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Study of the combined protective effect of adrenomedullin and exercise on induced fatty liver in male albino rats: A possible role of peroxisome proliferator-activated receptor-alpha

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***Abstract*---Background:** Adrenomedullin (AM) is a key antioxidant and anti-inflammatory agent vital for disease prevention throughout the body, while regular physical exercise helps prevent severe obesity and related complications like non-alcoholic fatty liver (NAFL). This

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study aimed to evaluate the protective effects of AM and regular exercise on fatty liver disease (FLD). **Methods:** This study involved 50 male rats divided into five groups: Group I received a normal diet for 8 weeks (control), Group II was given a high-fat high-cholesterol diet (HFHCD) to induce fatty liver, Group III received HFHCD plus daily subcutaneous adrenomedullin (50 ng/kg) for 8 weeks, Group IV received HFHCD plus moderate swimming exercise (1 hour/day, 5 days/week) for 6 weeks, and Group V received HFHCD along with both AM and exercise for 8 weeks. **Results:** In fatty liver-induced rats (GII): serum liver enzymes, lipid profile, hepatic MDA and NF-κB levels significantly increased, while hepatic PPAR-α and TAC significantly decreased compared to controls (GI). Treatment with A.M (GIII), exercise (GIV), or both (GV) significantly reversed these effects. GV showed the most improvement but not fully restoring levels to those of GI. **Conclusions:** AM and regular exercise together protect against FLD by reducing inflammation, fat content and liver damage.

***Keywords*---**Induced Fatty Liver, Adrenomedullin, Peroxisome Proliferator-Activated Receptor-Alpha, Male Albino Rats

Introduction

Fatty liver disease (FLD) is the most common chronic liver disorder worldwide with an estimated global prevalence of 25% of adults (Rinella and Charlton, 2016). It is a heterogeneous complex whose process is associated with multiple factors such as dyslipidemia, insulin resistance, obesity, oxidative stress (Younossi et al., 2023), imbalance of the microbes in the gut and may be genetic factors (Martin et al., 2021).

It is considered a spectrum of liver disorders ranging from simple steatosis to steatohepatitis and probably advanced hepatic fibrosis or cirrhosis (Argo and Caldwell, 2009). Steatosis is defined as when increased fat accumulation in 5% of hepatocytes (Friedman et al., 2018). The progression of FLD is associated with constant exposure to pathogenic factors, endocrinal diseases, lipid accumulation in the liver and oxidative stress (Petrakis et al., 2017).

On top of the hepatic steatosis, the pathogenesis of steatohepatitis is probably caused by mitochondrial dysfunction which may manifest as disrupted fatty acid oxidation, depressed bioenergetics of hepatocytes and increased oxidative stress due of increased production of reactive oxygen species (ROS) (Mantena et al., 2009).

Adrenomedullin (AM) is a 52-amino acid peptide that initially isolated from a human pheochromocytoma; also, it is produced by different tissues in humans; such as adrenal gland, cardiac atria, lung, kidney, blood vessels, bone, adipose tissue and from cells as endothelial cells, vascular smooth muscle cells, macrophages and monocytes (Hellenthal et al., 2022). It displays potent vasodilating and antioxidant effects; besides, it has angiogenic and growth factor

properties. Moreover, it can act as both a hormone and a cytokine "hormokine" (Schuetz et al., 2015).

AM is upregulated in liver diseases to counteract the disease process with its physiological actions including anti-oxidative effects (ElGendy et al., 2019). Also, it inhibits insulin secretion by pancreatic B cells and stimulates the synthesis and secretion of interleukin (IL)-6 which is involved in insulin resistance mechanisms (Linscheid et al., 2005).

The sedentary daily routine was reported higher in people predisposed to develop obesity, Type2 diabetes, FLD and metabolic syndrome (Chau et al., 2013). There is a strong association between increased hepatic triglyceride content and each hour spent sedentary during a day, while prospective studies identify sedentary behavior as an independent risk factor for FLD development and progression (Keating et al., 2017).

It was reported an increase in plasma AM concentration during three steps of submaximal cycle exercise (lasting 12 min) in healthy subjects (Krzeminski, 2016). Exercise plays a vital role in weight loss and reduces hepatic steatosis and its associated cardiovascular complications (Orci et al., 2016). In many of the published studies, the impact of exercise on the improvement of the liver fat content was seen even in patients who did not achieve weight loss, suggesting the exercise direct effects on the liver (Bacchi et al., 2013, Keating et al., 2017).

Also, it can improve insulin resistance in peripheral tissues, which results in a decrease in the free fatty acids (FFAs) flux to the liver and limit their new formation (Rabøl et al., 2011). Peroxisome proliferator-activated receptor-alpha (PPAR-α) is a ligand-activated nuclear receptor that is highly expressed in the liver (Kersten and Stienstra, 2017).

It is required for adipocyte (Phosphoenolpyruvate Carboxy kinase 1) PCK1 expression as any change in the activity or presence of these transcription factors leads to major changes in intracellular lipid levels (Kersten and Stienstra, 2017). It is involved in lipid metabolism by regulation of FFAs catabolism, lipogenesis and ketogenesis in response to feeding and starvation (Liu et al., 2019). In addition, it is an important regulator of the genes involved in β-oxidation in peroxisomes and mitochondria, transport of FFAs and gluconeogenesis in the liver (Llovet et al., 2023).

This work aimed to evaluate the potential effect of both AM and exercise in protection against induced fatty liver in male albino rats and the potential role of PPAR-α.

Material and Methods

This study was carried out on 50 healthy male albino rats ranging in weight between 200-250 g. The rats were housed in isolated animal cages in a standard animal laboratory room temperature with 12/12 h light/dark cycles with free access to tap water and pellet laboratory chow all over the period of the work. All procedures of handling of the rats were performed with the instructions of the

ethical committee of the Faculty of Medicine, Tanta University and following the ethical guidelines for investigation (Approval code: 35441/4/22).

Animals grouping

Rats were randomly divided into five equal groups (n=10): The rats were acclimatized for two weeks then randomly divided into five equal groups:
Group I (Control group): Rats were fed normal rat pellet chow, which contains 67% carbohydrates, 10% fat and 23% protein as the energy sources (overall calorie: 3.6 kcal/g) for 8 weeks as described by Xu et al. (Xu et al., 2010)
Group II (Induction of fatty liver): Rats were fed a high-fat high cholesterol diet (HFHCD) daily for 8 weeks. The HFHCD was prepared by mixing 2 g cholesterol and 10 g lard oil with 88 g normal pellet chow, it is composed of the following energy sources: 52% was provided by carbohydrates, 30% by fat and 18% by proteins (overall calories: 4.8 kcal/g) as previously described by Xu et al. (Xu et al., 2010)
Group III (Fatty liver + AM): Rats received HFHCD as group II with subcutaneous (S.C) injection of AM at a dose of 50 ng/kg/day for 8 weeks as described by ElGendy et al. (ElGendy et al., 2019)
Group IV (Fatty liver + Exercise): Rats were fed HFHCD as group II and submitted to regular moderate exercise. All rats were adapted to water before starting the experiment, then they were kept in shallow water for 1 hour 5 times a week. After that, the rats were floated 5 times a week for 1 hour for six weeks as described by Souza et al. (Souza et al., 2009)
Group V (Fatty liver + AM+ Exercise): Rats of this group were fed HFHCD as group II, received AM in the same dose as group III and submitted to regular moderate exercise as group IV for 8 weeks.

Drug preparation:
AM was obtained from Sigma Chemical Company and administered subcutaneously at 50 ng/kg/day for 8 weeks (ElGendy et al., 2019)

Body weight measurement:
It was measured before the starting and at the end of the experimental period, the animal was weighed after putting it in a closed plastic container (Nascimento et al., 2008). Then the results were written in a record for each rat (Elmarakby and Imig, 2010)

Biochemical analysis:
After 8 weeks, rats were anesthetized using intraperitoneal sodium pentobarbital (60 mg/kg) and sacrificed. sacrificed by decapitation and packed in a special package according to safety precautions and infection control measures and sent with hospital biohazards.
Blood was collected via cardiac puncture; liver tissues were excised, washed in cold PBS and prepared for biochemical and histological analyses (Shekarforoush et al., 2016).
Fasting blood samples were collected via cardiac puncture; serum and plasma were separated by centrifugation using plain and EDTA tubes.

Analysis of the level of the following parameters:

- **Liver enzyme markers (Serum aspartate aminotransferase (AST) and Serum alanine aminotransferase (ALT)):** were measured using colorimetric assay kits (Biodiagnostic Chemical Company, Giza, Egypt), according to the method described by Palipoch and Punsawad (Palipoch and Punsawad, 2013).
- **Fasting blood glucose (FBG):** the samples of plasma were obtained after 12 hours fasting according to the method described by Diamond et al. (Diamond et al., 2003)
- **Serum insulin:** was measured by determined using MyBioSource ELISA Kit procedure according to the method described by Chevenne et al. (Chevenne et al., 1994)
- **Serum total cholesterol (TC):** was measured using colorimetric assay kits (Biodiagnostic Chemical Company, Giza, Egypt), according to colorimetric method of Artiss (Artiss, 2000)
- **Serum triglycerides (TGs):** were measured using colorimetric assay kits (Biodiagnostic Chemical Company, Giza, Egypt), according to method of Mc Gowan et al. (McGowan et al., 1983).
- **Serum level of high density lipoproteins cholesterol (HDL-C) and low density lipoproteins cholesterol (LDL-C):** cholesterol was measured using enzymatic assay, according to method of by Ni et al. (Ni et al., 2010) After obtaining total cholesterol, HDL and triglycerides from respective kits, LDL-C was computed mathematically.
- **Hepatic TG and TC:** were measured according to method of Rodríguez-Sureda and Peinado-Onsurbe (Rodríguez-Sureda and Peinado-Onsurbe, 2005). Same enzymatic colorimetric methods as used in serum but applied to extracted liver lipids. Lipids are extracted using chloroform-methanol and reconstituted for assay.
- **Liver Malondialdehyde (MDA) level:** was measured in liver homogenate using colorimetric assay kits (Biodiagnostic Chemical Company, Giza, Egypt), according to method of Abarikwu et al. (Abarikwu et al., 2012)
- **Total Antioxidant Capacity (TAC) level:** was measured in hepatic homogenate using colorimetric assay kits (Biodiagnostic Chemical Company, Giza, Egypt), by colorimetric method Rezaei-Moghadam et al. (Rezaei-Moghadam et al., 2012), assay is based on the ability of antioxidants to reduce Fe^{3+}-TPTZ to Fe^{2+}-TPTZ complex.
- **Quantitative measurement of peroxisome proliferator-activated receptor alpha gene (PPARα): Relative gene expression by quantitative real-time polymerase chain reaction (RT-PCR) (Berger et al., 1999)**

Total RNA is purified using silica membrane-based spin columns. Chaotropic salts lyse cells and promote selective RNA binding. Impurities are washed away and RNA is eluted in nuclease-free water. Liver tissue is homogenized, lysed, passed through a spin column, treated with DNase, washed and eluted. RNA quality is confirmed by spectrophotometry. SYBR Green binds to double-stranded DNA and emits fluorescence upon excitation. During PCR amplification, fluorescence increases with the amount of DNA produced, enabling quantification. Real-time PCR is set up with cDNA, SYBR Green master mix and

primers. The reaction is run in a thermocycler with melt curve analysis. Relative expression is analyzed using ΔΔCt method.

- **Immunohistochemical examination of nuclear factor kappa-B (NF-kB) (Berger et al., 1999)**

Based on the principle of antigen-antibody interaction to examine targets one or more of its subunits (commonly p65/RelA) to detect their localization (usually nuclear vs cytoplasmic), which correlates with NF-KB activation. Also determination of NF-kB positivityby program of image J analysis.

Histopathological examination:
Pieces of the dissected livers were fixed in 10% buffered formalin for 24 hours then dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene and embedded in paraffin. Thin sections (5 µm) were cut and stained with hematoxylin and eosin (H&E) for routine light microscopic examination.

Statistical analysis:
Statistical analysis of the results was carried out depending on the following conventional standard equations:
Mean value (X) = $\sum x / n$,
where $\sum x$ = the sum of all observations and n = the number of observations. A One-way Analysis of Variance (ANOVA) was calculated when comparing more than two means, with Tukey-Kramer multiple comparison follow-up test calculation (when $p \leq 0.05$).

Results:

There was significant increase (P<0.001) of the body weight gain, fasting blood glucose, serum insulin levels in the group of induced fatty liver (GII) compared to control group (GI). On the other hand, they showed significant decrease (P<0.01) in the group of fatty liver + A.M (GIII) compared to (GII). Also, there was significant decrease (P<0.001) of them in the group of fatty liver + exercise (GIV) compared to (GII). Moreover, their levels showed significant decrease (P<0.001) in the group of fatty liver + A.M + exercise (GV) compared to (GII) although they were still higher than its level in (GI). **Table 1**

The induction of fatty liver (GII) caused significant increase (P<0.001) in the serum level of liver enzymes: (AST and ALT) compared to control group (GI). In (GIII), effect of A.M led to a significant decrease (P<0.001) of them compared to (GII). There was a significant decrease (P<0.05) in (AST and ALT) serum levels in group of fatty liver + exercise (GIV) compared to (GII). In addition, in (GV) at which both A.M + exercise were used, a significant decrease (P<0.001) of serum level of liver enzymes was detected compared to (GII) despite it was still higher than their levels in (GI). **Table 2**

By induction of fatty liver (GII), a significant increase (P<0.001) of the serum level of total cholesterol and triglycerides was found compared to control group (GI). They showed significant decrease (P<0.001) in the group of fatty liver + A.M (GIII) compared to the (GII). Furthermore, there was significant decrease (P<0.01) in their levels in (GIV) at which exercise has been done compared to (GII). The serum

level of them also decreased significantly (P<0.001) in the group of fatty livers + A.M + exercise (GV) compared to (GII) despite it being still more than its level in (GI). **Table 3**

The induction of fatty liver (GII) caused significant decrease (P<0.001) in the serum level of (HDL-C) compared to control group (GI). There was significant increase (P<0.001) in the group of fatty livers + A.M (GIII) compared to (GII). A significant increase (P<0.01) of it was found in the group of fatty liver + exercise (GIV) compared to (GII). Additionally, the serum level of (HDL-C) showed significant increase (P<0.001) in the group of fatty liver + A.M + exercise (GV) compared to (GII) but was still less than its level in (GI). **Table 3**

The serum level of (LDL-C) increased significantly (P<0.001) in the group of induced fatty liver (GII) compared to control group (GI). Interference with A.M (GIII) caused significant decrease (P<0.001) of it compared to (GII). Also, there was significant decrease (P<0.01) in the fatty liver + exercise (GIV) compared to the (GII). As well as, in (GV) at which both A.M + exercise were used, the serum level of (LDL-C) showed significant decrease (P<0.001) compared to (GII), despite it being still higher than its level in (GI).

Table 3
The hepatic level of total cholesterol and triglycerides increased significantly (P<0.001) by induction of fatty liver (GII) compared to control group (GI). They showed significant decrease (P<0.001) while using A.M as in (GIII) compared to (GII). There was significant decrease (P<0.01) in the group of fatty liver + exercise (GIV) compared to (GII). Additionally, significant decrease (P<0.001) in their levels was observed in the group of fatty livers + A.M + exercise (GV) compared to (GII), although they were still more than their levels in (GI). Table 4

In the group of induced fatty liver (GII), the hepatic level of (MDA) showed significant increase (P<0.001) compared to control group (GI). It decreased significantly (P<0.001) in the group of fatty liver + A.M (GIII) compared to (GII). Also, with exercise as in (GIV), a significant decrease (P<0.05) of it was detected compared to (GII). As well as, in the group of fatty liver + A.M + exercise (GV), the hepatic level of (MDA) showed significant decrease (P<0.001) compared to (GII) but was still higher than its level in (GI). **Table 4**

Induction of fatty liver (GII) led to significant decrease (P<0.001) in the hepatic level of (TAC) compared to control group (GI). In contrary, usage of A.M as in (GIII) caused significant increase (P<0.001) compared to (GII). There was a significant increase (P<0.05) in it by the effect of exercise as in (GIV) compared to (GII). Also, in the group of fatty liver + A.M + exercise (GV), the hepatic level of (TAC) showed significant increase (P<0.001) compared to (GII) despite it being still less than its level in (GI). **Table 4**

A significant decrease (P<0.001) in the level of hepatic gene expression of (PPAR-α) was found with induction of fatty liver in (GII) compared to control group (GI). In contrast, there was significant increase (P<0.001) of it in the group of fatty liver + A.M (GIII), compared to (GII). In (GIV), the effect of exercise produced significant increase (P<0.001) in it compared to (GII). Furthermore, significant increase

(P<0.001) in the level of hepatic gene expression of (PPAR-α) was observed in the group of fatty liver + A.M + exercise (GV) compared to (GII), despite it being still higher than its level in (GI) **Table 5**

In the group of induced fatty liver (GII), a significant increase (P<0.001) of the level of hepatic gene expression of (NF-κB) was detected compared to control group (GI). Injection of A.M in (GIII) produced a significant decrease (P<0.01) of it compared to (GII). Also, there was significant decrease (P<0.001) of the level of hepatic gene expression of (NF-κB) in the group of fatty liver + exercise (GIV) compared to (GII). In (GV) combination of both A.M + exercise resulted in significant decrease (P<0.001) in the level of hepatic gene expression of (NF-κB) compared to (GII), although it was still higher than its level in (GI). **Table 5**

Results of histopathological examination:

Control group :Liver sections of all animals in this group showed normal tissue architecture regarding inter-anastomosing network of hepatocytes arranged in single-cell thick plates separated from each other by vascular sinusoids. The liver is composed of lobules, each of which is a hexagonal structure consisting of a central vein surrounded by radiating hepatocyte plates. A functional unit defines an acinus in relation to terminal portal branches and terminal hepatic venules. Portal tracts surround the classical lobules, no apoptotic figures present. **Figure 1-A**

Group of induced fatty liver: Starting from 4 weeks of the HFHCD, the liver sections in all animals of this group showed an increase in lipid accumulation and pronounced steatosis in the liver, which was characterized by prominent deposition of lipid droplets in cytoplasm. The liver lobules of the control group were distinct, accompanied by cytological ballooning and mild to moderate lobular inflammatory cell infiltration. The degree of hepatic injury includes steatosis, degenerative changes, cytological ballooning and apoptosis of hepatocytes with infiltration of inflammatory cells in parenchyma. **Figure 1-B**

Group of fatty liver + AM: Hepatic sections in all animals of this group showed less marked degenerative signs with less accumulation of fat droplets, but there was still congestion of the central veins and inflammatory cell infiltration. **Figure 1-C**

Group of fatty liver + Exercise: Liver sections in all animals of this group showed near normal hepatocytes and no steatotic changes were detected. There were dilated sinusoids with lobular inflammatory infiltrate. **Figure 1-D**

Group of fatty liver + AM + Exercise: Hepatic sections in all animals of this group showed extremely lower histological changes when compared to that in the group of induced fatty liver. There was normal liver architecture with normal (clear) central veins and blood sinusoids in most of the hepatic lobules, but also mild parenchymal inflammatory cellular infiltrate was detected. Most of the hepatocytes appeared with normal colored eosinophilic cytoplasm and vesicular basophilic nuclei. **Figure 1-E**

Immunohistochemical examination of NF-κB:

Buffy yellow-stained NF-κB protein, located in the cytoplasm and/or the nucleus, identified NF-κB positive cells.
Figure 2-A displayed normal immunohistochemical expression of NF-κB in liver tissue of the control group. While **Figure 2-B** revealed prominent increase of NF-κB expression in hepatic tissue of the group of induced fatty liver.
The immunohistochemical expression of NF-κB showed reduction in hepatic tissue of all other groups: group of fatty liver + A.M, group of fatty liver + exercise and group of fatty liver + A.M + exercise (Highest degree of reduction) **Figures 2 (C, D, E).**

Discussion

Over the past decade, the global prevalence of Metabolic Non-Alcoholic Fatty Liver Disease (M-NAFLD) has markedly increased (Younossi et al., 2023), yet to date, limited pharmacological trials of its treatment exist (Ipsen et al., 2018). The pathogenesis of NAFLD is closely related to metabolic syndrome and insulin resistance; as NAFLD is thought to be the hepatic manifestation of metabolic syndrome, also it is presumed to be a polygenic genetic background to this disease (Anstee et al., 2013).

AM is increased during hepatic injury to counteract the effect of oxidative stress and apoptosis as it can act as both a hormone and a cytokine "hormokine" (Schuetz et al., 2015). Many studies reported that AM administration leads to the attenuation of oxidative stress in the liver (Yoshimoto and Hirata, 2005, Yildirim and Yurekli, 2010, MY Cheung and Tang, 2012).

In this study, the primary novel findings were that the exogenous AM application significantly attenuated the high-fat diet (HFD) induced inflammation and oxidative stress which were partially associated with the receptor-mediated AMP activated protein kinase (AMPK) pathway (Qian et al., 2022). The results about antioxidant effect of AM on the liver in this work are in agreement with other studies on different organs, through increasing the total antioxidant capacity (TAC) of the hepatic tissue including reduced glutathione (GSH), glutathione perioxidase (GPx) and superoxide dismutase (SOD) and through suppressing the expression of NADPH oxidase (Yoshimoto and Hirata, 2005). Strong evidence supports the hypothesis that AM possesses significant protective properties against end organ damage through its role by inhibiting oxidative stress (Abd-Ellatif et al., 2022).

In addition, AM was reported to be attributed to the prevention of some chronic diseases as D.M and CVDs (Kita and Kitamura, 2022), because it can participate in the regulation of many physiological functions such as vasodilation, angiogenesis, organ protection and tissue repair (Wang et al., 2023, Sacco et al., 2024).

The molecular mechanisms behind the protective action of AM in the liver are inhibition of the oxidative stress biomarkers and augmentation of the antioxidant

system. That was confirmed by the significant decrease in MDA and the significant increase in TAC in hepatic tissue (Kim et al., 2024).

A rat model of HFHCD had been reported that administration of AM could prevent the progression of NAFL in the rats, thus decreasing the injury of the liver. The endogenous antioxidant potential of AM could enhance the function of the mitochondria and antioxidant enzymes activity to decrease the apoptosis and oxidative stress; thus protects the liver against NASH development (Ye and Liu, 2022).

The results of the present study have shown that AM administration for 8 weeks was capable of producing alterations in liver functions; it was observed that AM injection as in both groups (GIII) and (GV) protected against hepatic function deterioration, as shown by improvement of them on comparison with (GII). Pintér et al. (Pintér et al., 2014) postulated that AM induces the downregulation of inflammatory cytokines in cultured cells. Current results in groups (GIII) and (GV) showed the attenuation of oxidative stress (decreased MDA and NOx) by AM, while greater increase in the cytokines was observed in the (GII) than in (GIII) and (GV).

Although, the precise mechanisms by which AM regulates redox systems have not yet been clarified, it can support the degradation of free radicals by activation of scavenger systems including non-enzymatic antioxidants (e.g., GSH) as well as enzymatic activities (e.g. SOD, catalase (CAT) and GSH-Px) (Oba et al., 2008). Kim et al. (Kim et al., 2024) have shown that AM elevated cellular GSH levels via an up-regulation of its rate-limiting synthetic enzyme (γ-glutamate-cysteine ligase) (γGCL). Besides, AM suppresses ROS production through different signaling pathways such as the activation of the (cAMP-protein kinase A) pathway in mesangial cells and the inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase via the nitric oxide-cGMP signaling pathway (Passaglia et al., 2014).

Histopathological examination of the liver of rats in (GIII) that injected by AM confirmed biochemical improvement in this study, as there was moderate hydropic degeneration of hepatocytes, portal inflammatory cells infiltration and slight cytoplasmic vacuolization of focal hepatocytes with some regenerative changes such as pyknotic nuclei and multinucleated cells. Exercise induces low amounts of ROS acutely, which positively stimulates oxidative damage-repairing enzymes and improves biological fitness (Golbidi et al., 2012).

Skeletal muscle as an endocrine organ which secretes cytokines and myokines on their contraction to communicate with liver and adipose tissues; and they are involved in an anti-inflammatory response (Catoire and Kersten, 2015). Besides, studies on animal models suggest exercise impacts liver mitochondrial function and can influence inflammation through up-regulation of antioxidant enzymes and anti-inflammatory markers (Farzanegi et al., 2019).

Furthermore, the oxidation of sulfhydryl groups of cytochrome and depletion of GSH occur; causing inadequate detoxification mechanism and accumulation of

MDA within hepatocytes causing increased permeability of mitochondrial membrane and cell death (Mustafa et al., 2013).

Recommendations were to be better understanding of AM physiology and determining the involved mechanisms contributing to metabolic control. Providing more relations between AM and the expression of PPAR-α gene that gives further promise for the continued investigation of this peptide in the therapeutic context of obesity and metabolic dysfunction.

Conclusions

The study highlights that AM has a significant protective role against FLD by exerting anti-inflammatory, reducing oxidative stress and lowering liver enzyme markers and blood levels of lipids caused by a high-fat diet. Moreover, regular physical exercise is crucial for promoting energy production, enhancing glucose metabolism, eliminating excess body fat, improving insulin sensitivity and reducing the risks of severe obesity. Combining daily exercise with AM offers enhanced protection against FLD and its complications in obese patients.

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Table 1: The body weight gain, level of fasting blood glucose and insulin

	Control (GI)	Induction of Fatty Liver (FL) (GII)	(Fatty Liver + Adrenomedullin) (FL + AM) (GIII)	FL + Exercise (GIV)	FL + AM + Exercise (GV)
Body weight gain (g)					
	124.1±16	**358.9±20.4**	**335.2±14.2**	**195.1±10.6**	**138.9±10.8**
F Test	**548.64**				
P Value					
GII Vs. GI	**GV Vs. GI**	**GIII Vs. GII**	**GIV Vs. GII**	**GV Vs. GII**	
<0.001*	**>0.05**	**<0.01***	**<0.001***	**<0.001***	
Level of Fasting Blood Glucose (FBG) (mg/dl)					
	151.4	**140.9**	**105.7**	**103.1**	
F Test	**125.5**				
P Value					
GII Vs. GI	**GV Vs. GI**	**GIII Vs. GII**	**GIV Vs. GII**	**GV Vs. GII**	
<0.001*	**>0.05**	**<0.05***	**<0.001***	**<0.001***	
Insulin (U/ml)					
	12.4±1.4	**27.9±0.9**	**26±1.1**	**18.9±1.1**	
F Test	**243**				
P Value					
GII Vs. GI	**GV Vs. GI**	**GIII Vs. GII**	**GIV Vs. GII**	**GV Vs. GII**	
<0.001*	**<0.001***	**<0.05***	**<0.001***	**<0.001***	

Data were presented as mean SD.* significant p value <0.05

Table 2: Serum level of Aspartate Aminotransferase (AST) and serum level of Alanine Aminotransferase (ALT)

	Control (GI)	**Induction of Fatty Liver (FL) (GII)**	**(Fatty Liver + Adrenomedullin) (FL + AM) (GIII)**	**FL + Exercise (GIV)**	**FL + AM + Exercise (GV)**
Serum level of Aspartate Aaminotransferase (AST) (U/ml)					
	122.9±4.5	232.7±15.3	152.6±9.4	214.8±13.2	122.9±4.5
F Test	223.99				
P Value					
GII Vs. GI	**GV Vs. GI**	**GIII Vs. GII**	**GIV Vs. GII**	**GV Vs. GII**	
<0.001*	>0.05	<0.001*	<0.01*	<0.001*	
Serum level of Alanine Aminotransferase (ALT) (U/ml)					
	30.1±3.4	104.9±10	63.4±3.8	94.9±7.6	
F Test	312.64				
P Value					
GII Vs. GI	**GV Vs. GI**	**GIII Vs. GII**	**GIV Vs. GII**	**GV Vs. GII**	
<0.001*	>0.05	<0.001*	<0.01*	<0.001*	

Data were presented as mean SD.* significant p value <0.05.

Table 3: Total Cholesterol (TC) (mg/dl), triglycerides (TGs) (mg/dl), high density lipoproteins cholesterol (HDL-C) (mg/dl) and low-density lipoproteins cholesterol (LDL-C) (mg/dl) in all studied groups

	Control (GI)	**Induction of Fatty Liver (FL) (GII)**	**(Fatty Liver + Adrenomedullin) (FL + AM) (GIII)**	**FL + Exercise (GIV)**	**FL + AM + Exercise (GV)**
Total Cholesterol (TC) (mg/dl)					
	167.7±26.4	267.8 ±21.2	176.6±14	229.9±6.3	167.7±26.4
F Test	47.971				
GII Vs. GI	**GV Vs. GI**	**GIII Vs. GII**	**GIV Vs. GII**	**GV Vs. GII**	**GII Vs. GI**
<0.001*	**>0.05**	**<0.001***	**<0.01***	**<0.001***	**<0.001***
Triglycerides (TGs) (mg/dl)					
	85±5		**192.9±12**	**165.2±7.5**	**177±6.9**
F Test	**233.52**				
P Value					
GII Vs. GI	**GV Vs. GI**		**GIII Vs. GII**	**GIV Vs. GII**	**GV Vs. GII**
<0.001*	**>0.05**		**<0.001***	**<0.05***	**<0.001***
High Density Lipoproteins Cholesterol (HDL-C) (mg/dl)					
	53.4±3.5		**40.7±4.2**	**47.5±2.7**	**43.5±3**
F Test	**45.346**				
GII Vs. GI	**GV Vs. GI**			**GIV Vs. GII**	**GV Vs. GII**
<0.001*	**>0.05**		**<0.001***	**<0.01***	**<0.001***
Low Density Lipoproteins Cholesterol (LDL-C) (mg/dl)					
	91.3±10.7		**181±9.2**	**153.2±6.5**	**165.5±9.6**
F Test	**190.79**				
P Value					
GII Vs. GI	**GV Vs. GI**		**GIII Vs. GII**	**GIV Vs. GII**	**GV Vs. GII**
<0.001*	**>0.05**		**<0.001***	**<0.01***	**<0.001***

Data were presented as mean ± SD.* significant p value <0.05.

Table 4: Hepatic level of cholesterol (mg/g), triglycerides (mg/g), MDA (nmol/g tissue) and TAC (mmol/g tissue) in all studied groups

	Control (GI)	**Induction of Fatty Liver (FL) (GII)**	**(Fatty Liver + Adrenomedullin) (FL + AM) (GIII)**	**FL + Exercise (GIV)**	**FL + AM + Exercise (GV)**
Hepatic level of total cholesterol (mg/g)					
	32.2±1		42.5±1.9	36.6±2.3	38.7±2.6
F Test	**37.538**				
P Value					
GII Vs. GI	**GV Vs. GI**		**GIII Vs. GII**	**GIV Vs. GII**	**GV Vs. GII**
<0.001*	>0.05		**<0.001***	**<0.01***	**<0.001***
Hepatic level of triglycerides (mg/g)					
	19.9±3.2		51.2±3.6	40.2±2.8	46.1±3.2
F Test	**187.46**				
GII Vs. GI	**GV Vs. GI**		**GIII Vs. GII**	**GIV Vs. GII**	**GV Vs. GII**
<0.001*	**>0.05**		**<0.001***	**>0.05**	**<0.001***
MDA (nmol/g tissue)					
	3.5±0.2	9.1±0.5	5.1±0.1	8.6±0.4	3.8±0.4
F Test	**589.67**				
P Value					
GII Vs. GI	**GV Vs. GI**		**GIII Vs. GII**	**GIV Vs. GII**	**GV Vs. GII**
<0.001*	**>0.05**		**<0.001***	**<0.05***	**<0.001***
TAC (mmol/g tissue)					
	40.8±1		29±2.4	33.6±1.7	31.4±1.4
F Test	**40.8**				
P Value					
GII Vs. GI	**GV Vs. GI**		**GIII Vs. GII**	**GIV Vs. GII**	**GV Vs. GII**
<0.001*	**>0.05**		**<0.001***	**<0.05***	**<0.001***

Data were presented as mean SD.* significant p value <0.05.

Table 5: Hepatic level of PPARα (%) and NF-κB expression (%) in all studied groups

	Control (GI)	**Induction of Fatty Liver (FL) (GII)**	**(Fatty Liver + Adrenomedullin) (FL + AM) (GIII)**	**FL + Exercise (GIV)**	**FL + AM + Exercise (GV)**
PPARα (%)					
	1.34±0.07	0.84±0.04	1.17±0.06	1.21±0.08	1.31±0.02
F test	248.01				
P Value					
GII Vs. GI	**GV Vs. GI**		**GIII Vs. GII**	**GIV Vs. GII**	**GV Vs. GII**
<0.001*	>0.05		**<0.001***	**<0.001***	**<0.001***
Hepatic level of NF-κB expression (%)					
	±0.4 4.1	44.7±2.3	41.8±2.1	28.2±2.4	5.6±0.9
F Test	1132.2				
P Value					
GII Vs. GI	**GV Vs. GI**		**GIII Vs. GII**	**GIV Vs. GII**	**GV Vs. GII**
<0.001*	>0.05		**<0.01***	**<0.001***	**<0.001***

Data were presented as mean SD.* significant p value <0.05.

Figure legends:

Figure 1: A) Control group; showing normal hepatocytes arranged in plates of one or two cell thickness, no evidence of steatosis, (H&E 400), B) Group of induced fatty liver; showing marked steatosis with intracellular fat accumulation causing clearing of cytoplasm with central small uniform nuclei, (H&E 200), C) Group of fatty liver + AM; showing hepatocytes with mild fatty changes in the cytoplasm with parenchymal inflammatory cellular infiltrate and congested central veins and blood sinusoids, (H&E 200), D) Group of fatty liver + Exercise; showing dilated sinusoids with lobular inflammatory infiltrate, No steatotic changes could be detected, (H&E 200), E) Group of fatty liver + AM + Exercise; showing clear central vein with normal appearance of hepatocytes, (H&E 400)

Figure 2: A) Control group; showed normal immunohistochemical expression of NF-κB in hepatic tissue, (magnification × 400), B) Group of induced fatty liver; showed prominent increase of immunohistochemical expression of NF-κB in hepatic tissue, (magnification × 200), C) Group of FL + AM; showed slight reduction of immunohistochemical expression of NF-κB in hepatic tissue, (magnification × 400), D) Group of FL + Exercise; showed reduction of immunohistochemical expression of NF-κB in hepatic tissue, (magnification × 200), E) Group of FL + AM + Exercise; showed prominent reduction of immunohistochemical expression of NF-κB in hepatic tissue, (magnification × 400)