Evening Primrose Oil Diminishes Fatty Degenerations And Down Regulates AGE/RAGE Axis In Fructose-Drinking Rats: Comparison To Fenofibrate.

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ABSTRACT

Increased consumption of dietary fructose constitutes one of the key players in the development of insulin resistance and metabolic syndrome. This study aimed to study the effect of evening primrose oil (EPO) in a meliorating insulin resistance, hyperlipidemia and hepatic fatty degeneration-promoted by fructose drinking in rats. As advanced glycated end products (AGE) and its receptor activation (RAGE) have been shown to be correlated with the hepatic component of metabolic syndrome, this work evaluated the effect of EPO on AGEs/RAGE axis in insulin resistant rats with metabolic syndrome induced by drinking fructose syrup. Rats were allocated into normal, fructose-drinking control, fenofibrate and EPO-treated (5 and 10 g/rat) groups. The present results highlighted the effectiveness of EPO in alleviating the severity of insulin resistance, hyperlipidemia, oxidative stress burden and inflammation. In addition, treatment with EPO diminished hepatic fatty degeneration and down regulated RAGE expression and suppressing AGEs/RAGE axis. The present study suggest the promising benefit of therapeutic utility of EPO on the hepatic component of metabolic syndrome induced by fructose drinking that is likely relies, at least in part, to suppression of AGEs/RAGE axis and its subsequent generation of reactive oxygen species (ROS) and inflammatory mediators.

Keywords: AGE; evening primrose oil; high-fructose; RAGE; rats

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INTRODUCTION

The environmental factors may play a role in pathogenesis of the metabolic syndrome which is strongly suggested by dramatic increase in its prevalence [1]. Increased consumption of dietary fructose acquired from high fructose corn syrup and sucrose, which represents the common sweetener in food industry have etiologic role [1]. Consumption of high fructose diet is strongly accompanied by high risk of obesity, non- alcoholic fatty liver disease (NAFLD) and non-alcoholic steato hepatitis (NASH) [2-5].

In animal models, fructose consumption and hyperglycemia leads to formation of advanced glycated end products (AGEs) and accumulation [6] with activation of its receptor [receptor for AGE (RAGE)] [7]; inducing generation of inflammation, features of metabolic syndrome and oxidative stress burden [1,8-10].

Fibrates are group of drugs act by activation of hormones peroxisome proliferator-activated receptor-α (PPAR-α), and effectively by improvement the disturbed lipid profile associated with metabolic syndrome [11]. Previous studies have implicated the nuclearPPAR-α in the pathogenesis of NAFLD. PPAR-α decreases liverfat by activating β-oxidation of fatty acids [12-14]. Fibrates have endothelial protective, antioxidant and anti-inflammatory actions which are observed in dyslipidemic patients and in subjects with glucose metabolism abnormalities [15].

Gamma-linolenic acid (GLA) is one of essential polyunsaturated fatty acids (PUFAs). Evening primrose oil (EPO) seems to be clinically the most effective source of GLA[16]. It also contains oleic acid, tocopherol, linoleic acid and Phenolic compounds. These essential fatty acids are important for different physiological functions in the human body and give the biological potency and effectiveness of EPO in reducing weight, lowering blood pressure, hypoglycemic and hypo-cholesteremic effects [17] and protection of the cardiovascular system [18 ,19].

With the increasing evidence that GLA plays an essential role in various metabolic disease processes [17], would be expected to modify the disease progression and provide a useful adjunctive strategy in the treatment of metabolic disorders. Therefore, the present study was conducted to test the effect of EPO in insulin resistant rats with metabolic syndrome induced by drinking fructose syrup; this comparison was done with respect to the insulin sensitizer, fenofibrate.

MATERIALS AND METHODS

Animals

Male Wistar rats (170-200 g) were allowed to acclimatize to the experimental conditions for one week before starting the experiment. Rats were placed in controlled hygienic conditions in stainless steel cages in normal light/dark cycle with water and regular diet given *ad libitum*. The experimental protocol was approved by the institutional research ethics committee at the Suez Canal University according to NIH-guide for the care and use of laboratory animals.

Drugs and chemicals

Fructose powder was obtained from Safety Company (Al-Asher men Ramadan City, Egypt). Fenofibrate was a gift from Sigma Pharmaceutical Company (Cairo, Egypt) and was dissolved in distilled water. EPO was purchased from the market in form of soft gelatin capsules (Primaleve capsules, Glaxo Smith Kline, Cairo, Egypt).

Induction of metabolic syndrome by fructose drinking

Rats were allowed to drink a10% fructose solution for 16 week to induce metabolic syndrome (10, 20). Fructose syrup was freshly prepared every two days and given to rats for 8 weeks, and then therapeutic regimens were launched and continued for extra 8 weeks along with fructose. Normal control rats were given bottle of drinking water instead of fructose.

Experimental design

Forty rats were allocated randomly into five experimental groups, eight rats each.
Group (i): Normal control group distilled water all over the experiment.
Group (ii): fructose-drinking rats,
Group (iii): fructose-drinking rats received fenofibrate (80 mg/kg/day) [21-23],
Groups (iv & v): fructose drinking-rats received EPO (5 or 10 g/rat/day), respectively [19].

Pharmacologic interventions started at the ninth week of the experiment and continued until the end of the study. Body weights (BWt) of rats were measured at the end of week 16 to determine the change in body weight during our experiment. At the end of the experiment, after overnight fasting of rats, blood glucose was determined employing a glucometer (Super Glucocard, Japan). Then, while the rats were under ether anesthesia, blood samples were obtained from the orbital sinus in dry centrifuge tubes and allowed to stand at room temperature for 30 min prior to centrifugation at 2000×g for ten min to obtain serum samples. Rats were then sacrificed by cervical dislocation under sufficient anesthesia. After that, a laparotomy was performed for each rat and the liver was isolated, cut from the largest hepatic lobule and prepared for histopathological staining with hematoxylin and eosin (H&E) and immune staining.

Fasting blood advanced glycated end products and insulin

Determination of serum levels of advanced glycated end products (AGEs) by using a rat AGEs ELISA kit (Sun Red, Shanghai) and rat insulin ELISA kit (Biorbyt Limited, Cambridge, UK) were used; measurements were done employing an ELISA reader. Insulin sensitivity index was determined by using a formula that is widely used for calculation of insulin resistance in rats [24, 25] or mice [26] after obtaining fasting glucose and insulin: HOMA-IR index formula; IRI=FBG×FINS/22.5(Matthews et al. 1985)[27].

Serum liver enzyme activities and lipid profile

Serum levels of liver enzymes (AST and ALT) as well as triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low- density lipoprotein cholesterol (LDL-C) were assayed using colorimetric kits from Bio diagnostics Company (Cairo, Egypt) following the methods reported by the manufacturer.

Hepatic level of malondialdehyde, reduced glutathione and tumor necrosis factor-α

Hepatic homogenates were used for determination of malondialdehyde(MDA) as a marker for lipid peroxidation as well as reduced glutathione (GSH) using colorimetric kits from Bio Diagnostics (Cairo, Egypt). The level of TNF-α was determined using ELISA kits from Cusabio (CSB-E11987r, USA).

Liver histopathological examination

Livers were dissected and fixed in a 10% phosphate-buffered paraformaldehyde solution. Tissues were dehydrated and embedded in paraffin and sectioned at 4-μm, stained with H&E and then examined blindly under a light microscope. The severity and extent of steatosis was observed for each case. The findings were classified into the following degrees: grade (0): No change; grade (1): mild steatosis, occasional ballooning degenerationinzone3, mild lobular inflammation with or without mildportal inflammation; grade (2): moderate steatosis in 33-66% of lobules, ballooning degeneration in zone 3 , moderate lobular inflammation with mild to moderate portal inflammation; grade (3): severe steatosis in>66%oflobules, marked ballooning especially in zone 3 , scattered lobular acute and chronic inflammation with moderate portal inflammation and perivenular and pericellular fibrosis limited to zone 3 [28].

Immuno histochemistry and image analysis for AGE receptors and iNOS

Anti-RAGE rabbit polyclonal antibodies (Biorbyt, Cambridge, UK) and rabbit polyclonal antibodies against iNOS were used for immunostaining (Cat. #RB-9242-R7, Thermo Fischer Scientific, Fremont, CA 94538, USA).

The staining process was then completed using Envision system (Dako DenmarkA/s, Glostrup, Denmark) following the manufacturer's instructions and finally sections were counterstained by Mayer's hematoxylin stain.

Analysis of data

The presentation of the data was done as mean ± standard error of the mean. Statistical analysis of data was performed using the one-way ANOVA followed by Tukey's post-hoc test. The level of significance was set at *P*<0.05. Every possible comparison between the study groups was recorded and showed in illustrations.

RESULTS

Table 1 demonstrates that fructose-drinking rats showed increase in the body weight percent compared to the normal rats in the control group (69.7±7.8 versus17.9±4.9). Treatment with fenofibrate or EPO (10 g/rat) significantly decreased the calculated value of increased percent of body weight in comparison with fructose-drinking control rats (48.9±2.4 and 47.2±4.8%, respectively, versus 69.7±7.8 % (P<0.05, Table 1).

Table 1: Effect of treatment with fenofibrate (80mg/kg) or EPO (5 or 10 g/rat) on body weight change and HOMA-IR index in fructose drinking insulin resistant rats

Groups	Baseline BWt (g)	Final BWt (g)	% Change in BWt	Blood Glucose (mg/dL)	Fasting insulin (mU/mL)	HOMA-IR index
Normal	198.2±5.2	232.7±5.3	17.9±4.9	90.5±2.3	42.16±3.8	9.39±0.8
Fructose-drinking	201.7±5.3	340.7±10.1*	69.7±7.8*	150.2±7.6*	103.3±7.6*	38.4±3.7*
Fructose-drinking+ Fenofibrate	201.2±2.9	293.7±4.9*#	48.9±2.4*#	115±5*#	54.6±5.3#	15.7±2*#
Fructose-drinking+ EPO (5 g/rat)	200±5	332.8±3.7*$	67.05±5.4*$	140±5.1*$	100.9±6.8*$	34.9±2.9*$
Fructose-drinking+ EPO (10 g/rat)	201.8±3.5	296.3±5.8*#¶	47.2±4.8*#¶	116.2±2.9*#¶	71.5±4.3*#$¶	20.4±1.1*#¶

Rats were drinking fructose syrup to induce insulin resistance and treated with various agents for 8 weeks (week 9-week 16). Percent change in BWt was calculated using a formula: % change BWt = [(final BWt- baselineBWt)/ baseline BWt] x 100.. HOMA-IR index: homeostatic model assessment–insulin resistance index. HOMA-IR index=[fasting glucose (mMol/L)×fasting insulin (µU/ml)]/22.5).Results are expressed as mean±S.E.M. and analyzed using one-way ANOVA followed by

Bonferroni's post-hoc test at *P*<0.05.*Compared to normal group, #Compared to fructose-drinking group, $Compared to fenofibrate group, ¶Compared to EPO (5 g/rat) group, *n*=8

Further, insulin levels and fasting blood glucose results highlighted increases in fructose-drinking rats in comparison with the normal control rats. Treatment with fenofibrate (80 mg/kg) or EPO (10 g/rat) decreased the elevated levels of fasting blood glucose and elevated insulin levels compared to levels in the fructose-drinking rats. Similarly, the calculated HOMA-IR index for the experimental groups indicated high index in fructose drinking rats in comparison with the normal rats (Table 1). Whereas, treatment with fenofibrate (80 mg/kg) or EPO (10 g/rat) reduced the calculated index by about 60% and48%, respectively in comparison with the fructose drinking rats(*P<0.05*, Table 1).

Table 2 shows serum liver enzyme activities in the experimental groups and shows elevation in AST and ALT activities in fructose-drinking rats in comparison with normal rats. Treatment with fenofibrate (80mg/kg) or EPO (5or10 g/rat) reduced *(P<0.05)* the level of the two enzymes in comparison with the fructose drinking rats (Table 2). Lipid profile measurement indicated greater serum levels of TG, TC and LDL- C but lower HDL-C value in fructose-drinking rats in comparison with normal rats (Table 2). Treatment with fenofibrate (80mg/kg) or EPO (5or10g/rat) reduced the elevated levels of TG, TC and LDL-C but enhanced HDL-C *(P<0.05)* in comparison to fructose- drinking rats (Table 2).

Table 2: Effect of treatment with fenofibrate or evening primrose oil (5 or 10g/rat) on serum liver enzymes and lipid profile in fructose-drinking rats

Group	AST (U/L)	ALT (U/L)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	TC (mg/dl)
Normal group	145.2±10.2	58.2±4.6	41.3±3.6	53±4.2	5.3±0.6	45.2±3.3
Fructose-drinking group	220.5±18.4*	90.3 ±7.1*	187.6±14.8*	28.5±1.9*	28.7±0.2*	90.4±7.2*
Fructose-drinking + Fenofibrate	178.3±11.9[#]	78.5±5.3[#]	68.5±5.3[#]	42.3±2.7[#]	8.9±0.6[#]	59.5±3.6[#]
Fructose-drinking + EPO (5 g/rat)	201.2±18.4[#$]	80.7±4.2[#]	112.3±9.8[#$]	33.7±2.1[#$]	18.5±1.1[#]	78.3±4.9[#$]
Fructose-drinking + EPO (10 g/rat)	164.4±12.9[#¶]	74.2±5.4[#]	89.7±6.4[#$¶]	40.8±3.7[#¶]	11.3±0.9[#¶]	63.2±4.3[#¶]

EPO: evening primrose oil, TC: total cholesterol, TG: triglycerides, LDL-C: low density lipoprotein cholesterol and HDL-C: high density lipoprotein cholesterol in fructose drinking fructose-drinking rats. Results are expressed as mean ± S.E.M and analyzed using one-way ANOVA followed by Bonferroni's post-hoc test at $P<0.05$. *Compared to normal group, #Compared to fructose-drinking group, $Compared to fenofibrate group, ¶Compared to EPO (5g/rat) group, $n= 8$.

Figure 1A demonstrates the greater MDA level (2.62-fold greater) in fructose- drinking rats compared to the normal rats. Treatment with fenofibrate (80 mg/kg) or EPO (5or10g/rat) reduced *(P<0.05)* the MDA level in comparison to the fructose-drinking rats. Reduced glutathione decreased significantly in fructose-drinking rats and then replenished again after treatment with the different implemented agents (f i g . 1 B)
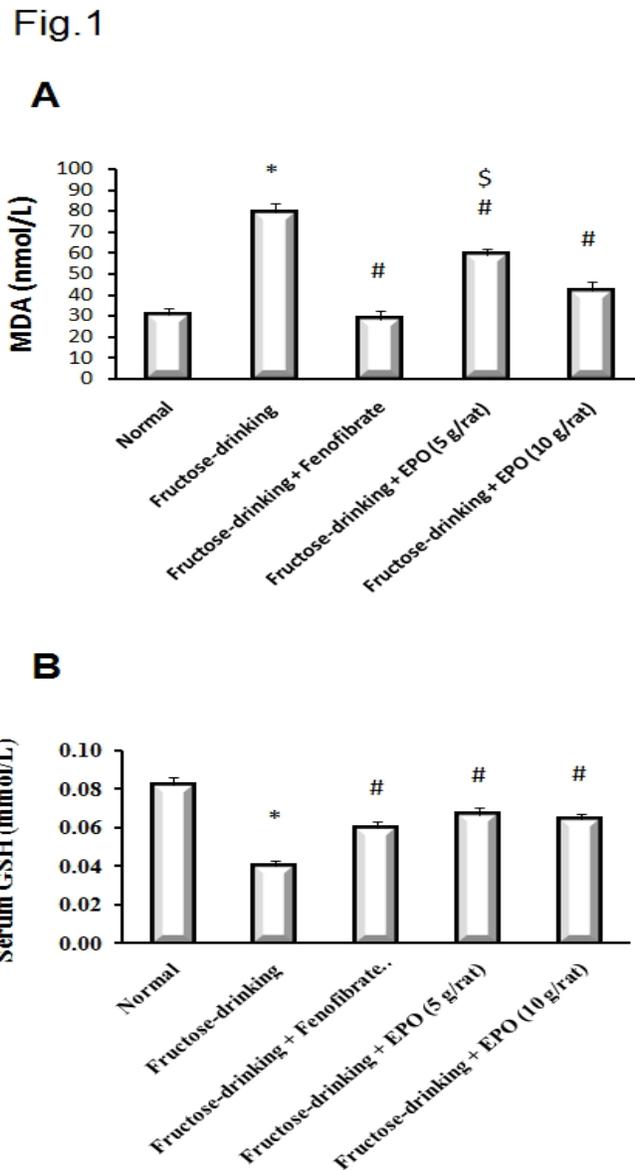

Fig.1

Fig 1 Effect of fenofibrate (80mg/kg) or EPO (5 or 10 g/rat) on serum (a: MDA "nmol/l", b: GSH "nmol/l") in fructose drinking insulin resistant rats. Results are expressed as mean± S.E.M. and analyzed using one-way ANOVA followed by Bonferroni's post-hoc test at *P*<0.05.*Compared to normal group,

#Compared to fructose-drinking group, $Compared to fenofibrate group, ¶Compared to EPO (5 g/rat) group, n=8

Fig 2 illustrates that serum TNF-α and AGE levels were greater in fructose- drinking rats in comparison to normal rats. Treatment with fenofibrate significantly lessened the high serum levels of TNF-α and AGE in comparison to the fructose-dinking control rats. Similarly, the two applied doses of EPO succeeded to lessen the elevated levels of these two markers when compared to the control rats (*P<0.05,* Fig 2)
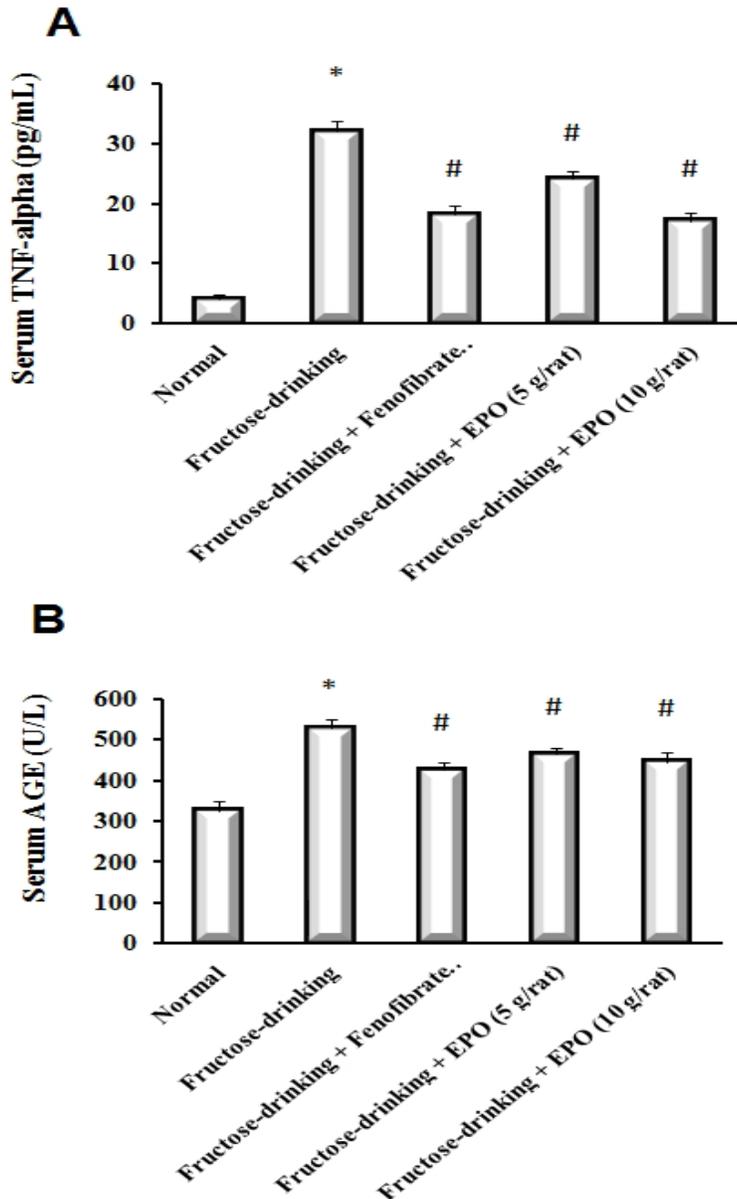

Fig 2 Effect of fenofibrate (80mg/kg) or EPO (5 or 10 g/rat) on serum (a: TNF-α" pg/ml", b: AGE "U/l") in fructose drinking insulin resistant rats. Results are expressed as mean± S.E.M. *P<0.05.**Compared to normal group, #Compared to fructose-drinking group, $Compared to fenofibrate group, ¶Compared to EPO (5 g/rat) group, n=8.

Figure 3 demonstrates hepatic sections stained with H&E for morphometric analysis. Sections from normal rats show normal architecture and normal branches of portal vein. However, sections from fructose-drinking rats showed disturbed architecture with abundant cytoplasmic clear vacuolation displacing nucleus (steatosisis morethan 66%), ballooning degeneration of some hepatocytes, scattered lobular inflammatory cells infiltration and moderate portal lymphocytic infiltrate with minimal early fibrosis in periportal areas and perivenular areas (Fig. 3). These deleterious effects associated with fructose consumption were ameliorated by administration of fenofibrate (80mg/kg) or EPO (5or10g/rat) compared to fructose-drinking control rats.

Figure 4, 5-I illustrates images for hepatic specimens stained for iNOS and RAGE. The area for immune staining for iNOS or RAGE in fructose-drinking rats was greater than the normal rats. Treatment with fenofibrate (80 mg/kg) or EPO (5 or 10 g/rat) resulted in decreases in areas for iNOS immunostaining compared to fructose-drinking rats (P<0.05, Fig 4-II).
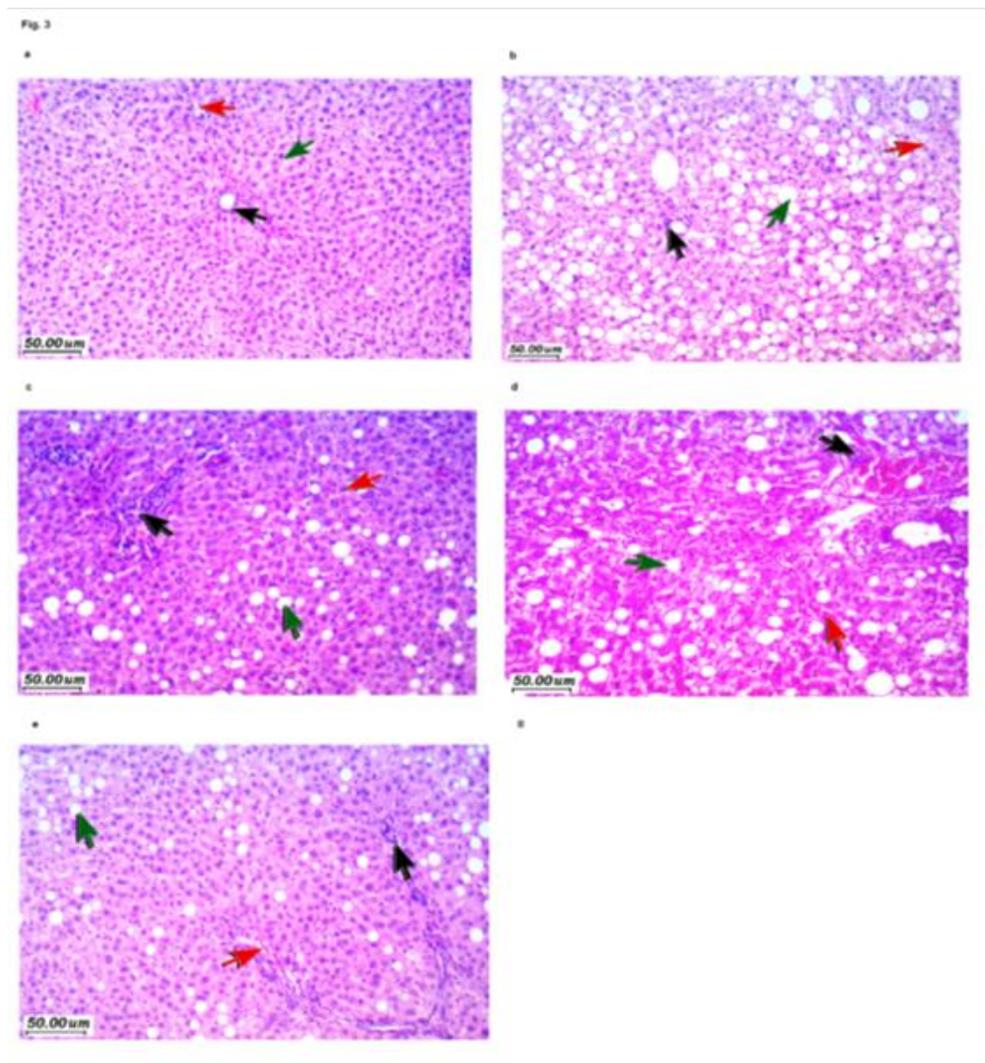

Fig 3 (I): Photomicrographs of hepatic sections stained with H&E for morphometric analysis. Sections from normal rats (a)preserved architecture with lobules formed of hepatocytes arranged in plates of one to two cell thickness showing abundant eosinophilic cytoplasm and central nucleus surrounded by blood sinusoids(green arrow), with normal portal areas (black arrow) and sinusoids (red arrow). Sections from fructose-drinking rats (b) show disturbed architecture with marked cytoplasmic clear vacuolation displacing nucleus (steatosis is more than66%), ballooning degeneration of some hepatocytes (green arrow) ,scattered lobular inflammatory cells infiltration and congested vessels (red arrow). Additionally, there is moderate portal lymphocytic infiltrate and minimal early fibrosis in periportal areas and perivenular areas (black arrow). On the other hand, rats' livers of fenofibrate treated group (c) showed preserved architecture with few

hepatocytes showed steatosis (green arrow), there is mild lobular focal inflammation with mild portal inflammation (black arrow) with few congested vessels (red arrow). However, rats' livers of EPO (5 g/rat) (d) showed moderate ballooning degeneration and steatosis (>33%) (green arrow), there is moderate lobular inflammation and portal inflammation (black arrow) with congested vessels (red arrow). EPO (10 g/rat) (e) showed preserved liver architecture with mild ballooning degeneration and steatosis (green arrow), there is mild lobular inflammation and portal inflammation (black arrow) with few congested vessels (red arrow). (H & E 200 ×).
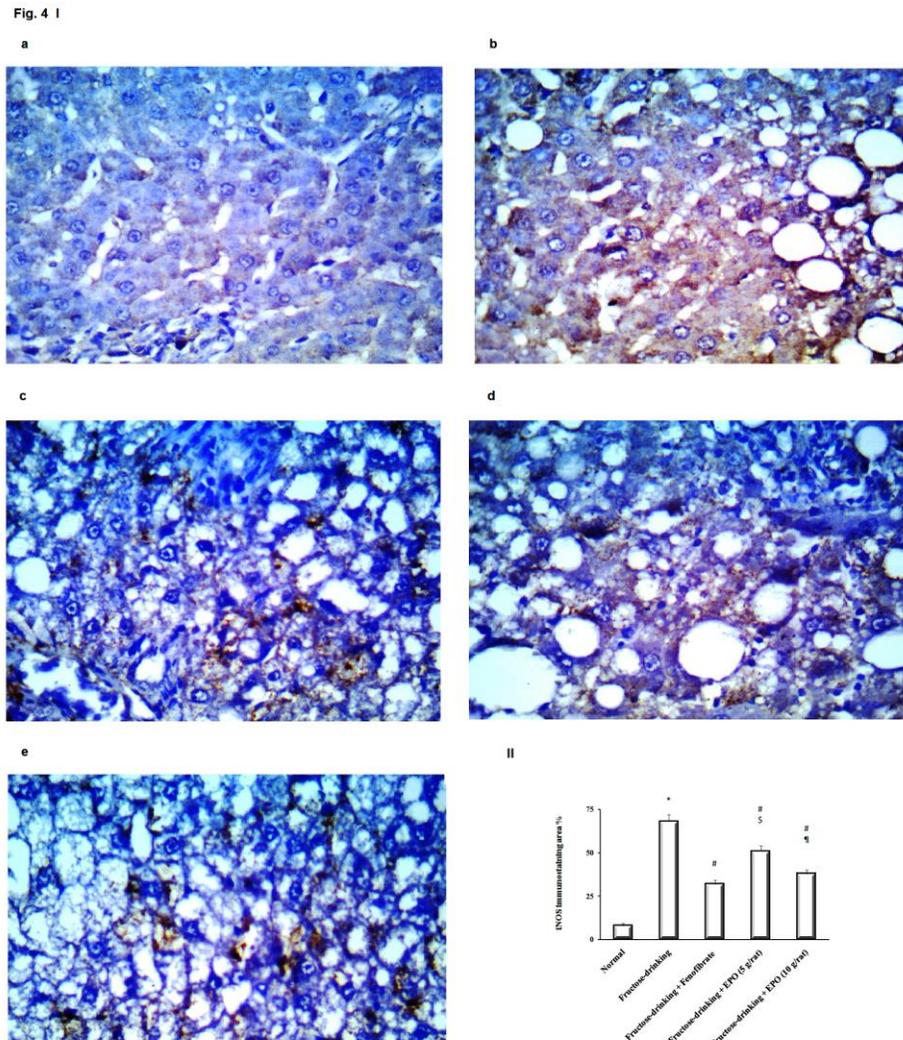

Fig 4-(I) Immunostaining for iNOS in the experimental groups. (II) Effect of fenofibrate (80mg/kg) or EPO (5 or 10 g/rat) on the percent area of iNOS immunostaining. Photomicrographs are captured at 40× magnification. Results are expressed as mean ±S.E.M. and analyzed using one-way ANOVA followed by Bonferroni's post-hoc test at $P<0.05$.*Compared to normal group, #Compared to fructose- drinking group, $Compared to fenofibrate group, ¶Compared to EPO (5 g/rat) group, n=8Moreover, treatment with fenofibrate (80 mg/kg) or the high dose of EPO (10 g/rat) downregulated the expression of RAGE as indicated by the decrease in immunostaining area ($P<0.05,$ Fig 5-II).
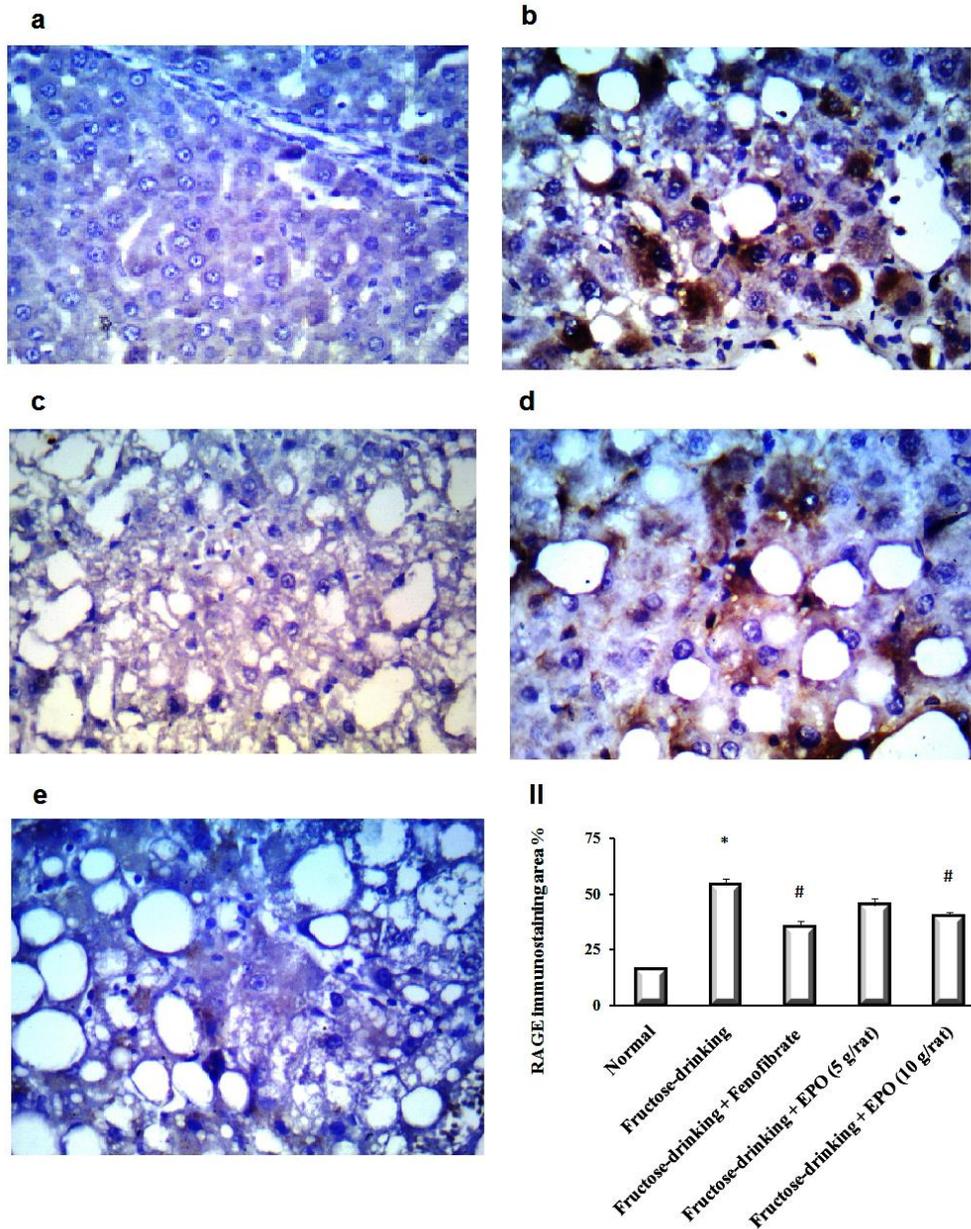
Fig. 5 I

Fig 5-(I) Immunostaining for RAGE in the experimental groups. (II) Effect of fenofibrate (80mg/kg) or EPO (5 or 10 g/rat) on the percent area of RAGE immunostaining. Photomicrographs are captured at 40× magnification. Results are expressed as mean ±S.E.M. and analyzed using one-way ANOVA followed by Bonferroni's post-hoc test at $P<0.05$.*Compared to normal group, #Compared to fructose-drinking group, $Compared to fenofibrate group, ¶Compared to EPO (5 g/rat) group, n=8.

DISCUSSION

Given the important crucial role of high fructose consumption in the pathogenesis of insulin resistance and NAFLD, research were undertaken to seek novel therapeutic agents that can be used for long-term administration. Although the traditional treatment for NAFLD is generally well tolerated, cases of myopathy, rhabdomyolysis and hepatotoxicity were reported [29, 30]. The interest seeking naturally micronutrients with antioxidant and anti-inflammatory properties with fewer or no adverse effects is

increasing. EPO has received importance as a promising dietary supplement because of its effectiveness as anti-inflammatory and anti-oxidant combined with its lipid lowering effect.

As AGE and the in receptor (RAGE) have a central role in the pathogenesis of NAFLD, the inhibition of the AGE–RAGE axis may be a new therapeutic target to be evaluated. Additionally, as one of the characteristic features of NAFLD is the correlation between the AGEs/RAGE axis and the development of oxidative stress and inflammatory process, our study was extended to evaluate the anti-oxidative and anti-inflammatory effect of EPO on hepatic fatty degeneration-promoted by fructose drinking in rats. In this regard, the effects of EPO were compared to fenofibrate as a standard reference drug.

In our rat model we demonstrated that fructose-fed diet was associated with increase in body weight and dyslipidemia and elicited impaired glucose tolerance. These results, consistent with reports by others [4, 31- 35], support the view that high consumption of dietary fructose is increasingly being identified as a causative environmental factor contributing to the development of a wide range of metabolic alterations, including obesity, and dyslipidemia. These alterations lead to visceral fat accumulation, fatty liver and NAFLD [36-38]. It was emphasized that increased fructose intake encourages weight gain, and impaired lipid metabolism by its ability to stimulate food intake and lower metabolism [36].

Our results revealed that fructose administration resulted in development of IR as noted by the observed elevation of HOMA-IR which is in accordance to other previous reports [32-34, 37]. Fructose-induced IR might be in part be related to hyperlipidemia as evident from the elevated serum levels of TGs and total cholesterol, LDL levels and the declined HDL level. Abnormal lipid profile is considered as another characteristic feature of metabolic syndrome, in which fructose acts as alipogenic sugar leads to acceleration of lipogenesis and accumulation of triglycerides [3,5,39],which inturn results in decreased insulin sensitivity and hepatic insulin resistance/glucose intolerance [31, 40]. Stanhope et al. [41] emphasized that excess fructose administration leads to increased de novo lipogenesis either directly [42, 43] via synthesis of fatty acids through gene expression of many enzymes, including acetyl-coenzyme A carboxylase and fatty acid synthase or indirectly by inhibiting fatty acid oxidation [44]. This dyslipidemia- induced IR could be clarified by Abdullaet al.[45] who highlighted tha thyperlipidemia down regulates insulin receptors and lowers insulin receptor mRNA levels in skeletal muscle; leading to reduced insulin stimulated glucose utilization and decrease in insulin sensitivity.

Evening primrose oil administration efficiently reduced the increased body weight, improved impaired glucose tolerance and a meliorated dyslipidemia. These results are in agreement with previous observations that indicate that EPO supplementation can effectively a meliorate the dyslipidemia and its consequences IR infructose-fed rats [46-49]. This lipid lowering effect might be attributed to the inhibitory effect of EPO on 3-hydroxy 3-methyl glutaryl coenzyme A reductase (HMG-Co-A reductase). PUFAs are potent inhibitors of HMG-Co-A reductase which considered the rate limiting enzyme in cholesterol synthesis via mevalonate pathway [50, 51]. Additionally, it was previously documented the ability of EPO to upregulate the activity of LDL receptor and its consequent increased clearance of LDL from the plasma [49].

Another principal causative factor that might be participated in the progression of fructose-induced decreased insulin sensitivity and impaired glucose tolerance is the generation and accumulation of AGE and activation of its receptor [receptor for AGE (RAGE)]. Fructose by its reducing capacity could adduct proteins via the Maillard reaction; yielding glycated/ fructated proteins that is ultimately converted to toxic AGEs. AGEs byits interaction with RAGE, play a central role in cellular organelles dysfunction and damage with ultimately development of IR [7, 52]. In harmony with previous studies documented that insulin resistance/glucose intolerance was positively correlated with activation of AGEs/RAGE axis [6, 35, 53, 54], the present results demonstrated that fructose drinking evoked protein glycation and mediated formation of AGE and activation of RAGE, which is implicated in insulin resistance/glucose intolerance and progression to the metabolic syndrome. Santos et al. [55] emphasized that AGEs formation which occurs slowly under physiological conditions, its generation increases considerably with hyperglycemia; participating in the development of IR and the progression to chronic diseases. The exposure of the liver to such enormous rising fructose consumption and activation of AGEs/RAGE axis have been associated with alteration of steatosis characterized

by the presence of hepatic fat accumulation with no evidence of hepatocellular injury to steatohepatitis [55] and development of hepatocellular injury and ultimately liver damage [7, 56, 57] as indicated by the elevated liver specific serum markers and confirmed by the histopathological assessment. In the current study, treatment with EPO through lowering glycated hemoglobin levels, down regulated RAGE expression and suppressing AGE-RAGE axis; provided effectiveness in reversing this scenario and ameliorating the liver tissue damage.

This improving outcome of EPO can be additionally being allocated to its anti- oxidant capacity. Some researchers emphasized that during the course of metabolic syndrome, AGEs/RAGE interaction has been involved in the progression of oxidative stress burden with its subsequent insulin resistance/glucose intolerance and pathogenic consequences on the proliferation and stimulation of hepatic stellate cells; indicating a functional association between formation of serum AGEs and the development of oxidative stress and development of liver injury [7,55,58]. Moreover, other studies strongly support the theory that oxidative process plays a critical role in IR/glucose intolerance after long-term fructose drinking and emphasized that continuous fructose ingestion-induced metabolic burden on the liver and is associated with ATP depletion; promoting development of oxidative stress[37 , 59, 60]. The present results agree with some published data that highlights the important link between high fructose consumption-induced activation of AGEs/RAGE axis and the development of oxidative stress burden as indicated by the significant increase in the oxidative stress biomarker and the decrease in the status of antioxidant enzymes [33, 58, 61, 62]. In agreement, many previous studies, documented that EPO is not only possess a powerful anti-oxidant effect, but also exhibit intense radical-scavenging activity; the current study demonstrated that EPO administration not only attenuated lipid peroxidation as evident by the significant decrease in MDA level, but also increased the antioxidant enzymes pool [16, 46, 63, 64]. These were emphasized by some research groups, authors revealed that EPO suppresses the formation of ROS by its Phenolic compounds which inhibit lipid peroxidation, chelate metal ions and stop the chain reactions [65-68].

Moreover, the increased expression of AGEs and activation of its receptor elicit inflammation and lead to formation of cytokines such as TNF-α and iNOS; which contribute to the progression of insulin resistance/glucose intolerance and play a role in liver damage [37,58,61,69, 70]. EPO administration by its known anti-inflammatory capacity [50, 71], effectively downregulated the increments in these inflammatory markers in many inflammatory conditions such as, cardiac injuries [18, 24] .

Collectively, our present findings provided effectiveness of EPO in counter acting the disrupting effects observed in hepatic component of metabolic syndrome induced by fructose drinking in rats, at least in part, by suppressing AGEs/RAGE axis and its subsequent generation of ROS and inflammatory mediators known to be involved in the pathogenesis of fatty degeneration and the progression of the metabolic syndrome. Conclusion: Inspite of the beneficial outcomes of EPO noticed in this study were almost comparable to fenofibrate known by its anti-inflammatory [22, 72] and antioxidant actions [15] and is implicated in reducing liver adiposity and IR [13,73], however, EPO might be preferable considering safety. Therefore, this study gives convincing evidence of the promising alternative modality of EPO administration and its benefit of therapeutic utility on the hepatic component of metabolic syndrome induced by fructose drinking. Hence, further studies are warranted to fully elucidate its clinical application.

REFERENCES

[1] X. Ouyang ,P. Cirillo , Y.Sautin , S.McCall, J. Bruchette, A. Mae Dieh , et al., Fructose consumption as a risk factor for non-alcoholic fatty liver disease, J. Hepatol. 48 (2008) 993–999. doi: 10.1016/j.jhep.2008.02.011.
[2] L. Tappy, K. Le , Metabolic effects of fructose and the worldwide increase in obesity, Physiol. Rev. 90 (2010) 23–46. doi: 10.1152/physrev.00019.2009.

[3] S.Zelber-Sagi,D.Nitzan-Kaluski,R.Goldsmith,M.Webb,L. Blendis,Z.Halpern,et al., Long term nutritional intake and the risk of nonalcoholic fatty liver disease (NAFLD): a population based study, J. Hepatol. 47 (2007) 711-717.

[4] H.Gao,T.Guan,C.Li,G.Zuo,J.Yamahara,J.Wang,etal.,Treatmentwith ginger ameliorates fructose-induced fatty liver and hypertriglyceridemia in rats: modulation of the hepatic carbohydrate response element-binding protein- mediated pathway, Evid. Based ComplementAlternat. Med. 2012 (2012) 570948-570960. doi: 10.1155/2012/570948.

[5] B. Fariba, S. Hassan, G. hahin, R. Yavar, Effects of dietary fructose, sucrose and lactoseininductionofnonalcoholicfattyliverinrat.WorldAppl.Sci.22 (2013) 368-373. DOI: 10.5829/idosi.wasj.2013.22.03.2101.

[6] A.Ardestani ,R.Yazdanparast, CyperusrotundussuppressesAGE formationand protein oxidation in a model of fructose-mediated protein glycoxidation. Int. J. Boil. Macromol. 41(2007) 572–578.

[7] Y.Kimura, H.Hyogo, S.Yamagishi, M.Takeuchi, T.Ishitobi, Y.Nabeshima, et al. , Atorvastatin decreases serum levels of advanced glycation endproducts (AGEs) in nonalcoholicsteatohepatitis(NASH)patients withdyslipidemia:clinical usefulness of AGEs as a biomarker for the attenuation of NASH, J. Gastroenterol. 45, (2010) 750–755. doi: 10.1007/s00535-010-0203-y.

[8] Z.Ackerman,M.Oron-Herman,M.Grozovski,T.Rosenthal,O.Pappo,G.Link,et al., Fructose-induced fatty liver disease: hepatic effects of blood pressure and plasma triglyceride reduction, Hypertension 45 (2005) 1012–1018.

[9] G.Kanuri,I.Bergheim,Invitroandinvivomodelsofnon-alcoholicfattyliver Disease (NAFLD), Int. J. Mol. Sci. 14 (2013)11963-11980. doi: 10.3390/ijms140611963.

[10] Q. Yin, J. Pei, S. Xu , D. Luo , S. Dong , M. Sun , et al. , Pioglitazone improves cognitive function via increasing insulin sensitivity and strengthening antioxidantdefencesysteminfructose-drinkinginsulinresistancerats,PloSone 20 (2013) e59313- e59323. doi: 10.1371/journal.pone.0059313.

[11] M. Kostapanos, A. Kei, M. Elisaf,Current role of fenofibrate in the preventionand managementofnon-alcoholicfattyliverdisease,WorldJ.Hepatol.27(2013) 470-478. doi: 10.4254/wjh.v5.i9.470.

[12] S. Chan, R. Sun, X. Zeng , Z. Choong, H. Wang , M. Watt, et al., Activation of PPARαameliorates hepatic insulin resistance and steatosisinhigh fructose–fed mice despite increased endoplasmic reticulumstress, Diabetes62(2013)2095-2105. doi: 10.2337/db12-1397.

[13] C. Larter, M. Yeh , J. Cheng, J. Williams, S. Brown, A. Dela Pena, et al., Activation of peroxisome proliferator-activated receptor alpha by dietary fish oil attenuates steatosis, but does not prevent experimental steatohepatitis because of hepatic lipoperoxide accumulation, J. Gastrol. Hepatol. 23 (2008)267–275.

[14] H.Schmilovitz-Weiss,E.Hochhauser,M.Cohen,Y.Chepurko,S.Yitzhaki,E. Grossman, etal., Rosiglitazone and bezafibrate modulate geneexpressionina rat model ofnon-alcoholicfattyliver disease- A historicalprospective.Lipids Health Dis. 12 (2013)41-48. doi: 10.1186/1476-511X-12-41

[15] M. Pruski, R. Krysiak, B. Okopien, Pleiotropic action of short-term metformin and fenofibrate treatment, combined with lifestyle intervention, in type 2 diabetic patients with mixed dyslipidemia, Diabetes Care 32 (2009)1421–1424. doi: 10.2337/dc08-2335.

[16] A. Riaz , R.Khan, A.Siddiqi,T. Mirza, Evaluationof weight reducing effectof evening primrose oil, J. Pharm. Nut. Sci. 1 (2011)15-19.

[17] M. Ismail, S. EL-Maraghy, N. Sadik, Study of theimmunomodulatory and anti- inflammatory effects of evening primrose oil in adjuvant arthritis. Af. J. Biochem. Res. 2 (2008)74-80.

[18] N.Abo-Gresha, E. Abel-Aziz, S. Greish,Evening primrose oil ameliorates platelet aggregationand improvescardiacrecoveryin myocardial-infarct hypercholesterolemic rats, Int. J. Physiol. Pathophysiol. pharmacol. 6 (2014) 23-36

[19] S.Zaitone,Y.Moustafa,S.Mosaad,N.El-Orabi,Effectofeveningprimroseoil and omega-3 polyunsaturated fatty acids on the cardiovascular risk of celecoxib in rats, Cardiovasc. Pharmacol. 58 (2011b) 72-79. doi:10.1097/FJC.0b013e31821c8353.

[20] L.Sánchez-Lozada, E.Tapia ,A. Jiménez ,P. Bautista ,M. Cristóbal ,T. Nepomuceno , et al., Fructose-induced metabolic syndrome is associated with glomerular hypertension and renal microvascular damage in rats, Am. J. Physiol. Renal Physiol. 292 (2007) 423-429.

[21]   T.Ogata, T.Miyauchi, S.Sakai, Y.Irukayama-Tomobe, G.Katsutoshi,I. Yamaguchi, Stimulation of peroxisome-proliferator-activatedreceptorα(PPARα) attenuates cardiac fibrosis and endothelin-1 production in pressure- overloaded rat hearts, Clin. Sci.103 (2002) 284S-288S.

[22]   T.Ogata, T.Miyauchi, S.Sakai, M.Takanashi, Y.Irukayama-Tomobe, I. Yamaguchi, Myocardial fibrosis and diastolic dysfunction in deoxycorticosterone acetate-salt hypertensive rats is ameliorated by the peroxisome proliferator-activated receptor-alpha activator fenofibrate, partly by suppressing inflammatory responses associated with the nuclear factor-kappa-B pathway, J. Am. Coll.Cardiol. 43(2004)1481-1488.

[23]   L. Tang , W. Wei , L. Chen , S. Liu , Effects of berberine on diabetes induced by alloxanandahigh-fat/high-cholesteroldietinrats,Ethnopharmacol.108(2006)109-115.

[24]   S.Zaitone,S.Essawy, Additionofalowdoseofrimonabanttoorlistattherapy decreases weight gain and reduces adiposity in dietary obese rats, Clin. Exp. Pharmacol. Physiol. 39 (2012)551-559. doi: 10.1111/j.1440-1681.2012.05717.x.

[25]   S. Zaitone, N. Hassan, N. El-Orabi, E. El-Awady, Pentoxifylline and melatonin in combination with pioglitazone ameliorate experimental non-alcoholic fatty liver disease, Eur. J. Pharmacol. 662 (2011a) 70-77. doi:10.1016/j.ejphar.2011.04.049.

[26]   D. Zaafar, S. Zaitone, Y. Moustafa, Role of metformin in suppressing 1, 2- dimethylhydrazine-induced colon cancer in diabetic and non-diabetic mice: effect on tumor angiogenesis and cell proliferation, PloS one 9 (2014) e100562- e100574.doi:10.1371/journal.pone.0100562.

[2 7] D.Matthews,J.Hosker,A. Rudenski,B.Naylor,D.Treacher,R.Turner, Homeostasis model assessment:insulinresistanceandbeta –cellfunctionfrom fastingplasmaglucoseandinsulinconcentrationsinmen, Diabetologia28 (1985) 412-419

[2 8] R.Lee,AutomatedquantificationofliversteatosisbyWSIbasedimageanalysis, 1st CongressoftheInt.AcademyofDigitalPathology,AnalyticalCellularPathol.34 (2011).

[2 9] C.Ho,T.Kuo,T.Chen,S.Tsay,F.Chang,S.Lee,Fenofibrate-induced acute cholestatic hepatitis, J. Chin. Med. Assoc. 67 (2004) 245–247.

[30]   M.Okada,F. Sano, I.Ikeda,J. Sugimoto,S. Takagi,H. Sakai,etal.,Fenofibrate- induced musculartoxicityisassociatedwith a metabolicshift limited to type-1 muscles in rats, Toxicol. Pathol. 37(2009) 517–520. doi:10.1177/0192623309336151.

[31]   P.Angelova,N.Boyadjiev,Areviewonthemodelsofobesityandmetabolic syndrome in rats, Trakia J. Sci. 1(2013) 5-12.

[32]   C. Chou, Y. Lai, T. Lin, T. Lee, T. Fang, Aliskiren prevents and ameliorates metabolicsyndromeinfructose-fedrats,Arch.Med.Sci.7(2011)882–888.doi: 10.5114/aoms.2011.25566.

[33]   R. Johnson, T. Nakagawa, L. Sanchez-Lozada , M. Shafiu , S. Sundaram , M. Le, et al.,Sugar,uricAcid,andtheetiologyofdiabetesandobesity,Diabetes62(2013) 3307-33015. doi: 10.2337/db12-1814.

[34]   M.Kho,Y.Lee,J.Cha,K.Choi,D.Kang,H.Lee,Gastrodiaelataameliorates high-fructosediet-inducedlipidmetabolismandendothelialdysfunction,Evid.BasedComplement Alternat. Med. 2014(2014)101624-101634. doi:10.1155/2014/101624.

[35]   A.Mahmoud,S.Elshazly,Ursodeoxycholicacidamelioratesfructose-induced metabolic syndrome in rats, PLoSOne 9(2014)e106993-e1069101. doi: 10.1371 / journal . pone. 0106993.

[36]   C.Roncal-Jimenez,M.Lanaspa,C.Rivard,T.Nakagawa,L.Sanchez-Lozada,D. Jalal,etal.,Sucroseinducesfattyliverandpancreaticinflammationinmale breederratsindependentofexcessenergyintake,Metabolism60(2011)1259–1270. doi: 10.1016/ j. metabol.2011.01.008.

[37]   Y.Yilmaz,Reviewarticle:fructoseinnon-alcoholicfattyliverdisease,Aliment. Pharmacol. Ther. 35(2012)1135–1144.

[38]   D.Cardinali, P.Bernasconi, R.Reynoso, C.Toso, P.Scacchi, Melatoninmay curtail the metabolic syndrome: studies on initial and fully established fructose- inducedmetabolic syndrome in rats,Int.J.Mol.Sci.14 (2013)2502-2514.doi:10.3390/ ijms 14022502.

[39]   M.AlQudah,Thehistologicexaminationofmalealbinoratsliverwhichwas exposed to hunger stress, World Appl. Sci. J. 16(2012)1427-1431.

[40] H. Kim, T. Okubo, L. Juneja, T. Yokozawa, The protective role of amla (Emblica officinalis Gaertn.) against fructose-induced metabolic syndrome in a rat model, Br. J. Nutr. 103 (2010) 502–512. doi: 10.1017/S0007114509991978.

[41] K.Stanhope,J.Schwarz,N.Keim,S.Griffen,A.Bremer,J.Graham,etal., Consuming fructose-sweetened, not glucose-sweetened, beverages increasesvisceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans, J. Clin. Invest. 119 (2009)1322–1334.

[42] L.Hudgins,T. Parker,D. Levine,M.Hellerstein,A dualsugar challengetestfor lipogenic sensitivity to dietary fructose, J. Clin. Endocrinol. Metab.96(2011)861–868. doi: 10.1210/jc.2010-2007.

[43] M. Maersk,A. Belza,H. Stodkilde-Jorgensen,S. Ringgaard, E.Chabanova, H.Thomsen, et al., Sucrose-sweetened beverages increase fat storage in the liver,muscle,andvisceralfatdepot:a6-morandomizedinterventionstudy, Am. J. Clin. Nutr. 95 (2012) 283–289. doi: 10.3945/ajcn.111.022533.

[44] C. Cox, K. Stanhope, J. Schwarz, J. Graham, B. Hatcher, S. Griffen, et al., Consumption of fructose-sweetenedbeverages for 10 weeks reduces net fat oxidation andenergy expenditure inoverweight/obesemenand women,Eur.J. Clin. Nutr. 66 (2012) 201–208. doi: 10.1038/ejcn.2011.159.

[45] M. Abdulla, M. Sattar, E. Johns, The Relation between fructose-induced metabolic syndrome and altered renal haemodynamic and excretory function in the rat, Int. J. Nephrol. (2011) 934659-934676 doi: 10.4061/2011/934659.

[46] A. El-kossi , M. Abdellah , A. Rashad, S. Hamed, The effectiveness of evening primrose oil and alpha lipoic acid in recovery of nerve function in diabetic rats, J.Clin. Exp. Invest. 2 (2011) 245-253.

[47] W. Harris, W Poston, C. Haddock, Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events, Atherosclerosis 193 (2007)1–10.

[48] A.Riaz, R.Khan, S.Ahmed, Assessment of anticoagulant effect of evening primrose oil, Pak. J. Pharm. Sci. 22 (2009) 355-359.

[49] M.Jamilian, M.Karamali, M.Taghizadeh, N.Sharifi, Z.Jafari, M.Memarzadeh, et al.,VitaminDand evening primrose oil administrationimproveglycemiaand lipidprofilesinwomenwithgestationaldiabetes,Lipids51 (2016)349-56.doi:10.1007/s11745-016-4123-3.

[50] U.Das,Essentialfattyacids andtheir metabolitescould function as endogenous HMG-CoA reductase and ACE enzyme inhibitors , anti-arrhythmic, anti- hypertensive, anti-atherosclerotic, anti-inflammatory, cytoprotective, and cardioprotective molecules, Lipids Health Dis. 7(2008)37-55. doi: 10.1186/1476-511X-7-37.

[51] V. Dhikav, K. Anand , R. Sudha, Omega-3 polyunsaturated fatty acid and cardiovascular disorders, JIACM 5 (2004)182–185.

[52] H.Unoki, S.Yamagishi, Advancedglycationendproductsandinsulinresistance, Curr. Pharm. Des. 14 (2008)987–989.

[53] A.Goldin,J.Beckman,A.Schmidt,M.Creager,Advancedglycationendproducts: sparking the development of diabetic vascular injury, Circulation 114(2006)597–605.

[54] A.Miller,K.Adeli,Dietaryfructoseandthemetabolicsyndrome.Curr.Opin Gastroenterol. 24 (2008) 204–209. doi: 10.1097/MOG.0b013e3282f3f4c4.

[55] J.Santos, I.Valentim, O.deAraújo, R.Ataide, M.Goulart,Developmentofnon- alcoholic hepatopathy : contributions of oxidative stress and advanced glycation endproducts, Int. J. Mol. Sci. 14(2013)19846-19866. doi: 10.3390/ijms141019846.

[56] K. Gaens, P. Niessen, S. Rensen, W. Buurman,J. Greve, A. Driessen, et al., EndogenousformationofN(ε)-(carboxymethyl)lysineisincreasedinfatty livers and induces inflammatory markers in an in vitro model of hepatic steatosis, J. Hepatol. 56 (2012)647–655. doi: 10.1016/j.jhep.2011.07.028.

[57] K. Kavanagh, A. Wylie, K. Tucker, T. Hamp, R. Gharaibeth, J. Cullen, Dietary fructose induces endotoxemia and hepatic injury in calorically controlled primates, Am. J. Clin. Nutr. 98 (2013) 349–357. doi: 10.3945/ajcn.112.057331.

[58] R. Sil, D. Ray, A. Chakraborti, Glycyrrhizin ameliorates insulin resistance, hyperglycemia, dyslipidemia and oxidative stress in fructose-induced metabolic syndrome-X in rat model, Indian J. Exp. Biol. 51(2013)129-138.

[59] M.Lane,S.Cha,Effectofglucoseandfructoseonfoodintakeviamalonyl-CoA signalinginthebrain,Biochem.Biophys.Res.Commun.382(2009)1–5.doi: 10.1016/j.bbrc.2009.02.145.

[60] J.Lim, M.Mietus-Snyder, A.Valente, J.Schwarz, R.Lustig,Theroleoffructosein the pathogenesis of NAFLD and the metabolic syndrome, Nat. Rev. Gastroenterol. Hepatol. 7 (2010)251–264. doi: 10.1038/nrgastro.2010.41.

[61] W.Nseir, F. Nassar, N. Assy, Soft drinksconsumptionand nonalcoholic fatty liver disease, World J. Gastroenterol.16 (2010)2579–2588.

[62] [62] L. Rodríguez, P. Otero, M. Panadero, S. Rodrigo, J. Álvarez-Millán ,C. Bocos, Maternalfructose intakeinduces insulin resistanceandoxidativestress inmale, butnotfemale,offspring,J.Nutr.Metab.2015(2015)158091-158099.doi:10.1155/2015/158091.

[63] W.Peschel,W.Dieckmann,M. Sonnenschein,A. Plescher,Highantioxidant potential of pressing residues from evening primrose oil in comparison to other oilseed cakes and plant antioxidants, Ind. crops and products 25(2007) 44- 54.

[64] R. El-Sayed, Y. Moustafa, M. El-Azab, Evening primrose oil and celecoxib inhibited pathological angiogenesis, inflammation, and oxidative stress in adjuvant- induced arthritis: novel role of angiopoietin-1, Inflammopharmacol. 22(2014)305-317. doi: 10.1007/s10787-014-0200-5.

[65] G. Czapski, K.Czubowicz, R.Strosznajder, Evaluationof the antioxidative properties of lipoxygenase inhibitors, Pharmacol. Rep. 64 (2012)1179–1188.

[66] A. Kiss, M. Derwinska, S. Granica, Quantitative analysis of biologically active polyphenols in evening primrose (Oenotheraparadoxa) seeds aqueous extracts, Pol. J. Food and Nut. Sci. 61(2011)109–113.

[67] Z.Kayaa,G. Eraslanb,Theeffectsofeveningprimroseoilonarsenic-induced oxidative stress in rats, Toxicol. Env. Chem. 95 (2013)1416-1423.

[68] S. Montserrat-de la Paz, A. Fernandez-Arche, M. Angel-Martın, M. Garcıa- Gimenez, Thesterolsisolated from eveningprimroseoil modulatethe releaseof proinflammatory mediators, Phytomedicine 19 (2012)1072–1076. doi: 10.1016/j.phymed.2012.06.008.

[69] Z. Madani , K. Louchami, A. Sener, W. Malaisse, Y. Ait, Dietary sardine protein lowers insulin resistance, leptin and TNF-alpha and beneficially affects adipose tissue oxidative stress in rats with fructose-induced metabolic syndrome, Int. J. Mol. Med. 29 (2012)311–318. doi: 10.3892/ijmm.2011.836.

[70] M. Mahmoud, M.El-Nagar, H.El-Bassossy, Anti-inflammatory effect of atorvastatinonvascularreactivityandinsulinresistanceinfructosefedrats, Arch. Pharm. Res. 35(2012) 155–162. doi: 10.1007/s12272-012-0117-8.

[71] L. Ferrucci, A. Cherubini, S. Bandinelli, B. Bartali, A. Corsi, F. Lauretani, et al., Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers, J. Clin. Endocrinol. Metab. 91(2006) 439–446.

[72] Q.Diep, K.Benkirane, F.Amiri, J.Cohn, D.Endemann,E. Schiffrin,PPAR alpha activator fenofibrate inhibits myocardial inflammation and fibroisin angiotensin II -infused rats, J. Mol. Cell Cardiol. 36 (2004)295-304.

[73] H.Lee, S.Choi,M. Park, Y.An, S.Seo,M. Kim, etal.,Fenofibratelowers abdominal and skeletal adiposity and improvesinsulin sensitivity in OLETF rats, Biochem. Biophys. Res. Commun. 296 (2002)293-299.