Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 1, 2024, pp. 345-362.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

BULL. PHARM. SCI. Assiut Univ.

ANTI-OBESITY ACTION OF GREEN TEA EXTRACT AND CURCUMIN: ROLE OF C1Q/TNF-RELATED PROTEIN-12 (CTRP-12) AND CASPASE-2

Mohamed A. F. Hussein[1], Manal A. M. Mandour[2], SaryKh. AbdElghaffar[3], Abdel-Raheim Mohamed A Meki[2,4], Michel E. Fakhry[2*]

[1]*Department of Biochemistry, Faculty of Pharmacy, Assiut University, PO Box 71526, Assiut, Egypt*
[2]*Department of Medical Biochemistry, Faculty of Medicine, Assiut University, PO Box 71526, Assiut, Egypt*
[3]*Department of Pathology, Faculty of Veterinary Medicine, Assiut University, PO Box 71526,Assiut, Egypt*
[4]*Department of Biochemistry, Faculty of Pharmacy, Sphinx University, PO Box 71515, New Assiut, Egypt*

Background: *The prevalence of obesity is increasing. It is regarded to be among of the world's biggest health issues and the primary factor in metabolic syndrome, a group of illnesses including hypertension, diabetes mellitus, and dyslipidemia. Many studies proved the efficacy of dietary polyphenols ascurcuminand green tea extractin ameliorating obesity and its correlated disorders.* ***Methods:*** *Our work was the first to explore the impact of green tea extract and curcumin on the insulin-sensitizing anti-inflammatory adipokine C1q/ TNF-related protein-12 (CTRP-12)serum level measured by ELISA and expression of caspase-2 in white adipose tissue invivo measured by qRT-PCR.* ***Results:*** *We found that both green tea extract and curcumin elevated serum CTRP-12 level. Moreover, they stimulated adipocytes apoptosis via increasing the expression of caspase-2 decreasing adiposity.* ***Conclusion:*** *The current study concludes that green tea extract and curcumin have prophylactic effect against obesity and obesity-related disorders*

Keywords: *high-fat diet, caspase-2, CTRP-12, green tea extract, curcumin*

INTRODUCTION

The prevalence of obesity is escalating. Four million deaths globally are due to increased adiposity. Most of these deaths were due to cardiovascular diseases[1]. Obesity is associated with many complications as impaired glucose tolerance, high blood pressure, diabetes mellitus, non-alcoholic fatty liverdisease (NAFLD), dyslipidemia, metabolic syndrome, cerebrovascular diseases, sleep apnea, gastroesophageal reflux disease, polycystic ovary syndrome, and osteoarthritis[2].

White adipose tissue (WAT) is an endocrine organ that produces many adipokines, including leptin, adiponectin, chemerin, apelin, visfatin, and C1q/ TNF-related protein-12 (CTRP-12), which have many roles in health and disease[3]. CTRP-12, also called adipolin, is an insulin-sensitizing and anti-inflammatory adipokine. Also called Family 132 Member A with Sequence Similarity (FAM132A)[4]. It belongs to the 16 member CTRP family, which also includes adiponectin. A signal peptide at the N terminus, a collagenous domain, a brief variable region, and a C-terminal globular domain that is homologous to complement components 1q (C1q) are the 4 domains present in proteins belonging to this family.[5]. Systemic

Received : 17/7/2023 & Accepted : 25/12/2023

*Corresponding author: Michel E. Fakhry, E-mail: mikelfakhry@aun.edu.eg

administration of recombinant CTRP-12 in obese rats ameliorated the insulin resistance and decreased the expression of the proinflammatory cytokines[6].

Caspase-2 is a cysteine-aspartic protease that is concerned in cell death (initiator) and many other procedures as oxidative stress response[7]. Mice lacking Caspase-2 were shielded against obesity caused by diets and its complications.[8] Furthermore, Mice lacking Caspase-2 were vulnerable to oxidative stress[9].

Green tea extract (GTE) is considered a substantial supply of polyphenols as catechins and flavonoids. It is prepared from *Cameliasinesis* leaves and buds[10]. Curcumin, a yellow-colored polyphenol, is extracted from turmeric *Curcuma longa* rhizome[11]. GTEand curcumin protect against obesity and ameliorate obesity-related disorders[12]. Their beneficial effects on obesity resides in the fact that they have strong antioxidant and anti-inflammatory properties[13]. A better understanding of their mechanisms will lead to establish strategies for obesity management and avoidance. Our study aimed to explore the effects of GTEand curcumin as anti-obesity agents.

MATERIAL AND METHODS

Experimental animals

The work was conducted on 60 maleSprague Dawley rats weighing about 110-130g. The animals were kept on a natural light-dark cycle, at a temperature of 25°C and a relative moisture level of 60–70%, with unlimited access to water and food. The rats were cared for and treated in accordance with the National Institutes of Health's protocol and the rules of the Animal House at Assiut University. The Assiut University Faculty of Medicine's ethics committee gave its approval to that research (IRB local approval number: 04-2023-300177).

Creating meals using an experimental approach and adding natural supplements

Following 2 weeks of acclimatization, the sixty animals were assigned and split into six groups at random. Each group had 10 animals at the beginning of the experiment. During the study, 15 rats died, and the experiment proceeded with 45 rats. The work continued for 8 weeks. First group was the control group (n = 7) which received the basal diet (ad libitum). The second group was carboxymethyl cellulose (CMC) group (n = 7) which received 2% CMC (vehicle of curcumin) in addition to basal diet. It was the second control group. The third group was the high-fat diet (HFD) group (n = 10) which received HFD. The fourth group was GTE group (n=7) which received HFD in addition to GTE instead of drinking water[15]. The fifth group was curcumin group (n = 7) which received HFD in addition to curcumin administered orally (80 mg/Kg body weight/day)[16]. The sixth group was mixture (Mix) group (n = 7) which received HFD in addition to GTE and curcumin (80 mg/Kg body weight/day) **(Fig. 1).**

HFD was prepared as described by Ragab et al.[14], **Table 1**. GTE was prepared by infusion of the commercial green tea (*Camelliasenesis*)[15]. Curcumin was prepared in the form of a suspension in 2% CMC and administered orally by gastric gavage in a dosage of 80 mg/Kg/day[16]. Curcumin was purchased from Dop, Turkey. Every week, the weight of rats was recorded to adjust the dose. Additionally, Lee index was also calculated weekly. If Lee index is greater than 300, the rat is considered obese[17].

Table 1: Composition of Diet.

Ingredient	Amount
Basal diet	550 g/Kg
Beef tallow	250 g/Kg
Roasted peanuts	50 g/Kg
Milk powder	50 g/Kg
Egg	50 g/Kg
Sesame oil	30 g/Kg
Sodium chloride	20 g/Kg

Amount of all ingredients of thehigh fat diet as described by Ragab et al.[14].

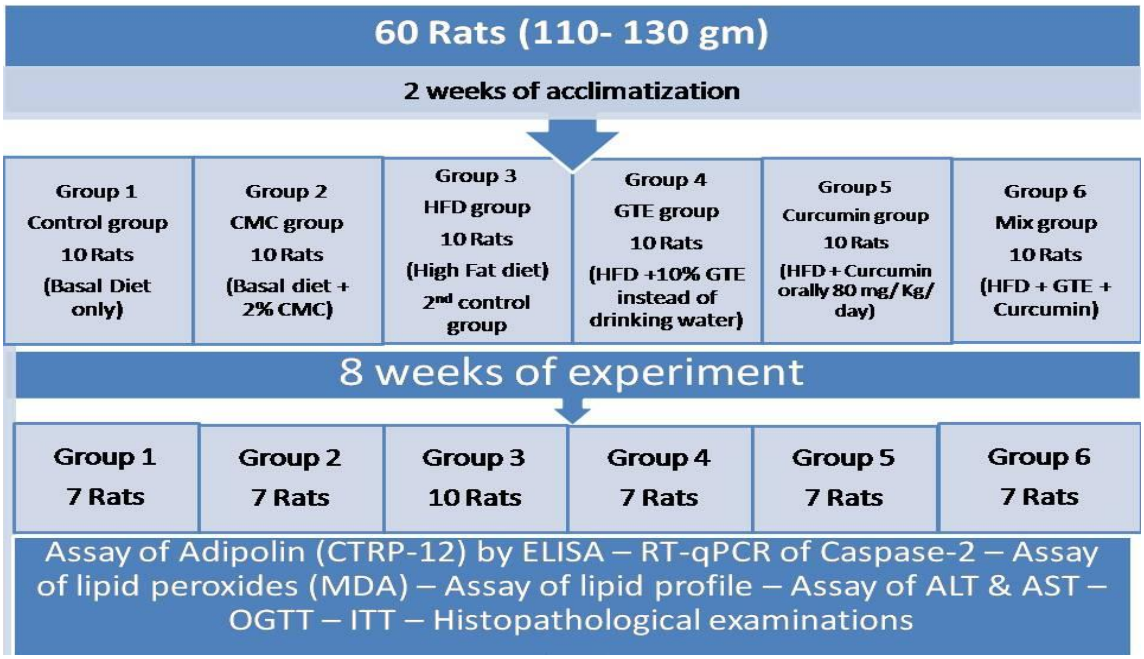

Fig.1: Summary of the study groups (numbers, doses, assessments).

Gathering and preparing of samples

Samples of fasting blood had been drawn from the rat's retro-orbital sinus, then serum was separated. Each sample was aliquoted into Eppendorf tubes and kept at -20°C. Epididymal fat had been collected in Eppendorf tubes which kept promptly in liquid nitrogen (snap freezing) to avoid the degradation of RNA and then kept at -80°C for gene expression analyses. Liver, visceral fat, and aorta were collected in 10 percent neutral-buffered formalin to be used for histopathology.

Determination of serum CTRP-12

Serum CTRP-12 was determined using ELISA kit (Cloud Clone Corporation, USA, Catalog No. R2050).

Determination of gene expression of caspase-2

Utilizing the Direct_zol™ RNA Miniprep kit (Zymo Research, USA, Catalog No. R2050), RNA has been extracted. The cDNA specimens required for qPCR were obtained using the cDNA synthesizing kit (High-Capacity cDNA Reverse Transcription Kit, Applied Biosystems, USA, Catalog No. 4368814) in accordance with the manufacturer's instructions. The PrimerQuest tool from Integral DNA Technologies® (Illinois, USA) was used to create the primers and were obtained from Thermo Scientific (Thermo, US). Caspase-2 gene primers were 5'-CTCTCCTTCCTTACTGTGGTTTC-3'(forward) and 5'-GCTGCTTGTCTCCTTCCTTTA-3'(reverse). While β-actin (reference gene) primers were5'-AGCCATGTACGTAGCCAT-3'(forward) and 5'-CTCTCAGCTGTGGTGGTGAA-3'(reverse).Master mix (Thermo, US) was prepared by adding the following components for each 20 µl reaction:template DNA, 10 µl SYBR Green qPCR Master Mix (2X), 1 µlforward Primer, 1 µlreverse Primer, and water nuclease-free. The thermal cycling apparatus (Application of Biosystems 7500 Fast Real-Time PCR System, United States of America, Catalog No. 4351104) has been configured to run at 95 °C for two mins, subsequently 40 cycles at 95 °C for twenty-five seconds, afterwards at 60 °C for a minute. At the conclusion of the responses, measurements are made to determine the variance among the reference and target Ct values for every single specimen. This variant of the Livak technique (ΔΔCt) is used to analyze the outcomes of the real-time PCR reaction.[18]

intra-peritoneal insulin tolerance test (IPITT)andOral glucose tolerance test (OGTT)

These experiments were performed during the last week of the expirement after 12 hrs of fasting and started with assessing blood glucose concentrations from an incision at the

tail' tip (time 0) using Smart Test™ glucometer (OK Biotech, Taiwan, Catalog No. 1062-2962-0003).

For OGTT, subsequently after determination of glucose concentration at time 0, a 20% glucose solution (2g/ kg body weight) was adminstered to the rats via a polyethylene gastric tue. Glucose concentrations were determined at 30, 60, 90 and 120 minutes following glucose adminstration. The results were analyzed by calculation the area under curve (AUC) of glucose tolerance test using the trapezoid method[19].

For IPITT, subsequently after determination of glucose concentration at time 0, insulin was injected intra-peritoneally by a dose of 1 IU/kg body weight. After that, glucose concentrations were determined at 30, 60, 90 and 120 minutes following glucose adminstration. The results were analyzed by calculation the area under curve (AUC) of glucose tolerance test using the trapezoid method[20].

Biochemical assays

Serum malondialdehyde (MDA) was estimated based on the fact that it forms 1:2 adduct with thiobarbituric acid which was measured colorimetrically at 532 nm[21]. Serum level of triglycerides was done using triglyceride kit (Diamond diagnostics, Egypt, Catalog No. 265). Serum level of total cholesterol was determined by using cholesterol kit (Spinreact, Spain, Catalog No. 41022). Utilizing an HDL-cholesterol assay (QCA, Spain, Catalog No. 993885), the amount of HDL-cholesterol in the blood was measured. Utilizing the Friedewald formula, the blood concentration of low density lipoprotein-cholesterol (LDL-cholesterol) was determined.[22]. Serum activity of alanine aminotransferase was determined using Spectrum ALT™ kit (Spectrum Diagnostics, Egypt, Catalog No. 264002). Serum activity of aspartate transaminase (AST) was determined using Spectrum Diagnostics AST™ kit (Spectrum Diagnostics, Egypt, Catalog No. 260002).

Histopathological examinations

To diagnose histopathological alterations, liver, visceral adipose tissue, and, the aorta were fixated in a 10% neutral-buffering formalin solution, buried in paraffin wax, sliced into 5 m-thick sections, and prepared for staining with hematoxylin and eosin (H&E) stains.[23]

Statistical analysis

The program GraphPad Prism 5 was used to conduct the statistical analyses. The information was shown as mean ± standard deviation. One-way ANOVA was used to examine substantial group variations, and Tukey's test was used to compare groups. Statistics are deemed significant when $P < 0.05$ is established.

RESULTS AND DISCUSSION

Results

Effect of HFD, GTE, and Cur on serum CTRP-12 level

Serum levels of CTRP-12 were substantially reduced in rats received HFD in contrast to control groups. Moreover, the rats received GTE and Cur separately or together showed a significant elevation in mean serum CTRP-12 in comparison with HFD group, but only the Mix treatment was statistically significant with control groups. It is worth to note that no statistically substantial variations existed in serum levels of CTRP-12 among control groups or between GTE and Cur groups **(Fig. 2A)**.

HFD, GTE, and Curresulted in elevation in caspase-2 gene expression

HFD group showed insignificant increase in caspase-2 relative expression of genes when contrasted to control groups. GTE, Cur, and Mix treatments led to a substantial rise in caspase-2 relative gene expression in comparison with control, CMC, and HFD groups. No statistically substantial variation existed among GTE and Cur groups. Mix group revealed a substantial rise in caspase-2 relative gene expression when contrasted with all other studied groups **(Fig. 2B)**.

Effect of HFD, GTE, and Cur on OGTT, fasting blood glucose level, and IPITT

Serum glucose levels were significantly higher in HFD group than control groups. The GTE, Cur, and Mix treated groups showed substantial decrease in serum glucose levels in

contrast to HFD group. The serum levels of glucose in GTE group did not show significant change but Cur group showed significant elevation in contrast to control groups. The Mix group demonstratedsubstantial reduction in serum glucose levels in comparison with Cur group. No substantial variation existed among GTE and Cur groups in serum glucose levels (**Fig. 3A).Fig. 3B and C** show OGTT and IPITT. They reveal impaired glucose and insulin tolerance in HFD group. On the other hand, Mix group showed the best glucose and insulin tolerance, then GTE, then Cur group.

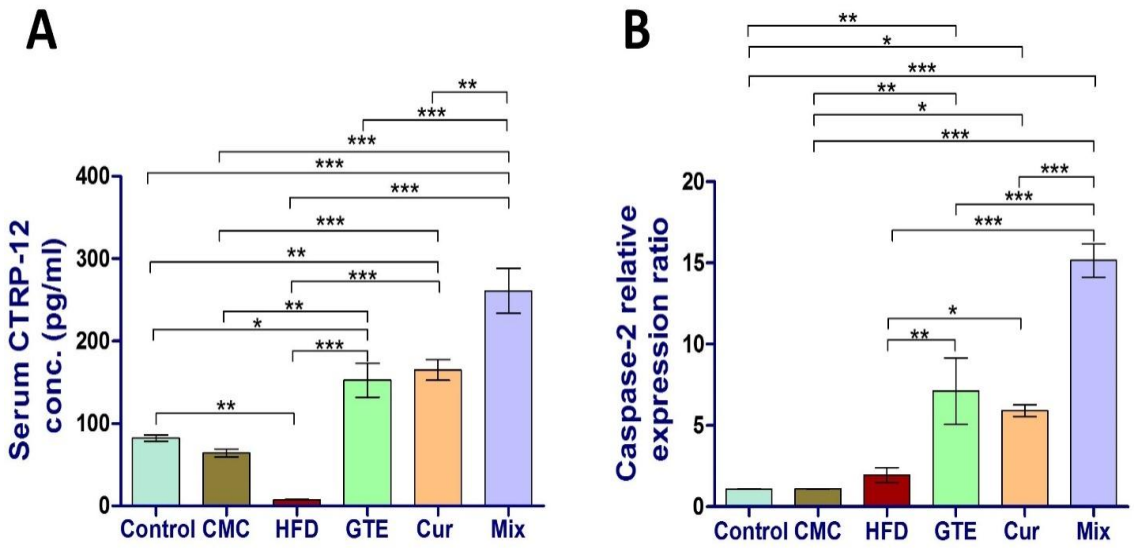

Fig. 2: Effect of HFD, GTE, and Cur on serum CTRP-12 level (A) and caspase-2 level relative gene expression ratio (B). Data represent mean ± S.E. ** *P*< 0.01, *** *P*< 0.001. (ANOVA with Tukey test for multiple comparisons).

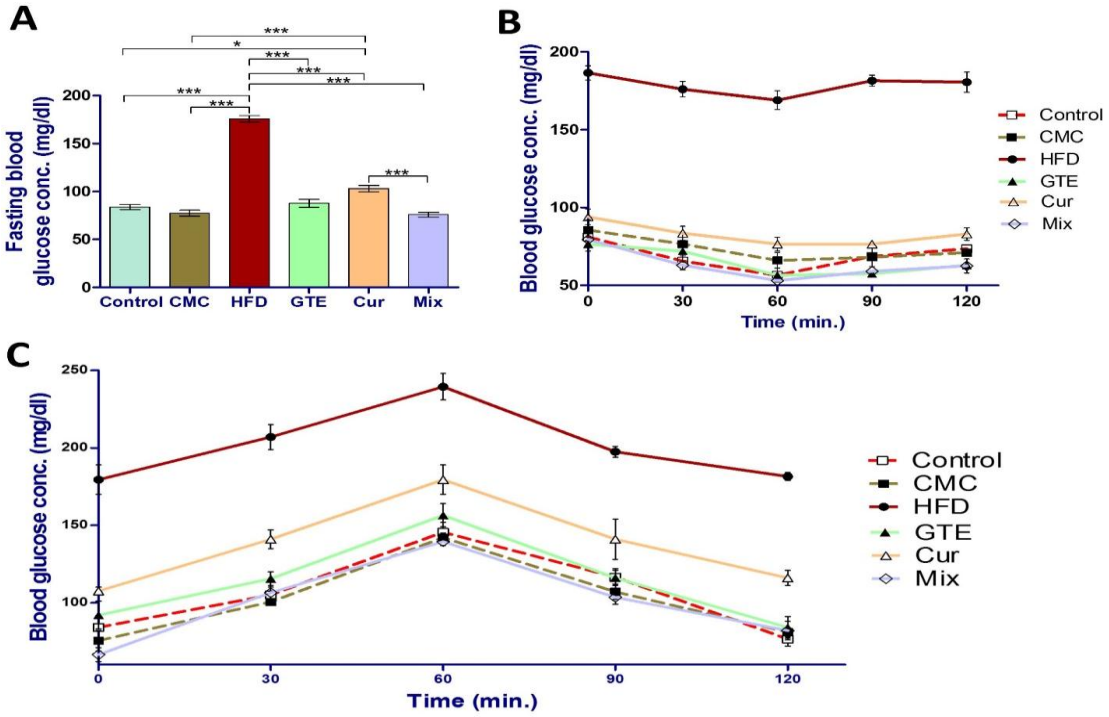

Fig. 3: Effect of HFD, GTE, and Cur on fasting blood glucose level (A), intra-peritoneal insulin tolerance test (B), and oral glucose tolerance test (C). Data represent mean ± S.E. * *p*< 0.05, *** *p*< 0.001. (ANOVA with Tukey test for multiple comparisons).

Effect of HFD, GTE, and Cur on body weight and Lee index

Group of HFD showed statistically significant increase in mean weight of body in contrast to control groups. All treated-groups demonstrated a significant reduction in mean weight of body in contrast to HFD group. Moreover, all treated groups (GTE, Cur, and Mix) showed significant elevation in weight of body in contrast to groups of control **(Fig. 4A)**. No statistical substantial variation existed among GTE and Cur groups. HFD treatment resulted in a statistically substantial rise in Lee index comparing with control groups. GTE, Cur, and Mix treatments led to a statistically substantial reduction in Lee index in comparison with HFD group. Cur and Mix therapy led to a statistically substantial difference in Lee index in comparison with control groups **(Fig. 4B)**. **(Fig. 4C)**. shows the gradual change of the mean body weight throughout the experiment in different studied groups.

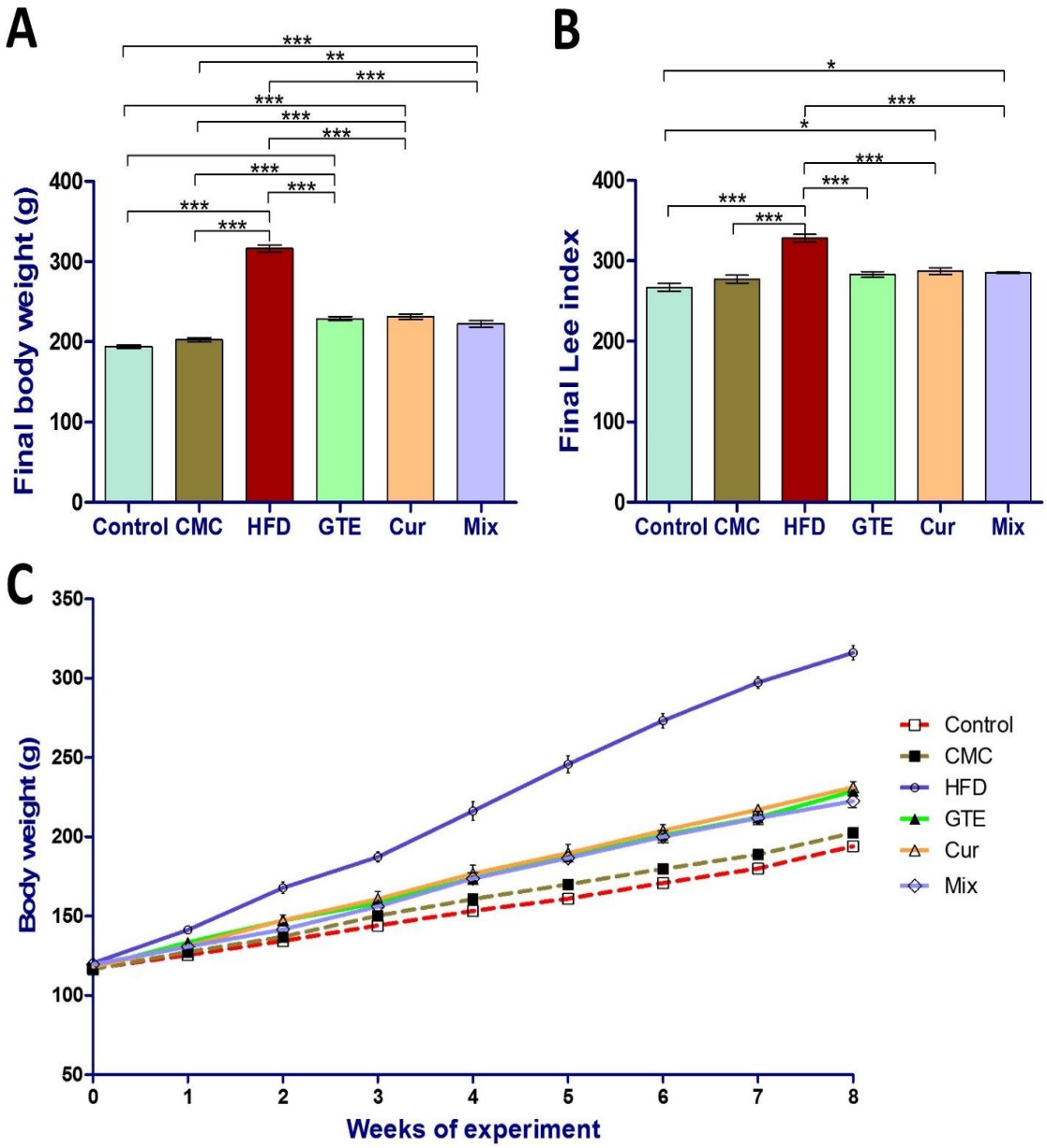

Fig. 4: Effect of HFD, GTE, and Cur on final body weight (A), final Lee index (B), and body weight over the course of experiment. Data represent mean ± S.E. * $p< 0.05$, ** $p< 0.01$, *** $p< 0.001$. (ANOVA with Tukey test for multiple comparisons)**.**

Ameliorating effect of GTE and Cur on liver enzymes, lipid peroxidation, and lipid profile

In comparison to control groups, the HFD group had greater serum ALT and AST activity. The liver enzyme activities of the GTE, Cur, and Mix groups were significantly lower than those of the HFD group. There was no statistically substantial variation between the GTE and Cur groups, showing that their effects on liver enzymes were equivalent. **(Fig. 5A).**

Ameliorating effect of GTE and Cur on liver

Serum triglycerides levels, LDL and total cholesterol were substantially greater in HFD group, but HDL levels were significantly lower than control groups. The serum triglycerides levels, LDL and total cholesterol in all groups received treatments showed a substantial decrease in contrast to HFD group. The serum levels of HDL in GTE and Cur group showed significant increase in mean serum HDL in contrast to HFD group. Mix group showed a statistically substantial increase in mean serum HDL in comparison with HFD, GTE, and Cur groups. Mix group showed a statistically significant alteration in mean serum HDL in contrast to control groups. There were insignificant differences between GTE and Cur groups in all studied serum lipid parameters **(Fig. 5B and C)**.

Serum MDA levels were greater in group received HFD than control groups. The groups received both GTE and Cur separately or together showed significant reduction in mean serum MDA in comparison with HFD group. No statistical variation existed among GTE and Cur groups in decreasing mean serum MDA level **(Fig. 5D).**

Histopathological findings and the HFD diet's effects

Liver examination from animals of control group demonstrated normal appearance of hepatocyte, central vein, and portal areas **(Fig. 6A).** HFD resulted in diffuse fatty degeneration in liver tissue and formation of different degrees of microvesicular and marcovesicularsteatosis in H&E stained-sections **(Fig. 6B – E)** when compared with control groups. Some cases showed local aggregation of mononuclear cells **(Fig. 6B)**, other showed vascular thrombosis **(Fig. 6D)**. On the other hand, GTE and Cur treatment protected rats from fatty liver. Liver sections were more or less similar to that of control groups where there was no accumulation of fat droplets **(Fig. 6F – H)**, only congestion of central vein **(Fig. 6H)** and sinusoids (not shown) and mononuclear cell reaction (not shown) at the portal areas in both GTE and Cur treated rats could be observed. The effect of both GTE and Cur on the histopathological examination of liver was comparable.

Histopathological examination of visceral adipose tissue showed enlarged adipocytes with accumulation of excessive amount of fat with abnormal shape **(Fig. 7B)** with evidence of fat necrosis in group of HFD contrasted to the control group **(Fig. 7C)**. GTE and Cur treatment prevented such abnormal changes in adipocytes where their size and shape were more or less as in control group **(Fig. 7D – F)**. The effect of both GTE and Cur on the histopathological examination of visceral adipose tissue was comparable.

Histopathological examination of liver and visceral adipose tissue in CMC group didn't demonstrate any difference when contrasted to control group (not shown). Histopathological examination of aorta did not show any abnormalities or any signs of atherosclerosis (not shown) which could be explained that the duration of experiment (8 weeks) was not enough to induce atherosclerosis.

Histopathological findings and the HFD diet's effects

Liver examination from animals of control group demonstrated normal appearance of hepatocyte, central vein, and portal areas **(Fig. 6A).** HFD resulted in diffuse fatty degeneration in liver tissue and formation of different degrees of microvesicular and marcovesicularsteatosis in H&E stained-sections **(Fig. 6B – E)** when compared with control groups. Some cases showed local aggregation of mononuclear cells **(Fig. 6B)**, other showed vascular thrombosis **(Fig. 6D)**. On the other hand, GTE and Cur treatment protected rats from fatty liver. Liver sections were more or less similar to that of control groups where there was no accumulation of fat droplets **(Fig. 6F – H)**, only congestion of

central vein **(Fig. 6H)** and sinusoids (not shown) and mononuclear cell reaction (not shown) at the portal areas in both GTE and Cur treated rats could be observed. The effect of both GTE and Cur on the histopathological examination of liver was comparable.

Histopathological examination of visceral adipose tissue showed enlarged adipocytes with accumulation of excessive amount of fat with abnormal shape **(Fig. 7B)** with evidence of fat necrosis in group of HFD contrasted to the control group **(Fig. 7C)**. GTE and Cur treatment prevented such abnormal changes in adipocytes where their size and shape were more or less as in control group **(Fig. 7D – F)**. The effect of both GTE and Cur on the histopathological examination of visceral adipose tissue was comparable.

Histopathological examination of liver and visceral adipose tissue in CMC group didn't demonstrate any difference when contrasted to control group (not shown). Histopathological examination of aorta did not show any abnormalities or any signs of atherosclerosis (not shown) which could be explained that the duration of experiment (8 weeks) was not enough to induce atherosclerosis.

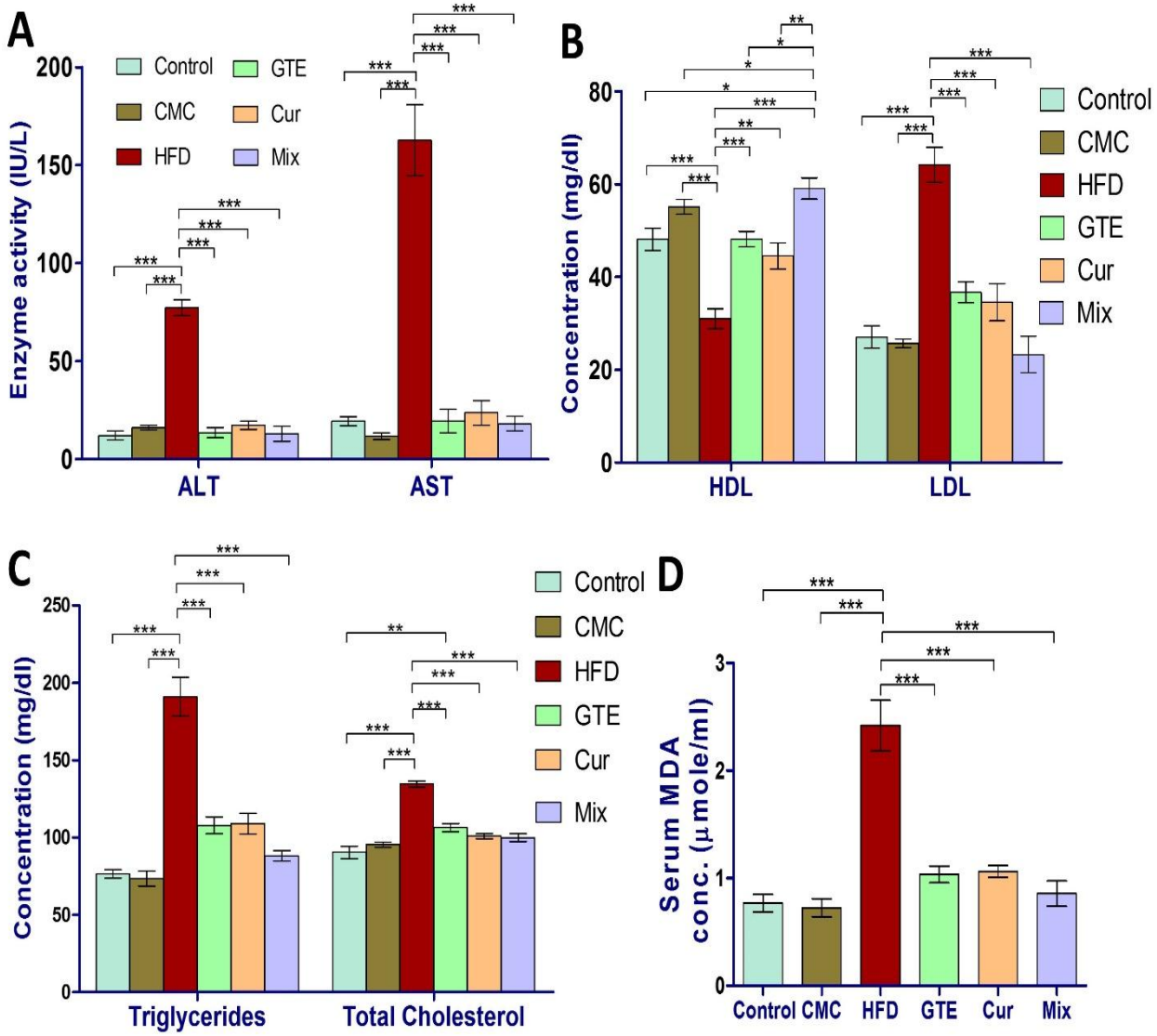

Fig. 5: Effect of HFD, GTE, and Cur on serum liver enzymes activities (A), serum level HDL and LDL (B), serum level triglycerides and total cholesterol (C), serum MDA level (D). Data represent mean ± S.E. * $p< 0.05$, ** $p< 0.01$, *** $p< 0.001$. (ANOVA with Tukey test for multiple comparisons).

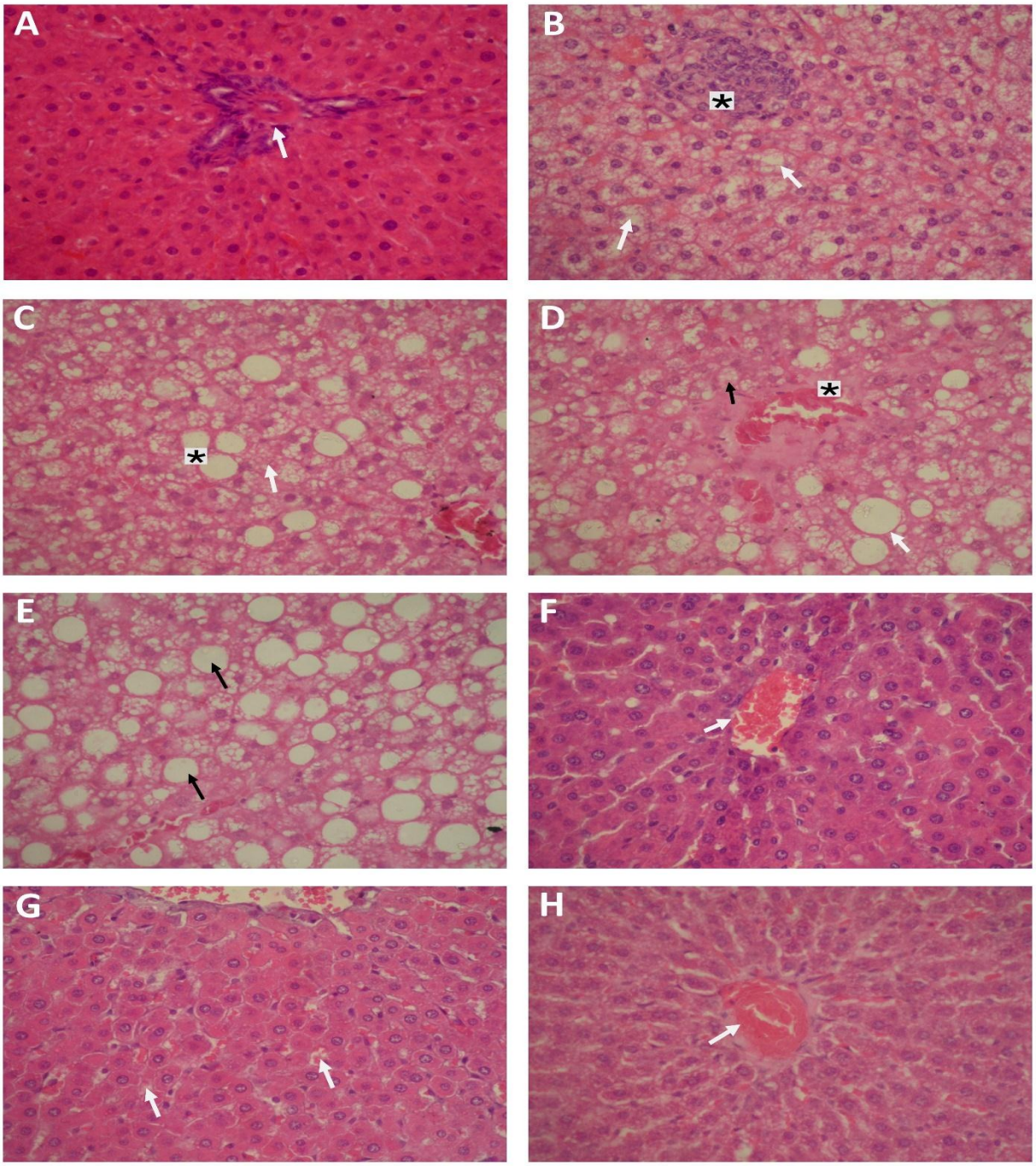

Fig. 6: Histopathological observations of the liver; sections stained with H&E. 10x40.: *A*,Control group showing portal area (arrow) and bile canals with normal histological appearance of hepatocytes, *B*, HFD group showing diffuse fatty degeneration of hepatocytes (arrows) with local aggregation of mononuclear cells (asterisk), *C*, HFD group showing both macrovesicular (asterisk) and microvesicular (arrow) steatosis in hepatocytes, *D*, HFD group showing vascular thrombosis (asterisk) with both macrovesicular (white arrow) and microvesicular steatosis (black arrow) in hepatocytes, *E*, HFD group showing diffuse macrovesicular steatosis (fatty change) (arrows) in hepatocytes, *F*, GTE group showing more or less normal histological appearance of hepatocytes with slight congestion of central vein (arrow), *G*, Cur group showing more or less normal histological appearance of hepatic lobule with slight congestion of central vein (arrow), *H*, Mix group showing more or less normal histological appearance of hepatocytes and portal area (arrow).

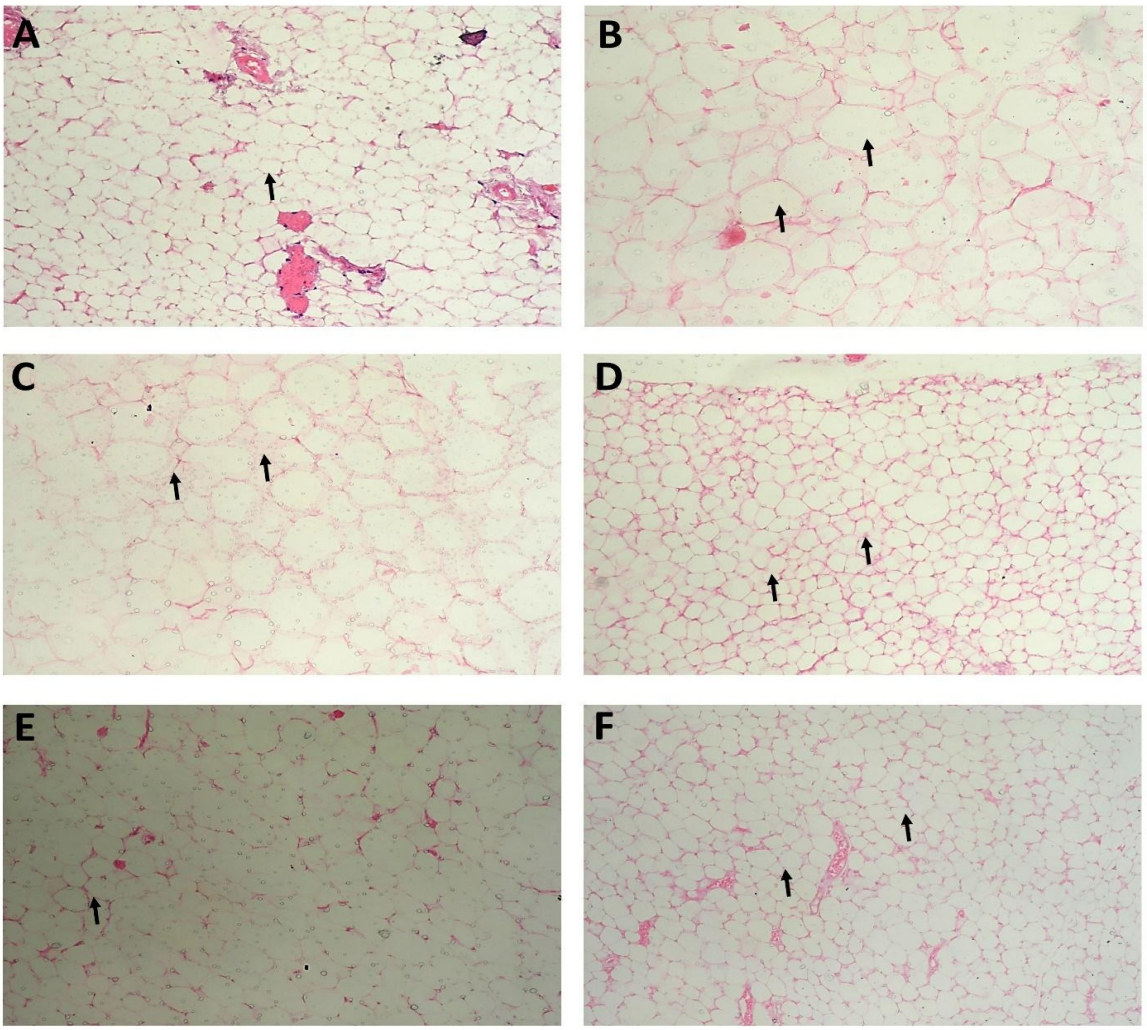

Fig. 7: Histopathological observations of the visceral adipose tissue; sections stained with H&E. 10x10.: *A*, Control group showing normal adipocytes with normal size and shape (arrows), *B*, HFD group showing abnormal adipocytes with large size and rounded shape (arrows), *C*, HFD group showing abnormal adipocytes with large size and abnormal shape with evidence of fat necrosis (arrows), *D*, GTE group showing more or less normal adipocytes (arrows) as control group, *E*, Cur group showing more or less normal adipocytes (arrows) as control group, *F*, Mix group showing more or less normal adipocytes (arrows) as control group.

Discussion

In the current work, we utilized HFD model to induce obesity in rats. HFD mimics the diet of fast food, Western diet, and the diet in the third world which are all rich in saturated fats and cholesterol. HFD was successful in inducing obesity (on Lee index), and its complications as diabetes mellitus, NAFLD, and dyslipidemia. HFD resulted in abnormal accumulation of fat and hence more free fatty acids (FFAs) are released from adipose tissue to plasma[24]. Rise in adiposity is the main driving factor of all complications of obesity. Enlarged adipose tissue secretes pro-proinflammatory cytokines. On the other hand, the anti-inflammatory adipokines level such as CTRP-12 decreases. The pro-inflammatory M1-marcophages replace the anti-inflammatory M2-macrophages which contribute with the overall inflammatory status in obesity[25]. All the previous changes are the main cause of insulin resistance, dyslipidemia and NAFLD[26].

Dietary polyphenols were strongly linked with their ability to prevent obesity and ameliorate its complications[27]. Our study aimed to explore new mechanisms for their beneficial actions **(Fig. 8)**. So, we focused on their action on CTRP-12 and caspase-2.

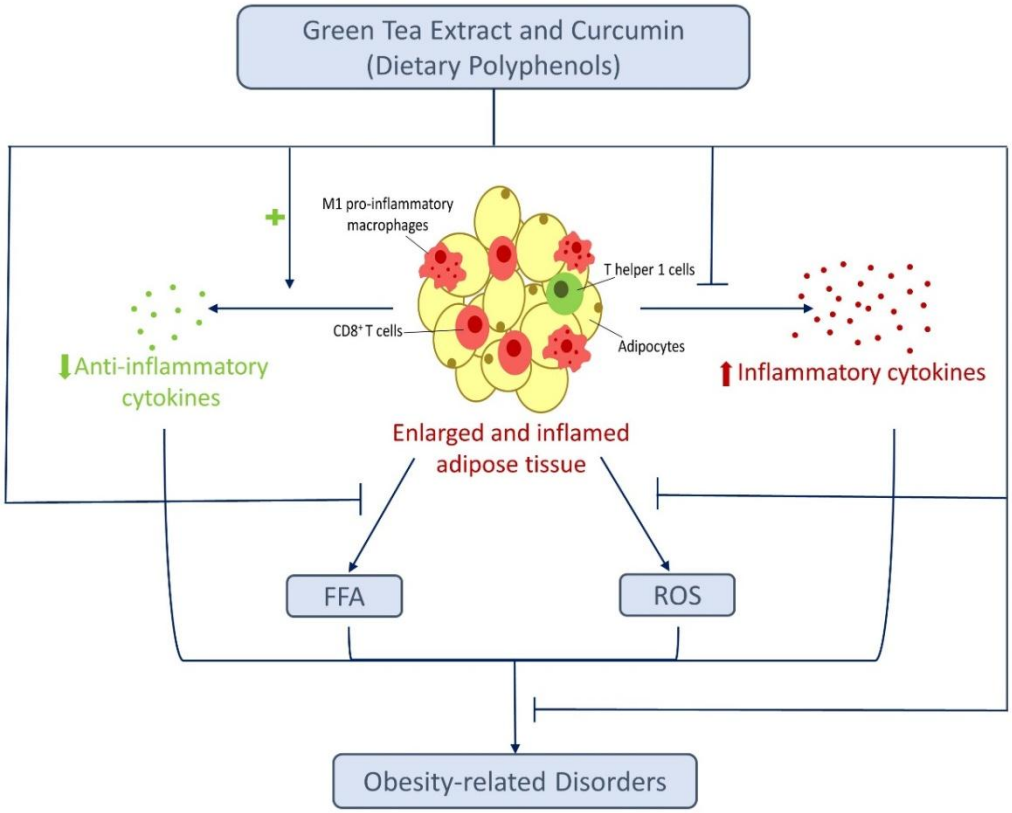

Fig. 8: Pathogenesis of obesity and anti-obesity action of green tea extract and curcumin. The Fig. shows how increased adiposity changes the balance of adipose tissue cytokine in favor of stimulating the secretion of inflammatory mediators. Accumulation of proinflammatory macrophages and $CD8^+$ T cells are a characteristic feature of the inflamed adipose tissue. These changes in turn enhance the release of free fatty acids (FFA) and formation of reactive oxygen species (ROS) and the subsequent obesity-related diseases. Green tea extract and curcumin guards against adipose tissue modeling which is the main driving factor of all complications of obesity.

CTRP-12 is an insulin-sensitizing anti-inflammatory adipokine[4]. In the present study, HFD resulted in decreasing serum CTRP-12 level. This findings is in line with that of Enomoto et al.[4]. Palmitic acid and endoplasmic reticulum stress inducers significantly reduced CTRP-12 transcript concentrations in cultured adipocytes. These data confirm that cellular stress and inflammation which accompany obesity are the main cause for the reduction of CTRP-12 levels[4]. It was found that CTRP-12 administration resulted in improvement of both insulin tolerance and glucose tests, a small reduction in body weight, but levels of fatty acids and triglycerides were not changed, they also found that it decreased adipocyte hypertrophy, diminished macrophage accumulation in epididymal adipose tissues, and caused reduction in expression of proinflammatory cytokines[4].

Moreover, CTRP-12 over-expression using adenoviral method in *ob/ob* mice increased the phosphorylation (activation) of Adipose tissue, liver, and insulin receptor substrate 1 and Akt. As a result, the expression of the gluconeogenic enzymes glucose-6-phosphatase and phosphoenol pyruvate carboxy kinase in the hepatocytes of ob/ob mice was reduced. This increased glucose absorption in adipose tissue. Following therapy with recombinant CTRP-12 in H4IIE cells—even in the lack of insulin—the gene expression of the aforementioned enzymes was inhibited. The suppression of gluconeogenesis was additive when insulin was present.[28].

Rosiglitazone (insulin sensitizing drug) stimulates both CTRP-12 expression in subcutaneous adipose tissue and secretion[29]. Additionally, metformin increases the level of CTRP-12 through AMP-activated protein kinase pathway activation[30].

GTE and curcumin treatment elevated serum CTRP-12 level by 12.2-, 13.8-fold respectively but there was no significant difference between them. This effect of GTE and curcumin is attributed to their anti-obesity effect. They reduce adipose tissue mass, circulating FFAs, endoplasmic reticulum stress, and macrophage infiltration[12]. These results affirm the positive impact of both GTE and curcumin in decreasing the metabolic complications of obesity. But it needs further research to elucidate the mechanism by which these substances increased the expression of CTRP-12. Furthermore, we found that the mixture treatment also elevated serum CTRP-12 level by 24-fold. This indicates that GTE and curcumin may act in a synergistic manner to produce their anti-inflammatory and insulin sensitizing effects through increasing serum CTRP-12 level.

In this research, HFD led to a modest uptick in the expression of the caspase-2 gene in the epididymal adipose tissue, which in turn led to apoptosis. Evidences of fat necrosis were also recognized histopathologically with the increase of amount of visceral adipose tissue **(Fig. 7C).** This outcome was in line with that of Jobgen et al.[31] who stated that caspase-2 gene expression was increased in rat WAT after HFD treatment. The elevated level of apoptosis in WAT results in the release of toxic free radicals and other inflammatory mediators to blood causing systemic oxidative stress and consequently the obesity-related complications. Furthermore, Alkhouri et al. reported that adipocyte apoptosis markedly increased in adipose tissue from both mice with HFD induced obesity and obese humans.Pathologic increase in apoptosis results in ataxia telangiectasia mutated protein activation, with subsequent development of hepatic steatosis, insulin resistance, and dyslipidemia[32].

In the current work, GTE, curcumin, and mixture therapy increased caspase-2 gene expression by 3.63, 3.01, and 7.72-fold respectively in comparison with control group. Ramachandran et al.[33] showed that curcumin up-regulated (>3 fold) caspase-1, -2, and -3 gene expression at both 25 μg/ml and 50 μg/ml doses in the MCF-7 cell line. Curcumin also induced the activation of caspase-2 in a dose-dependent manner together with caspase-8 and -9 in cutaneous T-cell lymphoma (HuT-78) cell line. Ejaz et al.[34] found that curcumin stimulated 3T3-L1 adipocyte apoptosis.

Green tea catechins induced apoptosis in murine 3T3-L1 preadipocyte and mature 3T3-L1 adipocytes as shown by DNA fragmentation and increased caspase-3 activity[35]. Many other natural products, as resveratrol, genistein, quercetin, were found to increase the rate of apoptosis in adipose tissue as a strategy to treat obesity[36].

Caspase-2-deficient mice showed diminished activities glutathione peroxidase and superoxide dismutase, and free radicals mediated cell damage. This is accompanied by decreased expression of the *FoxO1* and *FoxO3a* genes, that protect the cell from oxidative stress. So, Caspase-2 has anti-oxidant properties[9].

From the above findings, we can conclude that GTE and curcumin stimulated adipose tissue apoptosis and the number of adipocytes to decrease adiposity, the released toxic mediators and free radicals were scavenged by the strong antioxidant effect of both GTE and curcumin. Additionally, by increasing caspase-2 expression which guards against oxidative stress, this contributes in part to the antioxidant effect of GTE and curcumin. These findings suggest that curcumin and/or GTE may represent a valuable alternative therapy for the treatment of obesity by reducing adipocyte numbers by inducing apoptosis in adipocytes.

Insulin resistance is characterized by a lowered insulin response by peripheral tissues and it is a part of metabolic syndrome together with dyslipidemia, hypertension, and obesity[37]. HFD led to impaired glucose and insulin tolerance. Inflammation is the primary factor connecting insulin resistance and obesity[38] and accumulation of FFAs and their metabolites as acyl-CoA and diacylglycerol in blood[39]. Treatment with GTE, curcumin, and mixture reversed this impairment as shown from insulin and glucose tolerance tests, fasting blood glucose levels. These results were in line with that of Ortsäter et al.[40] and Maradana et al.[41]. The underlying mechanism is that GTE and curcumin decrease the mobilization of FFAs from adipose tissue to the circulation. Moreover, they decreased all the anti-

inflammatory adipokines in blood and the infiltration of macrophages to adipose tissue[12].

More than two-thirds of cases of NAFLD were suffering from obesity[42]. Hepatic steatosis, which results in an overabundance of triglycerides, and nonalcoholic steatohepatitis (NASH), which results in inflammation and fibrosis, are the causes of NAFLD. The multi hit theory might provide light on how NAFLD develops and how it leads to NASH.[43]. The first hit is insulin resistance and the consequent increase in lipogenesis and decrease in fat output from liver. This causes hepatic steatosis. On the other hand, disturbance in adipokines, mitochondrial dysfunction, oxidative stress, dysregulated liver apoptosis, pro-fibrogenic factors and pro-inflammatory cytokines and activation of hepatic stellate and Kupffer cells stimulate inflammation and fibrosis and hence steatohepatitis[44].

In the current work, HFD group revealed a substantial rise in serum activities of ALT and AST in comparison with control group. The increase in serum liver enzymes activities was suggested to be due to hepatocellular injury caused by excessive accumulation of lipids in liver causing NAFLD as shown in the liver histopathology analysis. Liver histopathology analysis in HFD group showed diffuse fatty degeneration in liver tissue and formation of different degrees of microvesicular and marcovesicular steatosis **(Fig. 6B-E)**. These results were in line with that of Huang et al.[45]. On the other hand, GTE, curcumin, and mixture groups revealed a substantial decrease in serum activities of ALT and AST, and protected rats from NAFLD as shown in the liver histopathology analysis where there was no accumulation of fat droplets **(Fig. 6F-H)**. These results were concordant with that of Y. Tan et al.[46], Maithili et al.[47], Zhao et al.[48], and Hassan et al.[49]. The protective impact of GTE and curcumin against NAFLD resides in their strong anti-inflammatory and antioxidant properties. Furthermore, GTE and curcumin improved glucose tolerance, insulin sensitivity, lipogram, and preventing all components of multi-hit hypothesis[50.51].

Conclusions

GTE and curcumin have anti-obesity properties as they reduced body weight and increased the rate of apoptosis in adipose tissue as they enhanced gene expression of caspase-2. They improved glucose and insulin sensitivity by elevating serum level of CTRP12. Furthermore, they reduced serum MDA level, improved lipid profile, and prevented NAFLD.

Acknowledgment

We acknowledge Hossam M. Omar and Sohair M. M. Ragab for their assistance in the high-fat diet model. We also acknowledge Walaa H. Kamel for her support regarding the histopathological part. Additionally, we acknowledge technicians at Medical Biochemistry Department, Animal House, and Medical Research Center (Faculty of Medicine, Assiut university) for their help throughout the experiments. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. GBD Obesity Collaborators, Afshin A., Forouzanfar M. H., *et al.*, "Health effects of overweight and obesity in 195 countries over 25 years", ***N Engl J Med,*** 377(1), 13-27(2017).
2. S. M. Fruh, "Obesity: risk factors, complications, and strategies for sustainable long-term weight management", ***J Am Assoc Nurse Pract,*** 29(S1), S3-S14(2017).
3. V. d. O. Leal and D. Mafra, "Adipokines in obesity", ***Clin Chim Acta,*** 419, 87-94(2013).
4. T. Enomoto, K. Ohashi, R. Shibata, A. Higuchi, S. Maruyama, Y. Izumiya, *et al.*, "Adipolin/C1qdc2/CTRP12 protein functions as an adipokine that improves glucose metabolism", ***J Biol Chem,*** 286(40), 34552-34558(2011).
5. A. Schäffler and C. Buechler, "CTRP family: linking immunity to metabolism", ***Trends Endocrinol Metab,*** 23(4), 194-204(2012).
6. B. Bai, B. Ban, Z. Liu, M. M. Zhang, B. K. Tan and J. Chen, "Circulating C1q complement/TNF-related protein (CTRP) 1, CTRP9, CTRP12 and CTRP13 concentrations in type 2 diabetes mellitus: in vivo regulation by glucose", ***PLoS One,*** 12(2), e0172271(2017).

7. M. Olsson, J. Forsberg and B. Zhivotovsky, "Caspase-2: the reinvented enzyme", ***Oncogene,*** 34(15), 1877-1882(2015).
8. M. Machado, G. Michelotti, M. Jewell, T. Pereira, G. Xie, R. Premont, *et al.*, "Caspase-2 promotes obesity, the metabolic syndrome and nonalcoholic fatty liver disease", ***Cell Death Dis,*** 7(2), e2096(2016).
9. S. Shalini, L. Dorstyn, C. Wilson, J. Puccini, L. Ho and S. Kumar, "Impaired antioxidant defence and accumulation of oxidative stress in caspase-2-deficient mice", ***Cell Death Differ,*** 19(8), 1370-1380(2012).
10. D. Bastos, L. Saldanha, R. Catharino, A. Sawaya, I. Cunha, P. Carvalho, *et al.*, "Phenolic antioxidants identified by ESI-MS from yerba mate (*Ilex paraguariensis*) and green tea (*Camelia sinensis*) extracts", ***Molecules,*** 12(3), 423-432(2007).
11. B. Kocaadam and N. Şanlier, " Curcumin, an active component of turmeric (*Curcuma longa*), and its effects on health", ***Crit Rev Food Sci Nutr,*** 57(13), 2889-2895(2017).
12. S. Wang, N. Moustaid-Moussa, L. Chen, H. Mo, A. Shastri, R. Su, *et al.*, "Novel insights of dietary polyphenols and obesity", ***J Nutr Biochem,*** 25(1), 1-18(2014).
13. M. Afzal, A. M. Safer and M. Menon, "Green tea polyphenols and their potential role in health and disease", ***Inflammopharmacology,*** 23(4), 151-161(2015).
14. S. M. Ragab, S. K. A. Elghaffar, T. H. El-Metwally, G. Badr, M. H. Mahmoud and H. M. Omar, "Effect of a high fat, high sucrose diet on the promotion of non-alcoholic fatty liver disease in male rats: the ameliorative role of three natural compounds " ***Lipids Health Dis,*** 14, 83(2015).
15. Z. Liu, Z. Chen, H. Guo, D. He, H. Zhao, Z. Wang, *et al.*, "The modulatory effect of infusions of green tea, oolong tea, and black tea on gut microbiota in high-fat-induced obese mice", ***Food Funct,*** 7(12), 4869-4879(2016).
16. M. A. El-Moselhy, A. Taye, S. S. Sharkawi, S. F. El-Sisi and A. F. Ahmed, "The antihyperglycemic effect of curcumin in high fat diet fed rats. Role of TNF-α and free fatty acids", ***Food Chem Toxicol,*** 49(5), 1129-1140(2011).
17. E. L. B. Novelli, Y. S. Diniz and C. M. Galhardi, "Anthropometrical parameters and markers of obesity in rats", ***Lab Anim,*** 41(1), 111-119(2007).
18. K.J. Livak and T.D. Schmittgen, "Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method", ***Methods,*** 25(4), 402-408(2001).
19. J. Matthews, D. G. Altman, M. Campbell and P. Royston, "Analysis of serial measurements in medical research", ***Br Med J,*** 300(6719), 230-235(1990).
20. K. T. Uysal, S. M. Wiesbrock, M. W. Marino, G. S. Hotamisligil, "Protection from obesity-induced insulin resistance in mice lacking TNF-α function", ***Nature,*** 389(6651), 610-614(1997).
21. H. Ohkawa, N. Ohishi and K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction", ***Anal Biochem,*** 95(2), 351-358(1979).
22. W. T. Friedewald, R. I. Levy and D. S. Fredrickson, "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge", ***Clin Chem,*** 18(6), 499-502(1972).
23. J. D. Bancroft and M. Gamble, "Theory and Practice of Histological Techniques, 6th ed., Churchill Livingstone", Philadelphia, 2008.
24. J. Liu, L. Han, L. Zhu and Y. Yu, "Free fatty acids, not triglycerides, are associated with non-alcoholic liver injury progression in high fat diet induced obese rats", Lipids Health Dis, 15, 27(2016).
25. Y. E. Kang, J. M. Kim, K. H. Joung, J. H. Lee, B. R. You, M. J. Choi, *et al.*, "The roles of adipokines, proinflammatory cytokines, and adipose tissue macrophages in obesity-associated insulin resistance in modest obesity and early metabolic dysfunction", ***PLoS One,*** 11(4), e0154003(2016).
26. U. Jung and M. S. Choi, "Obesity and its metabolic complications: the role of

adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease", ***Int J Mol Sci,*** 15(4), 6184-6223(2014).
27. T. Jin, Z. Song, J. Weng and I. G. Fantus, "Curcumin and other dietary polyphenols: potential mechanisms of metabolic actions and therapy for diabetes and obesity", ***Am J Physiol Endocrinol Metab,*** 314(3), E201-E205(2017).
28. Z. Wei, J. M. Peterson, X. Lei, L. Cebotaru, M. J. Wolfgang, G. C. Baldeviano, *et al.*, "C1q/TNF-related protein-12 (CTRP12), a novel adipokine that improves insulin sensitivity and glycemic control in mouse models of obesity and diabetes", ***J Biol Chem,*** 287(13), 10301-10315(2012).
29. B. K. Tan, K. C. Lewandowski, J. P. O'Hare and H. S. Randeva, "Insulin regulates the novel adipokine adipolin/CTRP12: in vivo and ex vivo effects", ***J Endocrinol,*** 221(1), 111-119(2014).
30. B. K. Tan, J. Chen, R. Adya, M. Ramanjaneya, V. Patel and H. S. Randeva, "Metformin increases the novel adipokine adipolin/CTRP12: role of the AMPK pathway", ***J Endocrinol,*** 219(2), 101-108(2013).
31. W. Jobgen, W. J. Fu, H. Gao, P. Li, C. J. Meininger, S. B. Smith, *et al.*, "High fat feeding and dietary L-arginine supplementation differentially regulate gene expression in rat white adipose tissue", ***Amino acids,*** 37(1), 187-198(2009).
32. N. Alkhouri, A. Gornicka, M. P. Berk, S. Thapaliya, L. J. Dixon, S. Kashyap, *et al.*, "Adipocyte apoptosis, a link between obesity, insulin resistance, and hepatic steatosis", ***J Biol Chem,*** 285(5), 3428-3438(2010).
33. C. Ramachandran, S. Rodriguez, R. Ramachandran, P. R. Nair, H. Fonseca, Z. Khatib, *et al.*, "Expression profiles of apoptotic genes induced by curcumin in human breast cancer and mammary epithelial cell lines", ***Anticancer Res,*** 25(5), 3293-3302(2005).
34. A. Ejaz, D. Wu, P. Kwan and M. Meydani, "Curcumin inhibits adipogenesis in 3T3-L1 adipocytes and angiogenesis and obesity in C57/BL mice", ***J Nutr,*** 139(5), 919-925(2009).
35. P. F. Hung, B. T. Wu, H. C. Chen, Y. H. Chen, C. L. Chen, M. H. Wu, *et al.*, "Antimitogenic effect of green tea (−)-epigallocatechin gallate on 3T3-L1 preadipocytes depends on the ERK and Cdk2 pathways", ***Am J Physiol Cell Physiol,*** 288(5), C1094-C1108(2005).
36. Y. Zhang and C. Huang, "Targeting adipocyte apoptosis: a novel strategy for obesity therapy", ***Biochem Biophys Res Commun,*** 417(1), 1-4(2012).
37. V. T. Samuel and G. I. Shulman, "The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux", ***J Clin Invest,*** 126(1), 12-22(2016).
38. M. Blüher, "Adipose tissue inflammation: a cause or consequence of obesity-related insulin resistance? ", ***Clin Sci,*** 130(18), 1603-1614(2016).
39. G. Boden, "Obesity, insulin resistance and free fatty acids", ***Curr Opin Endocrinol Diabetes Obes,*** 18(2),139-143(2011).
40. H. Ortsäter, N. Grankvist, S. Wolfram, N. Kuehn and A. Sjöholm, "Diet supplementation with green tea extract epigallocatechin gallate prevents progression to glucose intolerance in *db/db* mice", ***Nutr Metab,*** 9, 11(2012).
41. M.R. Maradana, R. Thomas and B.J. O'sullivan, "Targeted delivery of curcumin for treating type 2 diabetes", ***Mol Nutr Food Res,*** 57(9), 1550-1556 (2013).
42. M. Lazo and J.M. Clark, "The epidemiology of nonalcoholic fatty liver disease: a global perspective", ***Semin Liver Dis,*** 28, 339-350(2008).
43. S.A. Polyzos, J. Kountouras, C. Zavos and G. Deretzi, "Nonalcoholic fatty liver disease: multimodal treatment options for a pathogenetically multiple-hit disease", ***J Clin Gastroenterol,*** 46(4), 272-284 (2012).
44. U. J. Jung and M. S. Choi, "Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance,

dyslipidemia and nonalcoholic fatty liver disease", ***Int J Mol Sci,*** 15(4), 6184-6223 (2014).

45. C.-Z. Huang, Y.-T. Tung, S.-M. Hsia, C.-H. Wu and G.-C. Yen, "The hepatoprotective effect of *Phyllanthus emblica* L. fruit on high fat diet-induced non-alcoholic fatty liver disease (NAFLD) in SD rats", ***Food Funct,*** 8(2), 842-850 (2017).
46. Y. Tan, J. Kim, J. Cheng, M. Ong, W. G. Lao, X. L. Jin, *et al.*, "Green tea polyphenols ameliorate non-alcoholic fatty liver disease through upregulating AMPK activation in high fat fed Zucker fatty rats", ***World J Gastroenterol,*** 23(21), 3805-3814 (2017).
47. N. Maithilikarpagaselvi, M. G. Sridhar, R. P. Swaminathan, R. Sripradha, "Preventive effect of curcumin on inflammation, oxidative stress and insulin resistance in high-fat fed obese rats", ***J Complement Integr Med,*** 13, 137-143 (2016).
48. N. J. Zhao, M. J. Liao, J. J. Wu and K. X. Chu, "Curcumin suppresses Notch‐1 signaling: improvements in fatty liver and insulin resistance in rats", ***Mol Med Rep,*** 17(1), 819-826 (2018).
49. M. H. Hassan, E. A. Awadalla, A. El-Kader, E. A. Seifeldin, M. A. Mahmoud, A. R. Muddathir and A. Abdelsadik, "Antitoxic effects of curcumin against obesity-induced multi-organs' biochemical and histopathological abnormalities in an animal model", ***Evid Based Complement Alternat Med***, 2022, 9707278(2022).
50. Y. Tan, J. Kim, J. Cheng, M. Ong, W.G. Lao, X. L. Jin, *et al.*, "Green tea polyphenols ameliorate non-alcoholic fatty liver disease through upregulating AMPK activation in high fat fed Zucker fatty rats", ***World J Gastroenterol,*** 23(21), 3805-3814(2017).
51. N. J. Zhao, M. J. Liao, J. J. Wu and K. X. Chu, "Curcumin suppresses Notch‐1 signaling: improvements in fatty liver and insulin resistance in rats", ***Mol Med Rep,*** 17(1), 819-826 (2018).

Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 1, 2024, pp 345-362

نشرة العلوم الصيدليـــة
جامعة أسيوط

BULL. PHARM. SCI. Assiut Univ.

العمل المضاد للسمنة لمستخلص الشاي الأخضر والكركمين: دور البروتين ١٢ المرتبط بـ (CTRP-12 / TNF (c1q وكاسبيز - ٢

محمد عاطف فاضل حسين[١] – منال احمد محمد مندور[٢] – ساري خليل عبد الغفار[٣] – عبد الرحيم محمد عبد الحفيظ مكي[٢،٤] – مايكل عفت فخري عبد المسيح [٢*]

[١] قسم الكيمياء الحيوية، كلية الصيدلة، جامعة أسيوط ، صندوق بريدي ٧١٥٢٦، اسيوط، مصر

[٢]قسم الكيمياء الحيوية الطبية، كلية الطب، جامعة أسيوط، صندوق بريدي ٧١٥٢٦، أسيوط، مصر

[٣]قسم الباثولوجي، كلية الطب البيطري، جامعة أسيوط، صندوق بريدي ٧١٥٢٦، أسيوط، مصر

[٤]قسم الكيمياء الحيوية، كلية الصيدلة، جامعة سفنكس، صندوق بريدي ٥٧١٥١، أسيوط الجديدة، مصر

مقدمة

تمثل السمنة عامل خطر رئيسي لعدد من الأمراض المزمنة، بما في ذلك مرض السكري من النوع الثاني، وتصلب الشرايين و ارتفاع ضغط الدم و خلل في دهون الدم و تشمع الكبد ومتلازمة التمثيل الغذائي والأمراض القلبية الوعائية. و لوحظ في العديد من الدراسات زيادة معدل انتشار السمنة في السنوات الأخيرة. من الناحية الأخرى، أثبتت العديد من الأبحاث التأثير الإيجابي للعديد من المكونات الغذائية النباتية للحد من السمنة و الأمراض الناتجة منها مثل الكوركومين و مستخلص الشاي الأخضر.

منهجية العمل

تم إجراء هذه الدراسة على ٦٠ من ذكور الجرذان البيضاء التي تزن حوالي ١١٠- ١٣٠ جم، بقي منها ٤٥ جرذا في نهاية التجربة التي استمرت شهرين و تم تقسيم الجرذان إلى ٦ مجموعات، ثلاثة مجموعات ضابطة و مجموعة للكوركمين و مجموعة لمستخرج الشاي الأخضر و مجموعة لخليط الشاي الأخضر و الكوركمين.

تم قياس كل من: الأديبولين في مصل الدم بواسطة المقايسة الامتصاصية المناعية للانزيم المرتبط ELISA ومستويات التعبير الجيني لجين كاسبيز-٢ في دهون البربخ بواسطة تفاعل البوليميريز المتسلسل مسبوقاً بالنسخ العكسي (qRT-PCR) بالإضافة لقياس مستويات الدهون في مصل الدم و كذلك تم عمل اختبار تحمل الجلوكوز الفموي و اختبار تحمل الانسولين البريتوني كما تم عمل فحوصات نسيجية للكبد بواسطة الفحص المجهري الضوئي.

النتائج

خلصت نتائج هذه الدراسة أن المعالجة باشاي الأخضر و الكوركمين أدت إلى خفض وزن الجسم و زيادة معدل موت الخلايا المبرمج في الأنسجة الدهنية لأنهم أدوا إلى زيادة مستوى التعبير الجيني لانزيم كاسبيز-٢ و تحسين تحمل الجلوكوز و حساسية الانسولين و زيادة مستوى الأديبولين. بالإضافة إلى ذلك قاموا بتحسين وظائف الكبد و منع الكبد الدهني.

الاستنتاج

الدراسة الحالية استنتجت أن مستخلص الشاي الأخضر و الكوركمين لهم تأثير وقائي ضد السمنة و أثارها الضارة.