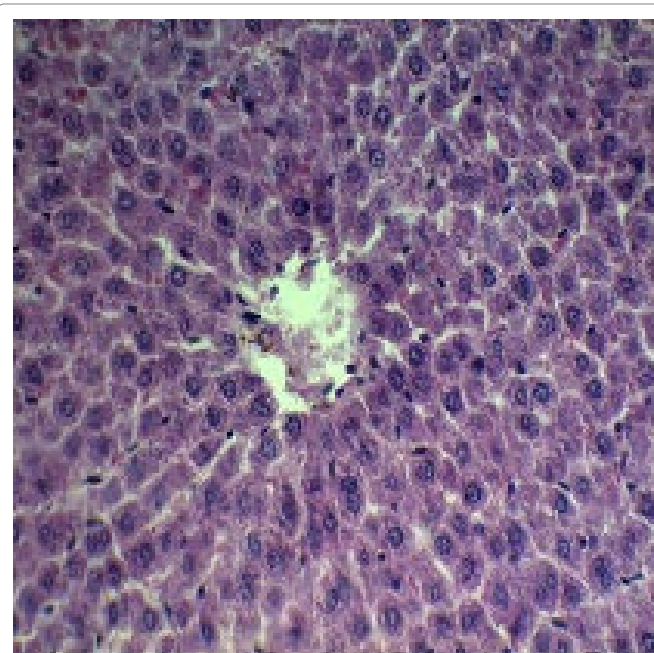


Figure 4: Section of the liver of normal rat showing normal liver architecture, with normal hepatocytes, no hydropic or fatty changes (H/E X400).

normal structure of aorta with minimal perivascular edema, as shown in Figures 21 and 22.

Results at the end of 12th week after fructose administration

Effect of therapeutic treatment with CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for 4 weeks, started at the end of 8th week till the end of 12th week, on body weight, serum glucose, insulin, IR, blood pressure, lipid profile, inflammatory cytokines, oxidative stress and liver enzymes of fructose enriched diet group at 12th week: Administration of CB1 antagonist (AM251) (1 mg/kg/day)

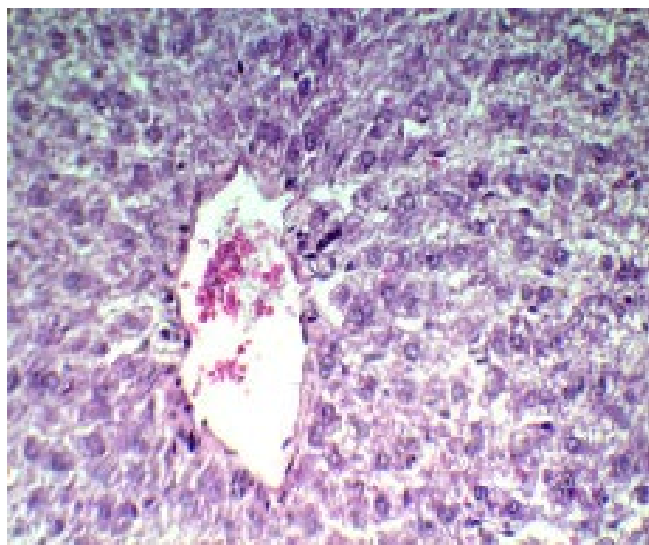


Figure 5: Section of rat liver showing congested dilated central vein with hydropic changes of liver hepatocytes (early changes of fatty liver) (H/E X400).

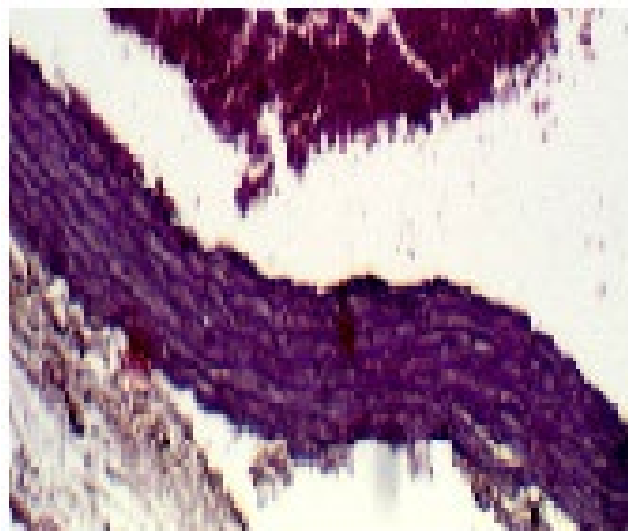


Figure 7: Section of rat aorta after administration of high fructose diet for 8 weeks showing hypertrophy of smooth muscle with perivascular edema (H/E X200).

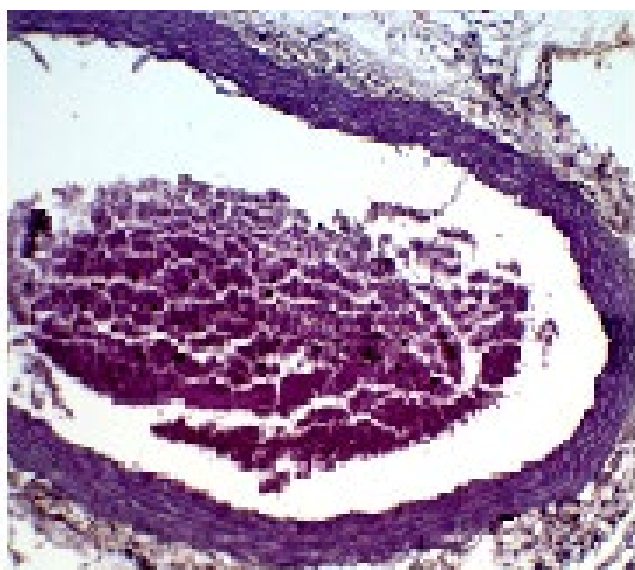


Figure 6: Section of the aorta of normal rat showing intact intima, no hypertrophy of smooth muscle or edema (H/E x200).

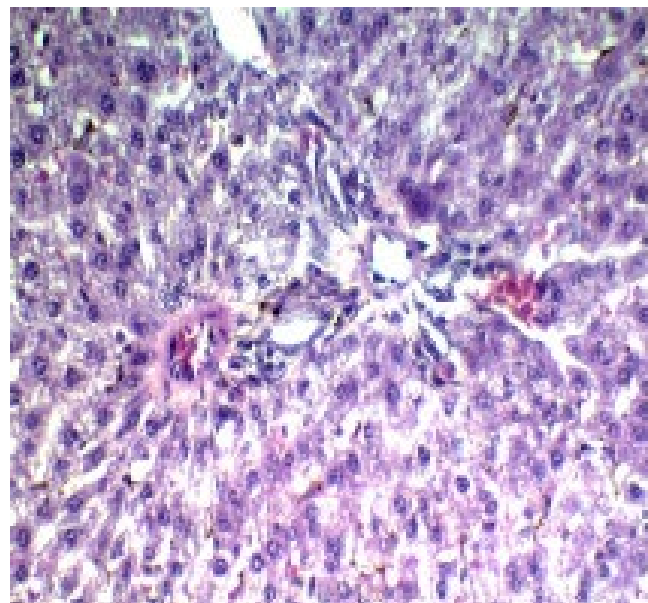


Figure 8: Section in rat liver after administration of high fructose diet plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for 8 weeks showing preserved portal tract with minimal hydropic changes of hepatocytes (H/E X400).

intraperitoneally for 4 weeks, started at the end of 8th week till the end of 12th week to fructose enriched diet for 12 weeks group, produced the following effects as shown in Tables 3 and 4 (Figures 23 and 24).

Effect of CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally, for the last 4 weeks, of fructose 12 weeks administration on serum glucose, insulin and IR: Administration of CB1 antagonist (AM251) (1 mg/kg/day intraperitoneally), started at the end of 8th week till the end of 12th week with fructose enriched diet for 12 weeks, the mean serum glucose, insulin and insulin resistance were 9.51 ± 0.14 mmol/L, 13.57 ± 0.28 I U/L and 5.71 ± 0.18 respectively.

There was significant decrease in serum glucose; insulin and insulin resistance in fructose plus CB1 antagonist (AM251) group in comparison to high fructose diet group at the end of 12th week. The mean serum glucose, insulin and insulin resistance values did not

return to the normal in high fructose diet plus CB1 antagonist (AM251) group, at the end of 12th week.

Effect of CB1 antagonist (1 mg/kg/day) intraperitoneally, for the last 4 weeks, of fructose 12 weeks administrations on body weight and BP: Administration of CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally, started at the end of 8th week till the end of 12th week with fructose enriched diet for 12 weeks, the mean microscopy was 214.50 ± 3.02 g at the end of 12th week. There was significant decrease in body weight of fructose diet plus CB1 antagonist (AM251) group when compared to either normal or high fructose diet at the end of 12th week. Administration of CB1 antagonist (AM251) (1 mg/kg/day), started at

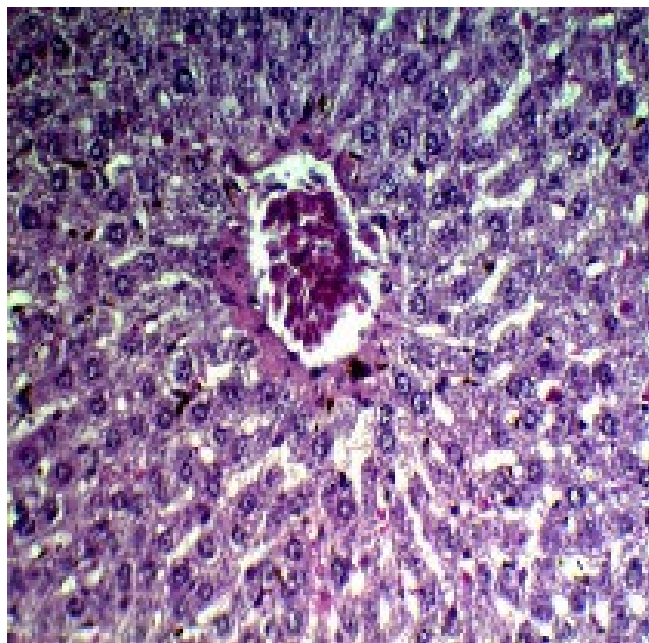


Figure 9: Section in rat liver after administration of high fructose diet plus CB1 antagonist (AM251), showing congested with normal hepatic architecture (H/E X200).

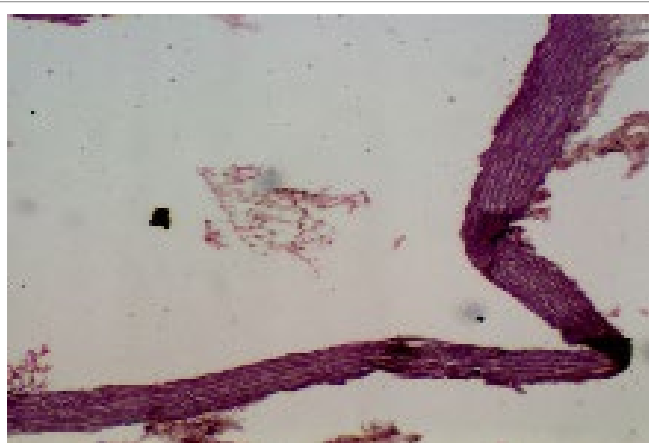


Figure 10: Section of rat aorta after high fructose diet administration with CB1 antagonist (AM251) intraperitoneally (1 mg/kg/day) for 8 weeks showing preservation of normal structure of the aorta with minimal perivascular edema (H/E X200).

the end of 8th week till the end of 12th week with fructose enriched diet for 12 weeks, the mean SBP & DBP were 141.50 ± 2.24 mmHg & 87.00 ± 1.63 mmHg, respectively at the end of 12th week.

There was significant decrease in both SBP & DBP in high fructose diet plus CB1 antagonist (AM251) group in comparison to high fructose diet group at the end of 8th week. The mean SBP & DBP values were not returned to the normal values in high fructose diet plus CB1 antagonist (AM251) group, at the end of 12th week.

Effect of CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally, for the last 4 weeks, of fructose 12 weeks administrations on lipid profile: Administration of CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for 4 weeks, started at the end of 8th week till the end of 12th week to fructose enriched diet for 12

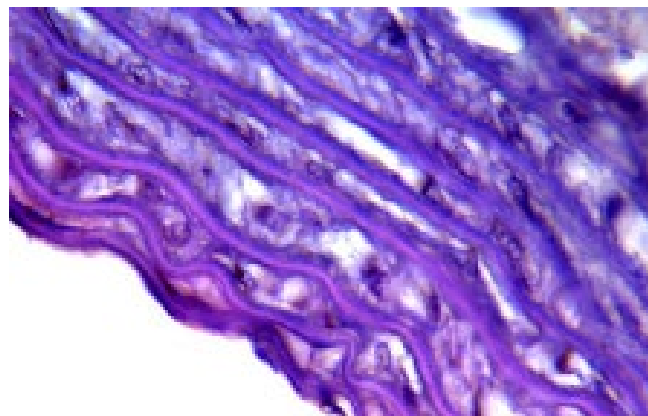


Figure 11: Section of rat aorta after high fructose diet administration with CB1 antagonist (AM251) intraperitoneally (1 mg/kg /day) for 8 weeks showing preservation of normal structure of the aorta with minimal perivascular edema (H/E X400).

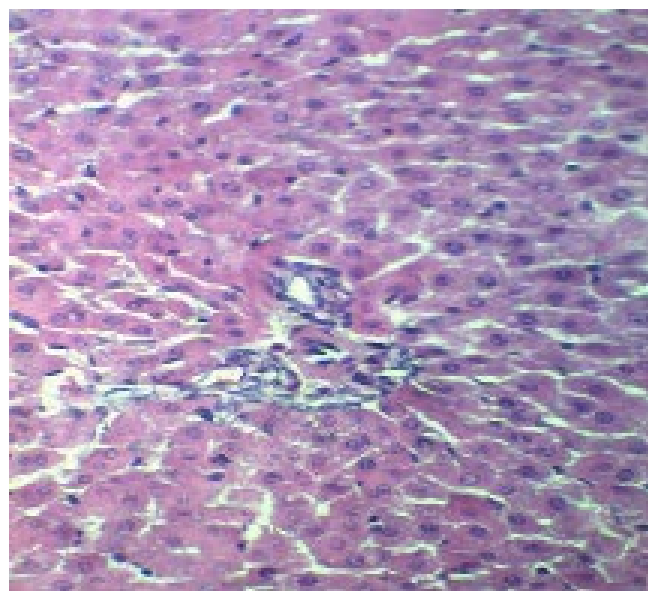


Figure 12: Section of normal liver at the end of 12th week showing normal portal tract with normal liver hepatocytes (H/E X400).

weeks group, the mean total cholesterol, triglyceride, HDL cholesterol & LDL cholesterol were 186.75 ± 2.59 mg/dl, 146.95 ± 6.88 mg/dl, 33.69 ± 0.60 mg/dl & 123.67 ± 5.59 mg/dl respectively.

There was significant decrease in serum triglyceride & and there was significant increase in HDL cholesterol, while there was no significant difference of total cholesterol and LDL cholesterol in fructose enriched diet plus CB1 antagonist (AM251) group in comparison high fructose group at the end of 12th week. The mean triglyceride, HDL cholesterol and LDL cholesterol were not returned to the normal values, in high fructose diet plus CB1 antagonist (AM251) group, at the end of 12th week.

Effect of CB1 antagonist (AM251) (1 mg/kg/day) intrapretonialy, for the last 4 weeks, of fructose 12 weeks' administrations on (TNF- α & NO): Administration of CB1 antagonist (1 mg/kg/day) i.p., for 4 weeks, started at the end of 8th week till the end of 12th week to fructose enriched diet for 12 weeks group, the mean TNF & NO were $88.85 \pm$

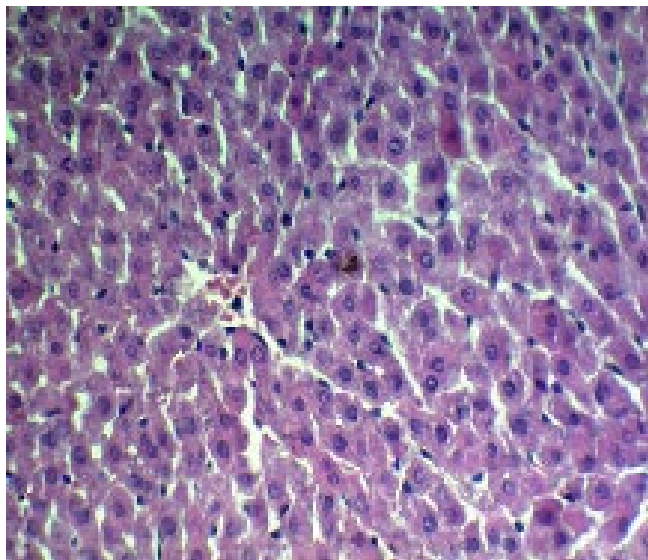


Figure 13: Section of normal liver at the end of 12th week showing normal portal vein with radiating normal hepatocytes (H/E X200).

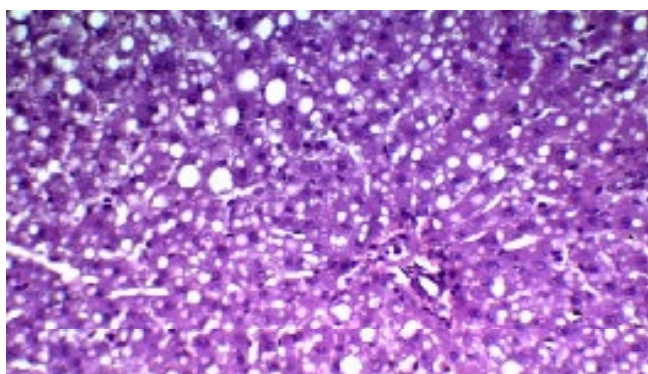


Figure 14: Section in rat liver after administration of high fructose diet for 12 weeks, showing loss of normal hepatic architecture with marked micro and macrovascular fatty changes (H/E X 200).

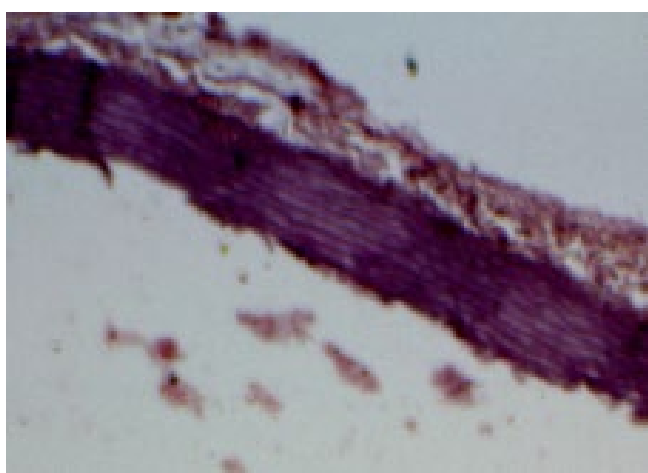


Figure 15: Section in the aorta of normal rat at the end of 12th week, showing normal vascular muscle fiber, intact endothelium with no perivascular edema (H/E X 200).



Figure 16: Section in the aorta of normal rat at the end of 12th week, showing normal vascular muscle fiber, intact endothelium with no perivascular edema (H/E X400).

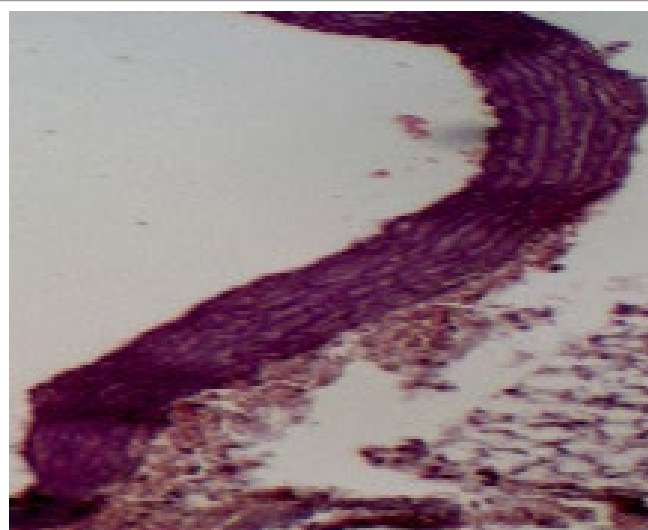


Figure 17: Section in rat aorta of high fructose diet for 12 weeks showing hypertrophy of vascular smooth muscle fiber with marked lymphocytic infiltration and perivascular edema (H/E X 200).

5.56 pg/mg & 5.69 ± 0.441 nmol/L respectively. There was significant decrease in TNF & NO in fructose enriched diet plus CB1 antagonist group in comparison to high fructose diet group at the end of 12th week. The mean serum TNF & NO returned to the normal values in high fructose diet plus CB1 antagonist group, at the end of 12th week.

Effect of CB1 antagonist (1 mg/kg/day) intraperitoneally, for the last 4 weeks, of fructose 12 weeks administrations on oxidative stress parameters (MDA, Catalase, GSH and SOD): Administration of CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally, for 4 weeks, started at the end of 8th week till the end of 12th week to fructose enriched diet for 12 weeks group, the mean serum MDA, Catalase, GSH & SOD were 1.95 ± 0.42 nmol/L, 94.55 ± 2.64 μ U/L, 32.02 ± 1.20 nmol/gPtn, and 1.32 ± 0.06 U/ml respectively at the end of 12th week.

There was significant decrease in MDA and significant increase in catalase, GSH and SOD in fructose enriched diet plus CB1 antagonist (AM251) group in comparison to High fructose diet group at the end of

12th week. The mean serum MDA, Catalase, GSH & SOD were returned to the normal values in high fructose diet plus CB1 antagonist (AM251) group, at the end of 12th week.

Effect of CB1 antagonist (1 mg/kg/day) intraperitoneally, for the last 4 weeks, of fructose 12 weeks administrations on liver enzymes (ALT & AST): Administration of CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally, for 4 weeks, started at the end of 8th week till the end of 12th week to fructose enriched diet for 12 weeks group, the mean serum ALT & AST were 31.02 ± 4.35 U/L & 22.50 ± 0.83 U/L respectively.

There was significant decrease in ALT & AST in fructose enriched diet plus CB1 antagonist (AM251) group in comparison to high fructose diet group at the end of 12th week. The mean serum ALT & AST were not returned to the normal values in high fructose diet plus CB1 antagonist (AM251) group, at the end of 12th week.

Histopathological results at the end of 12th week after administration of high fructose diet

Effect of fructose enriched diet for 12 weeks on liver structure: In the liver of normal rats, there was normal hepatic lobules, normal central hepatic vein with radiating normal hepatocytes with no evidence of hydropic or fatty changes as shown in Figures 12 and 25 after administration of high fructose diet for 12 weeks, there was changes in liver structure in the form of, marked congestion of central hepatic vein with marked hydropic, micro and macro vascular fatty changes of liver hepatocytes as shown in Figures 26 and 27.

Effect of fructose enriched diet for 12 weeks on structure of aorta: In the aorta of normal rats, the intima were intact, normal vascular smooth muscle fiber, with no evidence of hypertrophy or inflammation, as shown in Figures 15 and 16 after administration of high fructose diet for 12 weeks, there was hypertrophy of vascular smooth muscle fiber with marked subendothelial edema, and lymphocytic infiltration of vascular smooth muscle as shown in Figures 17 and 18.

Effect of CB1 antagonist (1 mg/kg/day) intraperitoneally, for the last 4 weeks, of fructose 12 weeks administrations on liver structure: Administration of high fructose diet for 12 weeks plus CB1 antagonist (1 mg/kg/day) intraperitoneally, for 4 weeks, started at the end of 8th week till the end of 12th week, produced preservation of the normal hepatic architectures with minimal hydropic changes of liver hepatocytes as shown in Figures 19 and 20.

Effect of CB1 antagonist (1 mg/kg/day) intraperitoneally, for the last 4 weeks, of fructose 12 weeks administrations on structure of aorta: Administration of high fructose diet for 12 weeks plus CB1 antagonist (1 mg/kg/day) intraperitoneally for 4 weeks, started at the end of 8th week till the end of 12th week, produced preservation of normal structure of aorta with minimal perivascular edema, as shown in Figures 21 and 22.

Comparison between studied drugs on structure of the liver at the end of 12th week after administration of high fructose diet: Administration of high fructose diet plus ALA or Telmisartan or plus Rosuvastatin or plus, CB1 antagonist (AM251), the normal hepatic architecture were preserved with minimal hydropic changes.

Histopathological results of the liver and aorta at the end of 12th week of high fructose diet administration:

1. Structure of normal liver and aorta at the end of 12th week shown above in Figures 12, 15, 16 and 27.

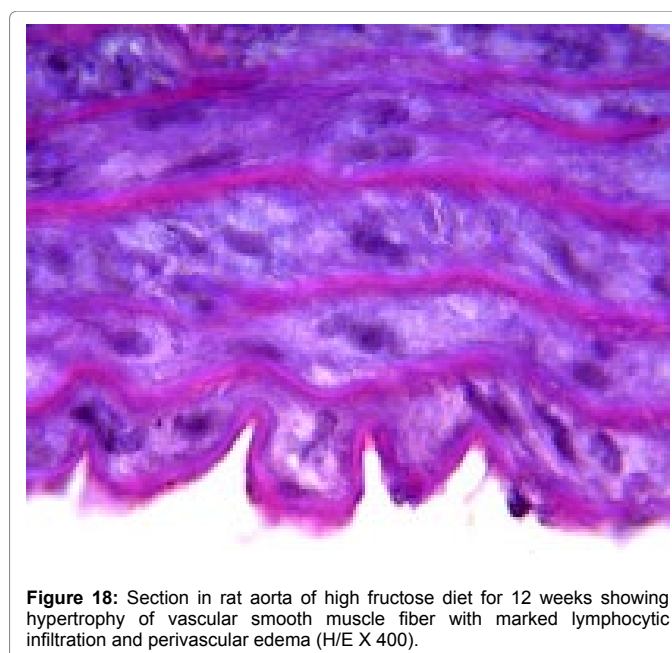


Figure 18: Section in rat aorta of high fructose diet for 12 weeks showing hypertrophy of vascular smooth muscle fiber with marked lymphocytic infiltration and perivascular edema (H/E X 400).

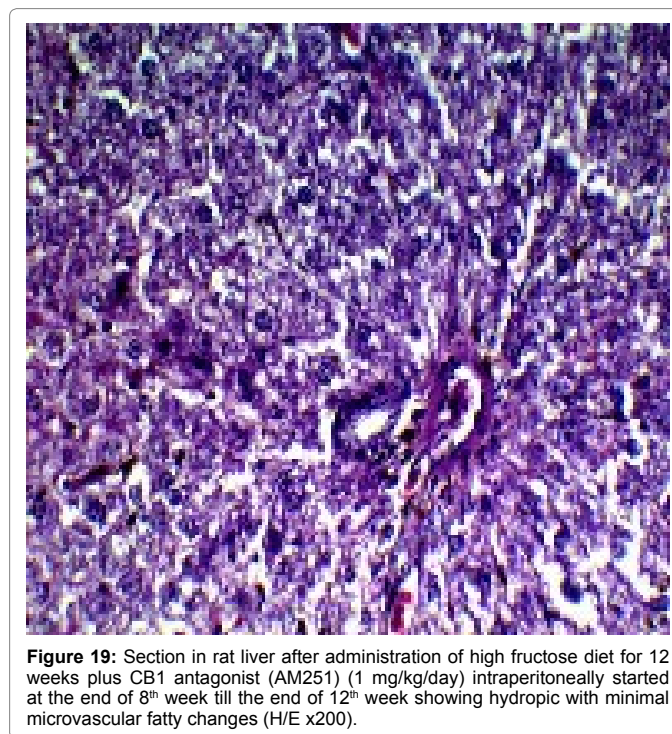


Figure 19: Section in rat liver after administration of high fructose diet for 12 weeks plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally started at the end of 8th week till the end of 12th week showing hydropic with minimal microvascular fatty changes (H/E x200).

2. Effect of high fructose diet for 12 weeks on the liver and aorta shown above in Figures 17 and 18.

Effect of high fructose diet for 12 weeks plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally, started at the end of 8th week till the end of 12th week after administration of high fructose diet on the liver and aorta shown above in Figures 18-22.

Discussion

In the present work, Prophylactic and therapeutic treatment with CB1-antagonist (AM251) produced significant decrease in serum

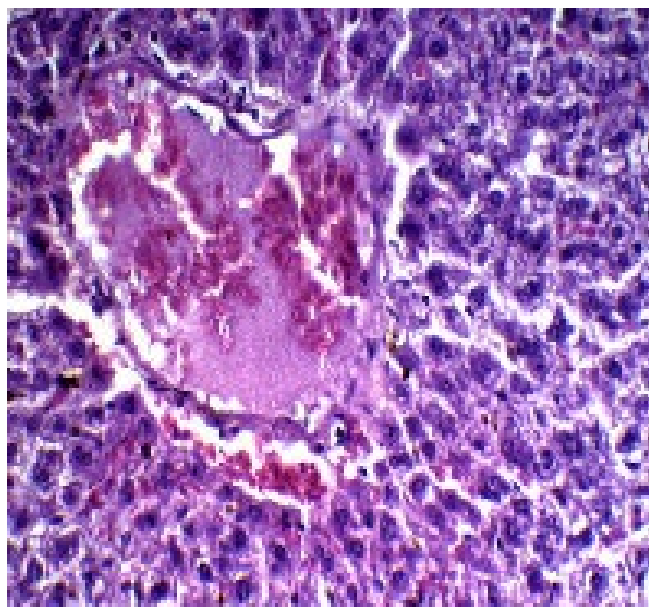


Figure 20: Section in rat liver after high fructose diet administration for 12 weeks plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally started at the end of 8th week till the end of 12th week showing marked central vein congestion with minimal hydropic and microvascular fatty changes (H/E X400).

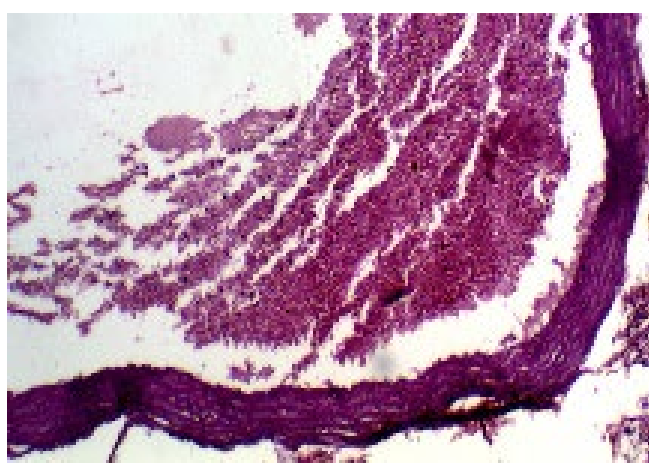


Figure 21: Section in rat aorta after high fructose diet administration for 12 weeks plus (AM251) (1 mg/kg/day) i.p. from the end of 8th week till the end of 12th week showing, perivascular edema, hypertrophy of smooth muscle with minimal lymphocytic infiltration (H/E X400).

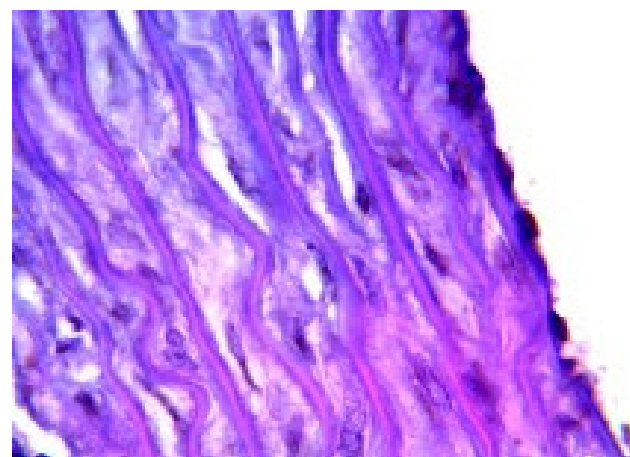


Figure 22: Section in rat aorta after high fructose diet administration for 12 weeks plus (AM251) (1 mg/kg/day) i.p. from the end of 8th week till the end of 12th week showing, perivascular edema, with minimal lymphocytic infiltration (H/E X400).

glucose, insulin, insulin resistance, blood pressure, lipid profile, oxidative stress, inflammatory cytokines, and liver enzymes of fructose diet rats. The serum triglyceride, oxidative stress, inflammatory cytokines and liver enzymes were returned to the normal values in prophylactic group only, while the previous parameters were improved but not returned to the normal values in therapeutic group. In addition, it decreased body weight to values less than corresponding normal group without significant affection on total cholesterol and LDL cholesterol.

The endocannabinoid system has been reported to play a key role in the regulation of food intake and in body weight control [23,24]. The endocannabinoid receptor CB1 is expressed in the central nervous system and peripheral tissues, particularly those involved in insulin

action, i.e. the liver, muscle, pancreas and adipose tissue. There is mounting evidence that the endocannabinoid system plays a role in regulating metabolism [25]. Activation of the cannabinoid receptor CB1 by the endogenous cannabinoid receptor ligands, anandamide and 2AG, favors the metabolic processes that lead to weight gain, lipogenesis, insulin resistance, dyslipidaemia and impaired glucose hemostasis [26].

The biological basis for the cannabinoid CB1 receptor blockade effect on weight modulation involves both central and peripheral mechanisms: central effects include anorexigenic stimuli, hormonal release modulation in the hypothalamus and decreased motivation for palatable food in the nucleus accumbens [13]. The peripheral effects are considered to be the stimulation of anorectic signals in the gastrointestinal tract, the inhibition of lipogenic enzymes in adipose tissue [27] and in the liver [28], all of which increase adiponectin levels, glucose uptake and oxygen consumption in the muscles and thus enhance thermogenesis [29]. Furthermore, AM 251 has been shown to significantly decrease the digestive utilization of protein [30].

Previous studies have also shown that the endocannabinoid system is involved in the regulation of glucose tolerance and whole-body insulin sensitivity. Acute CB1R agonism in male Wister rats caused reduced glucose tolerance, whereas acute CB1R antagonism was associated with enhanced glucose tolerance [31]. Moreover, chronic CB1R antagonism improved insulin sensitivity in rats with diet-induced obesity compared to pair-fed and ad libitum-fed controls [32]. Finally, chronic CB1 antagonism treatment of male obese Zucker rats reduced glucose-stimulated insulin secretion, whereas glucose tolerance was unchanged during an oral glucose tolerance test (OGTT) [33]. Chronic CB1R antagonism may improve glucose tolerance through an increased insulin-mediated glucose transport in skeletal muscles, as a 7-day treatment with the CB1R antagonist led to increase in vitro insulin stimulation of glucose transport activity in the soleus muscle of leptin-deficient obese mice [34].

Regarding the lipid metabolism, [30] it is found that the blockade of the cannabinoid receptors by AM 251 at a dose of 3 mg/kg in obese Zucker rats improves dyslipidemia, a major biochemical disorder associated with steatohepatitis leading to cardiovascular diseases. In fact, we have described that AM 251 leads to a more favorable

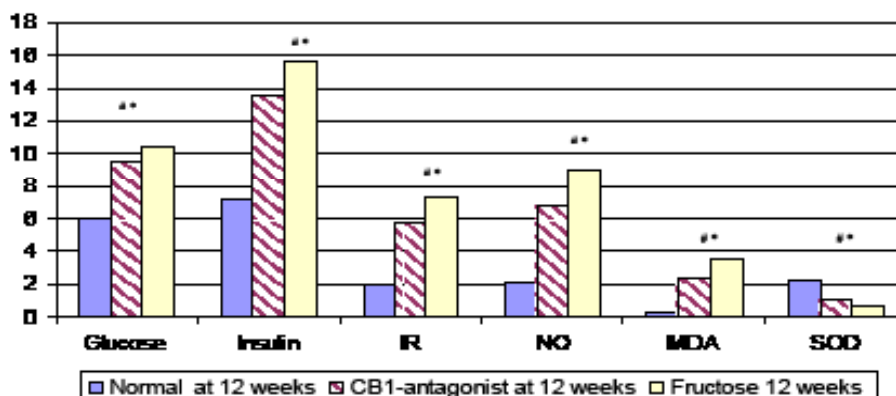


Figure 23: Effect fructose (60%) diet for 12 weeks plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for the last 4 weeks of fructose administration on serum NO (nmol/L), MDA (nmol/L), SOD (U/ml), glucose (mmol/L), insulin (μ U/L) and IR (index). at the end of 12th week.

Each value represents the Mean \pm S.E of 10 readings ($p < 0.05$; $n = 10$)

*=significant CB1 antagonist when compared to normal at the end of 12th week.

#=significant CB1 antagonist when compared to fructose 12 weeks at the end of 12th week.

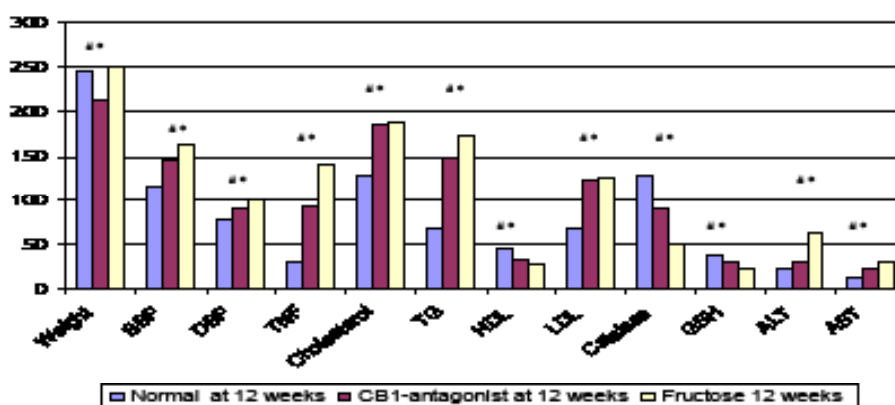


Figure 24: Effect fructose (60%) diet for 12 weeks plus CB1 antagonist (AM251) (1 mg/kg/day) intraperitoneally for the last 4 weeks of fructose administration on body weight (gm), blood pressure (mmHg), TNF- α (pg/mg), Catalase (μ U/L), GSH (nmol/gPtn), SOD (U/ml) lipid profile (mg/dl) and liver enzymes (U/ml) at 12th week.

Each value represents the Mean \pm S.E of 10 readings ($p < 0.05$; $n = 10$).

*=significant CB1 antagonist when compared to normal at the end of 12th week.

#= significant CB1 antagonist when compared to fructose 12 weeks at the end of 12th week.

lipid profile, producing a significant increase in HDL cholesterol and a significant decrease in triglycerides. These results have been confirmed in other studies in which rimonabant which have the same pharmacological action of AM251, were administered at different doses [32]. The effect on the lipid profile could be mediated by changes in the levels of adipokines, and several studies have reported a positive correlation between adipokines and HDL cholesterol levels [24]. In a similar way to what has been reported by other authors for rimonabant, this general improvement of the plasma lipid profile may be mediated by restoration, following AM 251 treatment, of the structure and metabolic function of the main organs and tissues involved in lipid and glucose metabolism, among which the liver is especially relevant [35].

In Merroun et al. study, [30] the obese Zucker rats were found to have higher plasma levels of urea, bilirubin and transferases compared with the lean animals. The decrease achieved by treatment with AM 251 suggests a normalizing effect of this drug on liver damage in

Zucker rats. Most of the effects of AM251 (3 mg/kg) were not observed in pair-fed obese animals, highlighting the additional beneficial effects of treatment with AM 251.

Endocannabinoid and CB1 receptors have also been identified in pancreas β cells [36], which suggest that these receptors play a role in the control of insulin secretion. Furthermore, CB1 receptors seem to be involved in the stimulation of insulin secretion in a state of hyperglycemia [24]. CB1 antagonists have a direct influence on the pancreatic cells, reducing insulin secretion [37] and improving glucose tolerance and insulin sensitivity [38].

Li et al. reported that CB1 receptors modulate insulin secretion by regulating glucose-induced calcium transients [39]. In their experimental conditions, they observed a slight improvement in insulin levels and HOMA-IR index when AM 251 was injected at a dose of 3 mg/kg. These results are in agreement with those described by other authors [40], who observed a significant decrease in insulin and glucose levels when rimonabant was administered at a dose of 10 mg/kg for 10

weeks. The CB1 receptor antagonist decreases insulin levels, increases glucose homeostasis and improves insulin sensitivity. This outcome could be related to plasma levels of adiponectin and the activation of the 5' adenosine monophosphate-activated protein kinase (AMPK) in the liver, leading to reduced production of hepatic glucose.

As regard to effect of CB1 antagonist on inflammatory cytokines, it was reported that, there is increasing evidence to suggest that cannabinoids modulate the immune system and represent an important target for the treatment of inflammatory disorders [41]. It can be explained by the fact that, the CB1 receptor (CB1-R) is mainly localized in the central nervous system but is also abundant in peripheral tissues including liver, skeletal muscle, myocardium, endothelial cells, gastrointestinal tract and adipose tissue [10,42]. These facts explained also the effect of CB-antagonist on liver tissues and enzymes.

In clinical trials, treatment of overweight patients with CB1-anatognists resulted in significant weight loss and improvement of multiple cardio-metabolic risk factors, such as waist circumference, systolic blood pressure, HDL-cholesterol, triglyceride levels and insulin sensitivity [38,43].

In addition, Tiyerili et al. reported that, CB1-R antagonist has athero protective effects by down-regulation of the AT1 receptor, reduced NADPH oxidase activity, decreased vascular oxidative stress and thus improved endothelial function in hypercholesterolemic ApoE^{-/-} mice [44].

Several studies have shown the pro-inflammatory status of ApoE^{-/-} mice. Judkins et al. demonstrated increased superoxide production, reduced nitric oxide bioavailability and an early atherosclerotic plaque formation [45]. It had been previously shown that ApoE^{-/-} mice display significantly higher levels of aortic superoxide production [46]. Continuous treatment with CB1 antagonist over the entire course of the diet led to a marked improvement of endothelial function, but was not sufficient to significantly alter atherosclerotic plaque formation, collagen content or macrophage infiltration. The combined effect of

apolipoprotein E deficiency and cholesterol challenge may have been too great of a burden to be sufficiently inhibited by CB1 receptor antagonism.

In addition, it is known that glucose uptake by muscle and adipose tissue is improved in mice lacking ApoE when fed a diabetogenic diet. The improved insulin sensitivity observed in CB1 treated animals may have caused better muscle metabolism. In general, ApoE^{-/-} mice exhibit lower body weight and insulin levels than ApoE^{+/+} mice when fed a diabetogenic diet [47].

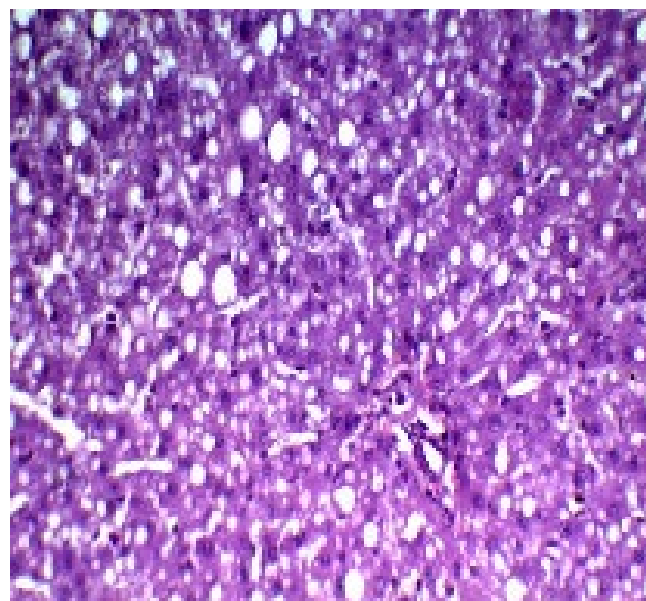


Figure 26: Section in rat liver after administration of high fructose diet for 12 weeks, showing loss of normal hepatic architecture with marked micro and macro vascular fatty changes (H/E X200).

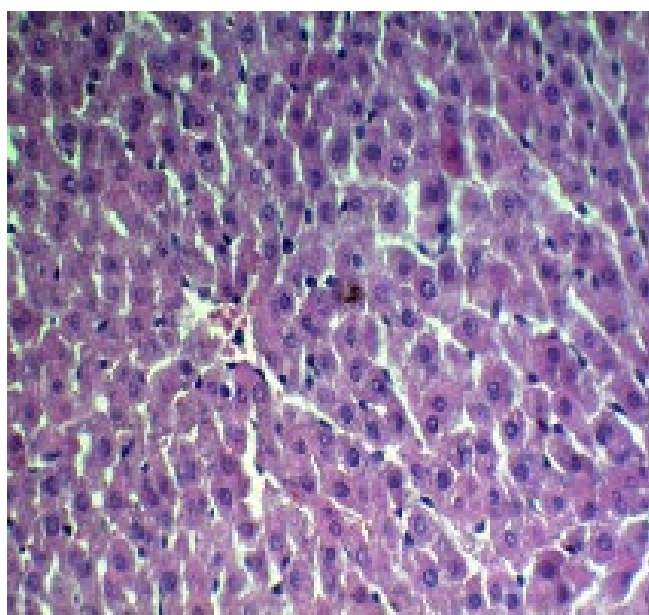


Figure 25: Section of normal liver at the end of 12th week showing normal portal vein with radiating normal hepatocytes (H/E X200).

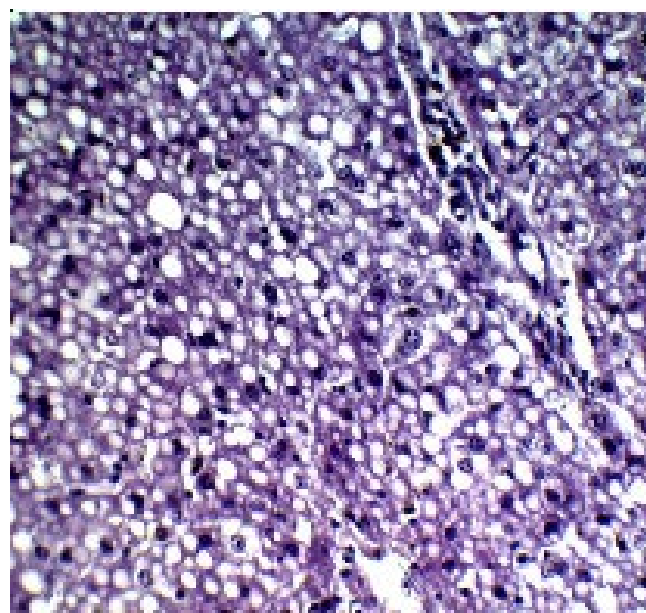


Figure 27: Section in rat liver after high fructose diet administration for 12 weeks, showing loss of normal hepatic architecture with marked micro and macro vascular fatty changes (H/E X400).

CB-1 antagonist- treatment improved insulin sensitivity. It is known that CB1-R^{-/-} mice have lower plasma insulin levels and do not develop diet-induced insulin resistance [48]. The inhibition of CB1 receptors may prevent the impaired insulin sensitivity induced by high energy feeding observed in wild-type animals. This finding may be related to the low adiposity of CB-1 antagonist-treated mice fed the high-fructose diet, as insulin sensitivity was shown to correlate with the level of fat storage [49].

An important effect of AT1 receptor stimulation by angiotensin II is the activation of NADPH oxidase [50,51]. In conjunction with this, findings show that CB-1 antagonist reduces angiotensin II-mediated NADPH oxidase activity in vivo and in vitro. Importantly, it had been demonstrated that CB-1 antagonist decreases AT1 receptor expression in the aortic wall and cultured VSMCs. It may be speculated that CB-1 antagonists leads to improved endothelial function by down regulation of the AT1 receptor and thus decreased vascular oxidative stress [44]. Other studies have also shown interactions between AT1 receptor and CB1 receptor functions. Turu et al. highlighted that the CB1 receptor is activated following AT1 receptor stimulation. Physiologically, both are involved in generation and inactivation, respectively of ROS and oxidative stress [52,53].

Furthermore, CB1-R activation modulates adenylate cyclase activity in most tissues and regulates calcium and potassium channels. Evidence suggests that cannabinoids can activate mitogen activated protein kinases (MAPK), e.g., p38 MAPK and c-Jun N-terminal kinase (JNK) through specific phosphorylation [52]. These signaling pathways play an important role and have an impact on cardiac and vascular function [54]. Sugamura et al. demonstrated a greater CB1-R expression in lipid-rich atherosclerotic plaques compared to fibrous plaques. Interestingly, they showed after CB1-R antagonism, a significant increase in cytosolic cAMP levels, inhibited phosphorylation of c-Jun N-terminal kinase and a significant decrease in the production of pro-inflammatory mediators such as IL1b, IL6, IL8, TNF alpha and MMP-9 in macrophages [55]. All the authors summarized a benefit for the progression of atherosclerosis through an anti-inflammatory process by CB1-R blockade.

In the present work, comparing studied prophylactic and therapeutic drugs. It was found that, CB1-antagonist (AM251) can be used as prophylactic and therapeutic treatment of metabolic syndrome especially when metabolic syndrome associated with obesity (was the best and only effective drug in reduction of body weight or hypertriglyceridemia).

Telmisartan can be used as therapeutic and prophylactic drug in metabolic syndrome especially when associated with hypertension since it decrease blood pressure to near the normal value and improve other components of metabolic syndrome.

Rosuvastatin can be used as prophylactic and therapeutic drug in treatment of metabolic syndrome especially when associated with dyslipidemia as hypercholesterolemia or hypertriglyceridemia.

ALA can be used as prophylactic and therapeutic drug in treatment of metabolic syndrome especially when associated with other diseases related to oxidative stress.

There was drug had upper hand on TNF, NO, oxidative stress markers or liver enzymes. All studied drugs improved histopathological structure of the liver in aorta in both prophylactic and therapeutic groups with no drug had the upper hand of improvement of these histopathological changes in the liver and aorta. Unfortunately, there are no previous studies in literature that compared studied drugs as

done in the present study. Thus, this study needed to be confirmed in subsequent studies.

It is hypothesized that, adding drugs to each other can potentiate or synergize the beneficial effect on treatment of metabolic syndrome. In short, there was no only single drug (of studied drugs) can be used alone in prophylaxis or therapeutic treatment of metabolic syndrome.

Conclusion

The results of the present study concluded that:

- Fructose (60%) diet produced manifestations of metabolic syndrome without significant increase of body weight and this effect was duration dependent.
- Prophylactic treatment by CB1-antagonist (AM251) significantly improved and returned serum triglyceride, inflammatory, oxidative stress parameters and liver enzymes to the normal values while it significantly improved blood pressure, glucose, insulin, insulin resistance and lipid profile but not significantly returned to the normal values. Interestingly, it significantly decreased body weight to values less than corresponding normal group.
- Therapeutic treatment with CB1-antagonist (AM251) improved all studied manifestations of metabolic syndrome but not returned to normal values except telmisartan which return the blood pressure nearly to the normal value in therapeutic group.
- The studied drug produce significant improvement of histopathological changes of the liver and aorta induced by high fructose diet with no drug had the upper hand in this improvement.

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