The Possible Role of Orthoboric Acid on Adipogenesis in Adult Male Albino Rat. Histological and Immunohistochemical Study

Original Article

Reda Ahmed Hasan, Mona Hussien Raafat, Mohamed Abd ElRahman Ahmed Mekawy and Samir Nairooz Ibrahim

Department of Histology and Cell Biology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

ABSTRACT

Background: Obesity is a worldwide problem which leads to increased morbidity and rate of deaths. Clinical use of some therapeutic options for the prevention of overweightness is limited due to their dangerous side effects. Orthoboric acid is a new safe and beneficial treatment for prevention of high fat diet induced obesity.

Aim: Study the effect of orthoboric acid on adipogenesis in rats.

Materials and Methods: The study included 30 adult male albino rats. The rats were divided into 4 main groups. Group I: control. Group II (High Fat Diet ″HFD″ group): IIa (HFD 8 weeks), IIb (HFD 12 weeks). Group III (HFD and orthoboric acid group) for 8 weeks. GroupIV (HFD for 12 weeks but starting from the 8th week the rats were given orthoboric acid daily for 4 weeks). Orthoboric acid was used at dose of 2.5 mg/rat given orally via intragastric tube. Body weights of all rats were recorded weekly during the experiment. Adipose tissues pecimens were collected at the end of experiment and processed for H&E, oil red O stain and antiβ - catenin immunohistochemistry. Morphometric and statistical analysis were also done.

Results: HFD group showed significant increase in body weight and in size of adipocytes compared to the control group meanwhile, groups of orthoboric acid showed a significant decrease in body weight and size of adipocytes relative to HFD group. As regard effect of orthoboric acid on adipogenesis, groups treated with orthoboric acid showed positive immunohistochemical reaction for β- catenin with subsequent decrease in lipid accumulation confirmed with less optically dense oil red O staining compared to HFD group.

Conclusions: Low dose of oral orthoboric acid was able to reduce body weight. Therefore, it can be considered as a suitable treatment for obesity due to its vital role in inhibition of adipogenesis.

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Key Words: Adipogenesis, high fat diet, orthoboric acid, rats.

Corresponding Author: Reda Ahmed Hasan, PhD, Department of Histology and Cell Biology, Faculty of Medicine, Ain Shams University, Cairo, Egypt, **Tel.**: +2 02 2636 7961, **E-mail:** hania2012.ra@gmail.com

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INTRODUCTION

Overweight or obese people were found to estimate about 2.1 billion people worldwide, and 2.8 million deaths are caused by obesity every year[1]. Obesity can be reached when the body mass index (BMI) exceeds 30 kg/m2and abdominal obesity is considered if the waist circumference exceeds 102 cm for men and 88 cm for women[2].

In humans, there are two main sites for distribution of adipose tissue: visceral; around the internal organs and subcutaneous; just under the skin around the belly, thighs, and rear. It has been noticed that visceral abdominal fat more dangerous than subcutaneous fat as it contributes to insulin resistance and inflammation reducing 8 years from the life expectancy[3].

In developed countries obesity leads to increased morbidity and rate of deaths as it causes serious associated diseases, such as type II diabetes, high blood pressure, cardiovascular disorders and metabolic syndrome[4,5]. Metabolic syndrome or prediabetes can be defined as a complex of metabolic abnormalities including obesity especially visceral obesity with its associated comorbidities including, insulin resistance, hyperlipidemia, hypertension, prothrombotic and proinflammatory states[6]. As regard development of obesity, uncontrolled increased number and size of adipocytes thought to be contributing factors in process of adipogenesis[7].

Although several therapeutic options have been offered to the market for the treatment of obesity, their clinical use is restricted due to their dangerous side effects including high blood pressure, cardiovascular complications, liver diseases and psychiatric illnesses[8]. Therefore, it was necessary to discover a new, safe, and efficient alternative treatment for obesity. The new approaches in treatment of overweightness concentrate on aiming certain pathways involved in adipogenesis to decrease storage of lipid and adipocyte proliferation.

Boron compounds are naturally occurring elements and found in various human tissues. They are present in two forms; Orthoboric acid which is hydrogen borate and Borax which is sodium borate. There are several types of foods rich in these compounds specially orthoboric acid, including fruits like raw red apple with peel and raw banana,

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nuts like almonds and peanuts, cereal grain products like enriched white bread and instant white rice. They are involved in several pathways, including psychological activities[9], hormonal regulation[10], bone development[11], and wound healing[12]. Orthoboric acid is a weak inorganic acid with antiseptic properties. It′s also called boric acid or boracic acid.

Low concentrations of boric acid doesn′t pose any toxicity. However, boric acid is poisonous if ingested or inhaled in large quantities. Recently, researches revealed that swallowed usage of orthoboric acid as a dietary enhancement resulted in short- term and long-standing loss of weight[13,14].

AIM

This study was carried out to study the effect of orthoboric acid on adipogenesis in adult male albino rats.

MATERIALS AND METHODS

A-Animals

Thirty adult male albino rats (Wister strain) weighing 200-250 gm were used in this study for experimental grouping.

The rats were maintained in the Medical Research Center in Faculty of Medicine Ain Shams University. They were kept in plastic cages with mesh wire covers and kept under proper environments of light, temperature, and humidity with free access of water and food. The practical work was performed according to the guidelines of Animal Care and the Scientific Research of Ethical Committee of the Faculty of Medicine, Ain Shams University.

B- Orthoboric acid (B.A)

Was obtained from Sigma–Aldrich Chemical Co. (Cairo, Egypt). It was available in the form of powder. It has been given to the rats in a dose of 2.5 mg/rat daily diluted in 3 ml tap water orally via intragastric tube[14].

C- Antibodies for immunohistochemistry

Primary antibody: anti-β-catenin and secondary antibody: conjugated goat anti-rabbit IgG were purchased from Dako Denmark A/S company.

D- Oil red O

Was obtained from Sigma–Aldrich Chemical Co. (Cairo, Egypt). It was available in the form of liquid. Its concentration was 0.5% in isopropanol.

Experimental protocol

Animals were kept for one week before starting the experiment for acclimatization. Rats were randomly divided into four main groups.

Group I (Control)

Included ten rats that were further subdivided into 2 subgroups, five animals each:

- Subgroup Ia: rats in this group drank standard tap water and they were fed with standard diet for 8 weeks.
- Subgroup Ib: rats in this group drank standard tap water and they were fed with standard diet for 12 weeks.

Group II (High Fat Diet ″HFD″)

Included ten rats that were further subdivided into 2 subgroups, five animals each:

- Subgroup IIa: rats drank standard tap water and they were fed with HFD for 8 weeks.
- Subgroup IIb: rats drank standard tap water and they were fed with HFD for 12 weeks.

The HFD was composed of 60% standard rodent diet and 40% beef tallow[15]. Standard rodent diet was purchased from forage and beef tallow was purchased from the butcher. The standard rodent diet and the beef tallow were then mixed together manually.

Group III (HFD 8 weeks and Orthoboric acid)

This group included five rats that were fed with HFD concomitantly with orthoboric acid at dose of 2.5 mg/rat daily diluted in 3 ml tap water supplied orally via intragastric tube for 8 weeks[14].

Group IV(HFD 12 weeks and Orthoboric acid)

This group included five rats that were fed with HFD for 12 weeks but starting from the 8th week the rats were given orthoboric acid at dose of 2.5 mg/rat daily diluted in 3 ml tap water orally via intragastric tube for 4 weeks.

Body weights of all rats were recorded every week during the experiment.

Sample collection

At the end of the experiment, all animals were sacrificed by cervical dislocation after inhalation of ether as an anesthesia. The adipose tissue was harvested from inguinal adipose tissue. Half of the number of the specimens were fixed in 10% buffered formalin to be handled for the paraffin technique, while the other half was frozen to be processed for staining with oil red O stain. Adipose tissue was stained by Hematoxylin and Eosin (H&E) and oil red O in all groups. Immunohistochemical stain (anti β - catenin) was also performed for all groups. The bodies of the dead animals were discarded in the incinerator after being sacrificed.

Preparation of adipose tissue specimens for immuno-histochemical staining (anti-β-catenin)

Immunostaining was performed to verify occurrence of process of adipogenesis. Sections were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton-X 100/phosphate-buffered saline (PBS) solution then exposed to 1% bovine serum albumin (BSA) for blocking. Sections were incubated overnight at 4 °C with the primary antibody:

anti-β-catenin. Then, sections were treated with secondary antibody conjugated goat anti-rabbit IgG for 1 hour at room temperature and samples were visualized with light microscope (LSM 700; Zeiss, Heidelberg, Germany)[16]. Positive results recorded brown cytoplasmic and nuclear reaction of cells. Negative and positive controls were used.

For negative control staining, some sections were incubated with PBS instead of the primary antibody. No immunoreactivity was present in these sections.

Positive tissue control

A section of control intestine was immunostained for β-catenin-positive cells (according to data provided by the antibody manufacturer).

Oil Red O staining

Adipose tissue was fixed in4% natural buffered formalin overnight. Cryostat cut sections,10 µm thick, were rinsed in water for 1-10 minutes then rinsed in 60% isopropyl alcohol. They were stained in the working solution of Oil Red O for 10 minutes. Extra stain was removed in 60 % isopropyl alcohol. Then sections were cleaned well in water, counterstained with Hematoxylin, washed and then mounted in glycerol jelly[17].

Morphometric and statistical Study

An image analyzer Leica Q win V.3 program installed on a computer in the Department of Histology and Cell Biology, Faculty of Medicine, Ain Shams University, was used. The computer was connected to a Leica DM2500 microscope (Wetzlar, Germany). Measurements were taken from three different slides obtained from each animal. Five haphazardly selected non overlapping fields were examined for each slide.

The following parameters have been quantitated

1. Body weights of all rats were documented weekly during the experiment.
2. Size of normal shaped adipocytes using 20X objective lens.
3. The optical density of anti-β-catenin stained sections using 20X objective lens.
4. The optical density of the lipid droplets in Oil red O stained sections to quantify the lipid accumulation using 20X objective lens.

Statistical analysis

Morphometric measured parameters recorded for each group were revised and the results were expressed as mean ± SD. Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS), software program, version 20 (IBM corporation, Armwork, North castle, Westchesten Country, New York, USA). Statistical difference among groups for each parameter was determined using one-way analysis of variance (ANOVA) followed by post hoc least significance difference (LSD) for comparison between more than two groups. Regarding the probability, the significance of data was determined by the *P value*[18] where:

- $P \geq 0.05 \rightarrow$ non-significant (NS)
- $P < 0.05 \rightarrow$ significant (S)

RESULTS

Histological results

1-H&E results:

Both subgroup Ia (control 8weeks) and Ib (control 12 weeks) showed nearly similar histological results. The histological examination of H&E stained sections of inguinal adipose tissue of rats of groupI(control)showed that it was formed of white adipose tissue. It contained polygonal adipocytes, variable in sizes with well-defined cell boundaries. The adipocytes were unilocular with single large unstained vacuole and thin rim of cytoplasm at the periphery. The nucleus is flat and eccentric in position giving the signet ring appearance (Figure 1A).

Examination of H&E sections of subgroup IIa (HFD 8 weeks) showed that it was formed of white adipose tissue. Large adipocytes could be seen and some adipocytes coalesce together forming one large adipocyte. Inflammatory cellular infiltration among blood vessels could be noticed (Figures 1B,1C).

Examination of H&E sections of subgroup IIb (HFD 12 weeks) showed that it was formed of white adipose tissue. Very large adipocytes could be seen and some adipocytes coalesce together forming one huge adipocyte. Inflammatory cellular infiltration and dilated, congested blood vessels could be noticed between adipocytes (Figure 1D).

Examination of H&E sections of group III (HFD and BA 8weeks) showed that it was formed of white adipose tissue. It contained variable sized adipocytes which were irregular in shape. Corrugated cell boundaries could be seen. The adipocytes were unilocular with single large unstained vacuole and thin rim of cytoplasm at the periphery. Large number of small sized adipocytes could be noticed (Figure 1E).

Examination of H&E sections of group IV (HFD and BA 12weeks) showed that it was formed of white adipose tissue. It contained polygonal adipocytes variable in sizes with clear cell boundaries. The adipocytes were unilocular with single large unstained vacuole and thin rim of cytoplasm at the periphery. The nucleus was flat and eccentric in position giving the signet ring appearance (Figure 1F).

Oil red O staining results

Examination of oil red O stained sections of Group I (control) showed nearly the same histological results. The sections contained polygonal adipocytes of variable sizes. The adipocytes were unilocular, each cell contained lightly

stained single large lipid droplet occupying whole of the cytoplasm, while the nuclei were counterstained blue with Hematoxylin stain (Figure 2A).

Examination of oil red O stained sections of subgroup IIa (HFD 8 weeks) revealed polygonal adipocytes of variable sizes. Large sized adipocytes could be seen, and some adipocytes coalesce together forming one large adipocyte. The cytoplasm showed dark reddish colour of single large lipid droplet, while the nuclei were counterstained blue with Hematoxylin stain (Figure 2B).

Examination of oil red O stained sections of subgroup IIb (HFD 12 weeks) revealed irregular adipocytes of variable sizes. Very large sized adipocytes could be seen, and some adipocytes coalesce together forming one huge adipocyte. The cytoplasm showed dark reddish colour of single large lipid droplet, while the nuclei were counterstained blue with Hematoxylin stain (Figure 2C).

Examination of oil red O sections of group III (HFD and BA 8weeks) and group IV (HFD and BA 12 weeks) revealed polygonal adipocytes of variable sizes. Increased number of small sized adipocytes could be noticed. The cytoplasm showed lightly stained single large lipid droplet, while the nuclei were counterstained blue with Hematoxylin stain. Lipid droplets were stained less optically dense relative to subgroups IIa and IIb (Figures 2D,2E).

Immunohistochemical results

Immunohistochemical staining for β – Catenin of sections of subgroup Ia and Ib (control 8&12 weeks) revealed a positive β –Catenin reaction in some adipocytes, which exhibited brown colour in the cytoplasm and nucleus (Figures 3A,3B).

Immunohistochemical staining for β – Catenin of sections of subgroup IIa and IIb (HFD 8&12 weeks) revealed a very faint β –Catenin reaction in all adipocytes (Figures 3C,3D).

Immunohistochemical staining for β – Catenin of sections of group III (HFD and BA 8 weeks) revealed a strong positive β –Catenin reaction in some adipocytes which exhibited brown colour in the cytoplasm and nucleus (Figure 3E).

Immunohistochemical staining for β –Catenin of sections of group IV (HFD and BA 12 weeks) revealed a strong positive β –Catenin reaction in most of adipocytes which exhibited brown colour in the cytoplasm and nucleus (Figure 3F).

Histomorphometric Results

1-Mean body weight of experimental rats

Both subgroups IIa& IIb (HFD) exhibited a significant increase (P<0.05) in the mean body weight when compared to all other groups (319.30± 20.3 gm and 321.50± 26.4 gm) respectively. However, both Group III (HFD and BA 8weeks), 266.70 ± 22.2 gm and Group IV (HFD and BA 12weeks), 289.10± 11.5 gm manifested significant decrease (P<0.05) when compared to both subgroups IIa and IIb (HFD)and a significant increase (P<0.05) compared to Group I (control), 260.60 ± 5.4 gm. Group III (HFD and BA 8weeks) manifested a significant decrease in the mean body weight compared to Group IV (HFD and BA 12weeks) (Histogram 1A).

2- Mean size of adipocytes

Subgroups IIa and IIb (HFD) showed a significant increase in size of adipocytes (P<0.05) 13256.10±1802.3 μm^2 and 15193.60± 2660.6 μm^2 respectively when compared to subgroup Ia (control 8weeks), 2490.9±337.8295 μm^2 and subgroup Ib (control 12weeks), 3368.8 ± 151.8455 μm^2. Meanwhile, Group III (HFD and BA 8weeks), 1016.5 ±273.7 μm^2 and Group IV (HFD and BA 12weeks), 1977.3±454.9 μm^2 manifested a significant decrease (P<0.05) when compared to all other groups of the experiment (Histogram 1B).

3 -The mean optical density of oil red O staining

Group III (HFD and BA 8 weeks), 51.03 ± 3.16 and Group IV (HFD and BA 12weeks), 57.72± 3.74showed a significant decrease (P<0.05) in mean optical density of oil red O when compared to subgroup IIa (HFD 8weeks), 75.53 ±2.06 and subgroup IIb (HFD 12weeks),78.08 ±3.29. Meanwhile, both subgroups IIa and IIb (HFD)manifested a significant increase (P<0.05) when relative to Group I (control)50.02± 3.12 (Histogram 1C).

4 -The mean optical density of β - Catenin antibody

Group III (HFD and BA 8weeks) 87.18 ± 6.4 and Group IV (HFD and BA 12weeks) 74.67 ± 7.4 showed a significant increase(P<0.05) in mean optical density of β - Catenin antibody, when compared to both subgroups IIa and IIb (HFD) 64.066 ± 3.05 and 64.111 ± 3.59 respectively. subgroups IIa and IIb showed a significant decrease (P<0.05) when compared to both subgroups Ia and Ib (control 8 and 12 weeks) 72.557 ± 6.3715 and 71.528 ± 5.952 respectively (Histogram 1D).

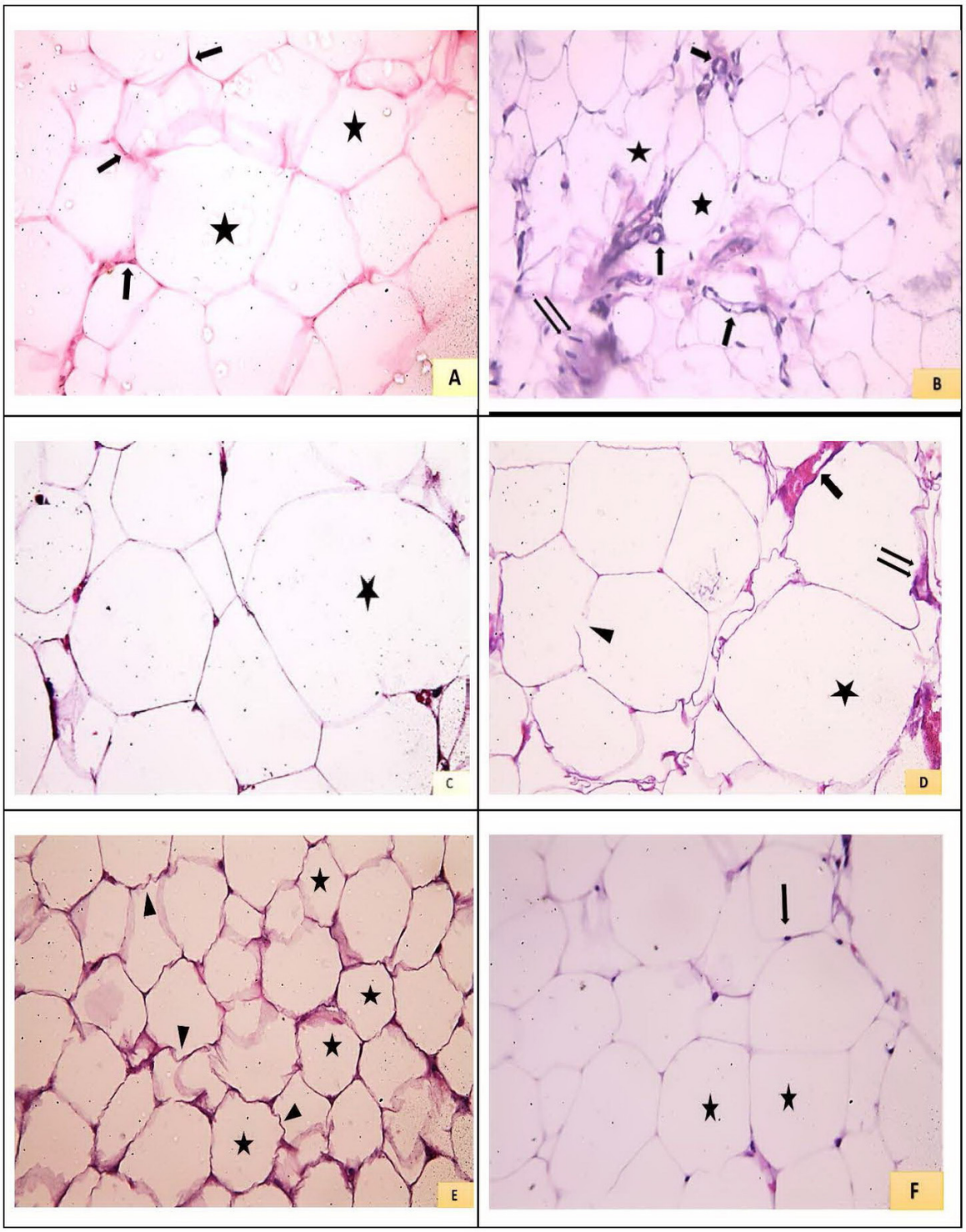


Fig. 1[A-F]: Photomicrographs of adipocytes in inguinal adipose tissue showing [A]Group I (control) unilocular polygonal adipocytes of variable sizes with clear cell boundaries. Each cell contains a single large unstained vacuole (*) and thin rim of cytoplasm at the periphery. The nucleus is flat and eccentric in position giving the signet ring appearance (↑). [B] subgroup IIa (HFD 8 weeks) large adipocytes and coalescence between few adipocytes (*). Inflammatory cellular infiltration (↑↑) among blood vessels can be seen (↑).[C]subgroup IIa (HFD 8 weeks)large adipocytes (*) are noticed.[D]subgroup IIb (HFD 12 weeks) very large adipocytes (*) and coalescence between adipocytes (arrowhead)can be noticed.Inflammatory cellular infiltration (↑↑)and dilated congested blood vessels can be seen (↑).[E]GroupIII (HFD and BA 8weeks) unilocular adipocytes of variable sizes with corrugated cell boundaries(arrowhead). Each cell contains a single large unstained vacuole and thin rim of cytoplasm at the periphery. Notice small sized adipocytes (*).[F]Group IV (HFD and BA 12weeks) unilocular polygonal adipocytes of variable sizes (*).The nucleus flat and eccentric in position (↑). H&E (A,C,D,E X400)(B X100)

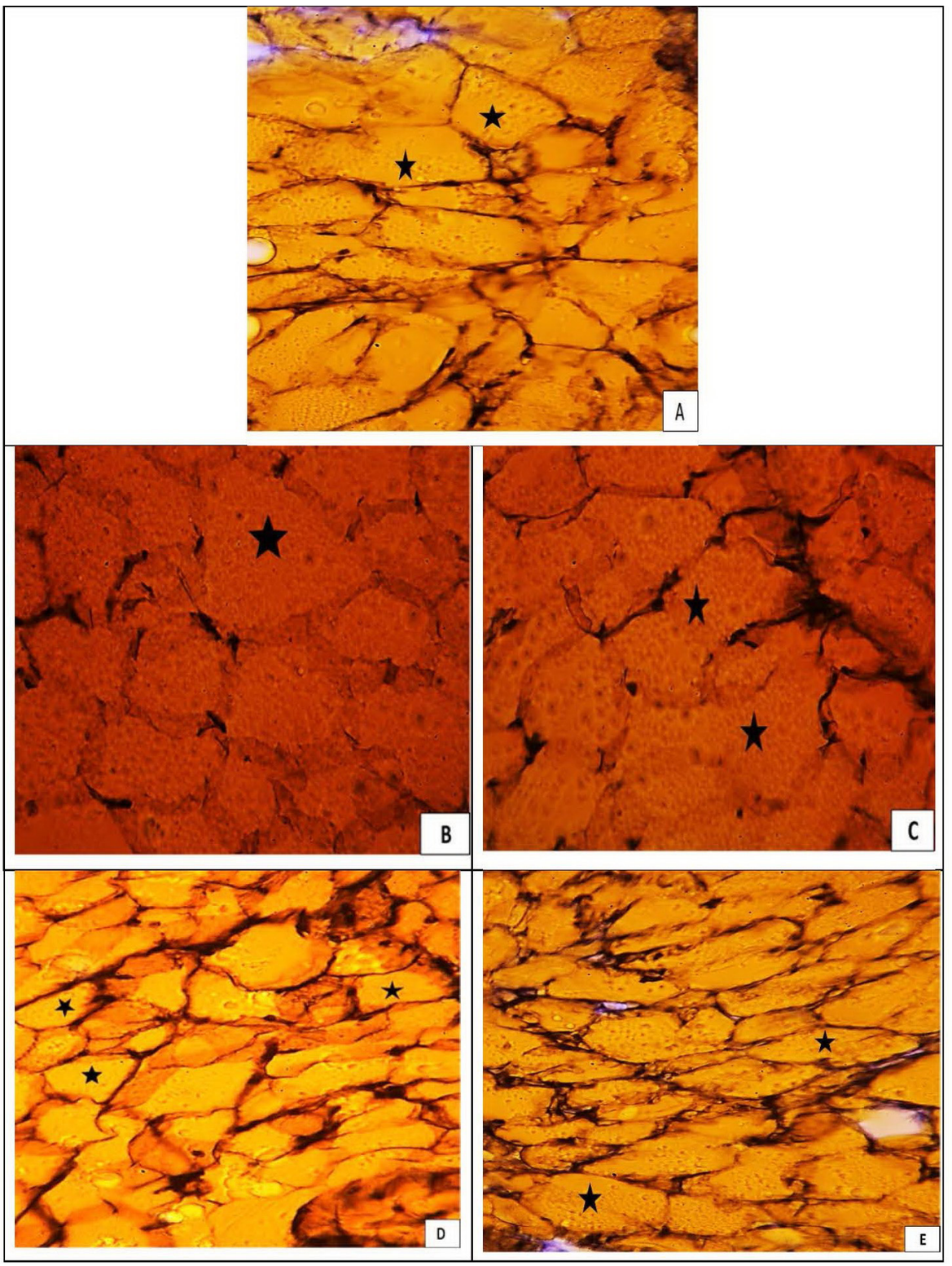


Fig. 2 [A-E]: Photomicrographs of adipocytes in inguinal adipose tissue showing [A]Group I (control)polygonal adipocytes of variable sizes with lightly stained single large lipid droplet occupying whole of the cytoplasm. The nuclei were counterstained blue with Hematoxylin stain. Notice small sized adipocyte with less optically dense stain(*).[B] subgroup IIa (HFD 8 weeks) dark reddish colour of single large lipid droplet, The nuclei were counterstained blue with Hematoxylin (*) is seen.[C] subgroup IIb (HFD 12 weeks)very large sized adipocytes. The cytoplasm showed dark reddish colour of single large lipid droplet, while the nuclei were counterstained blue with Hematoxylin stain (*).[D&E] Group III(HFD and BA 8weeks)and Group IV (HFD and BA 12weeks) showing small sized adipocyte. The cytoplasm showed lightly stained single large lipid droplet, while the nuclei were counterstained blue with Hematoxylin stain (*). (Oil red O stain X400)

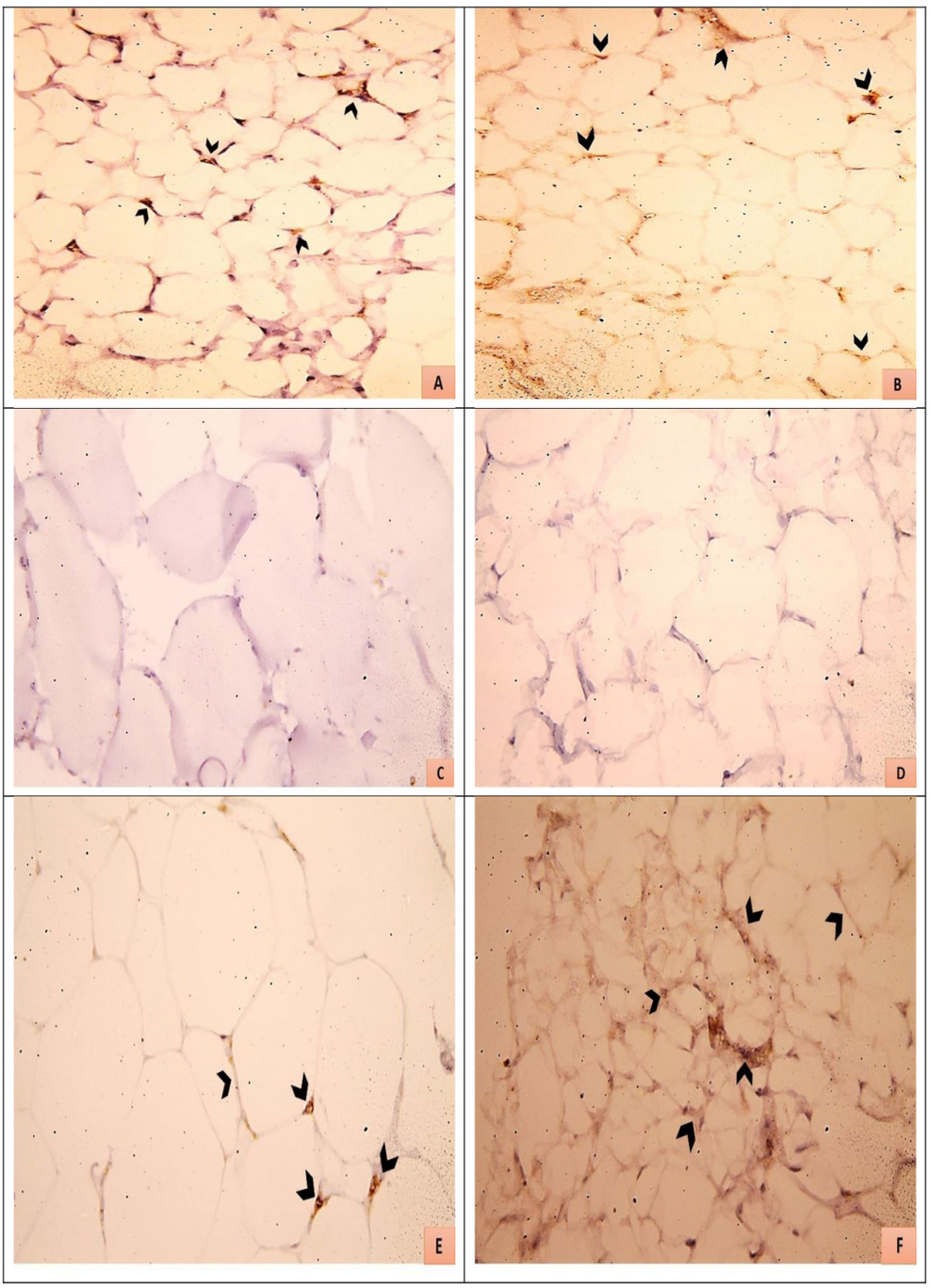
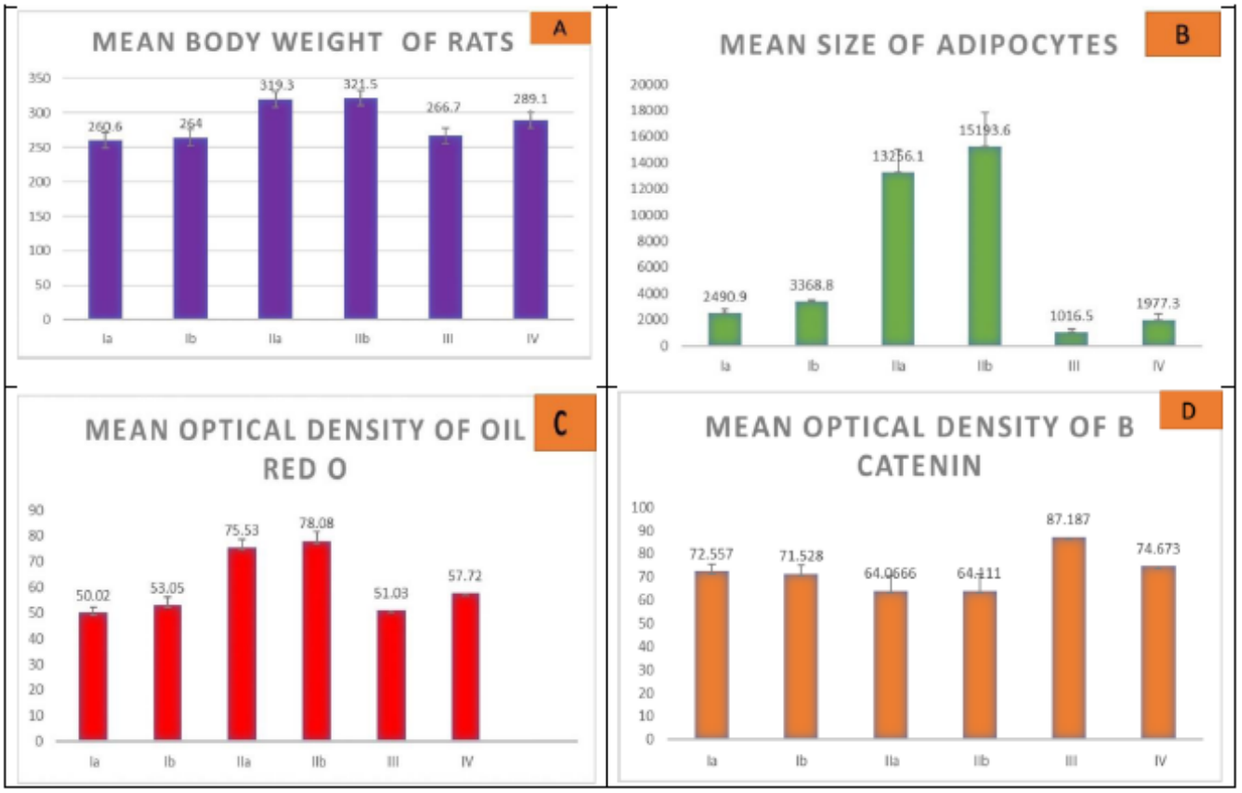


Fig. 3[A-F]: Photomicrographs of adipocytes in inguinal adipose tissue showing [A]subgroup Ia (control 8 weeks) and[B]subgroup Ib (control 12weeks) showing positive β –Catenin cytoplasmic and nuclear reaction in some adipocytes (bifid arrow).[C]subgroup IIa (HFD 8 weeks) and[D]subgroup IIb (HFD 12weeks) showing a very faint β –Catenin reaction in all adipocytes. [E] Group III (HFD and BA 8 weeks) showing strong positive β –Catenin cytoplasmic and nuclear reaction in some adipocytes (bifid arrow). [F] Group IV (HFD and BA 12weeks) showing strong positive β –Catenin cytoplasmic and nuclear reaction in most of adipocytes (bifid arrow). β -Catenin immunohistochemical stain (A,B,C,D,E X400) (F X100)



Histogram 1 [A-D]: Showing [A] mean body weight of rats, [B] mean size of adipocytes, [C] mean optical density of oil red O and [D] mean optical density of β - Catenin.

DISCUSSION

Obesity is a worldwide problem which leads to increased morbidity and rate of deaths. Therapeutic options present in the markets nowadays for treatment of overweightness have limited clinical use due to their dangerous side effects. So, it was necessary to discover a new, safe, and efficient alternative treatment for obesity which is a challenge for scientists[8].

This study was carried out to assess the outcome of orthoboric acid on the structure of inguinal fat pad of rat in an experimental obese model induced by HFD to evaluate its role in inhibition of adipogenesis.

When epididymal and inguinal fat were examined in a study aimed to postulate HFD induced changes in fat cell size and cytokine/chemokine expression in visceral and hypodermal fat tissue in obesity prone (OP) and obesity resistant (OR) rats, it has been noticed that there were increased ratio of large adipocyte (>5000 μm^2) in adipose tissue of OP rats and increased ratio of small adipocytes (<4000 μm^2) in OR rats[19]. In the current study subgroup IIa and IIb (HFD) 8 and 12 weeks respectively when stained with H&E revealed the same results as there were large adipocytes and some adipocytes coalesce together forming one large adipocyte which were confirmed by statistical results that showed increased mean size of adipocytes in rats of HFD group relative to control group. In parallel with these results some authors noticed a phenotypic shift in epididymal white adipose tissue from anti-inflammatory (M2) to pro-inflammatory (M1) macrophage which was obvious on the 5th week of the experiment. While on the end of the 9th week, metabolic inflammation was obvious[20]. In the current study there were similar results cause an inflammatory cellular infiltration and congested blood vessels have been noticed.

Other authors approved similar results as they found increased size and area of adipocytes sampled from perigonadal fat of male mice treated with HFD. Over nutrition is a critical cause of increased size and area. Fat cells are characterized by their size, area and number indicating fat cell hyperplasia and hypertrophy which is encouraged by excessive storage of energy due to excess food intake. These actions leading to metabolic syndrome in which fatty acid dysregulation occurs. This is also referred as a "healthy" fat extension[21]. In accordance with the same results other authors confirmed that mice with excessive fat extension persist metabolically healthy, signifying that extension of fat is not essentially come in relation with metabolic syndrome[22].

Process of adipogenesis require consecutive stimulation of numbers of pro-adipogenic transcription factors, including CCAAT/enhancer-binding proteins (C/EBPs) and peroxisome proliferator- activated receptor γ (PPAR γ)[23]. C/EBPs regulate expression of genes responsible for fat cell differentiation. During the very early

stage of adipogenesis C/EBP β and C/EBP δ are expressed meanwhile there is a reduction in their expression in the terminal stages. Both C/EBP β and C/EBP δ encourage the expression of C/EBP α and PPAR γ, which perform a chief role in control of the late stages of adipogenesis. PPAR γ is a principal controller of adipogenesis[24]. In the present study, subgroup IIa (HFD 8 weeks) when examined using oil red O staining to demonstrate fat accumulation inside fat cells, large densely stained lipid droplet inside adipocyte and coalescence between adjacent adipocytes could be observed. The same results present with the other HFD subgroup IIb (HFD 12 weeks) in which lipid droplets showed increased optical density of oil red O stain determining more lipid accumulation hence, stimulation of adipogenesis process.

Induction of Wnt/β–Catenin signalling pathways were postulated by some authors to inhibit adipogenesis in preadipocytes[16]. Wnt signalling pathway perform a vital role in adipogenic differentiation. It was found that there were several types of Wnt proteins. Wnt10b activates canonical Wnt signalling which in turn downregulate the expression of C/EBP α and PPAR γ resulting in inhibition of adipogenesis in preadipocytes[25]. Other researches proved that canonical Wnt 3a increases levels of β – Catenin hence causes dedifferentiation of both 3T3-L1 and human adipocytes[26]. In the current study when subgroup IIa and IIb (HFD 8, 12 weeks) processed for Immunohistochemical staining for β–Catenin. They revealed a very faint β–Catenin reaction in all adipocytes confirming the same results reached by some authors who have found that Knockdown of β–Catenin may induce spontaneous adipogenesis[27].

Meanwhile, Immunohistochemical staining for β – Catenin of sections of groupIII (HFD and BA 8weeks) revealed a strong positive β – Catenin cytoplasmic and nuclear reaction in some adipocytes while, group IV (HFD and BA 12 weeks) revealed a strong positive β –Catenin cytoplasmic and nuclear reaction in most of adipocytes indicating activation of wnt / β – Catenin signalling pathway in case of inhibition of adipogenesis which in turn induced by BA. Similar results have been reported when retinoids found to regulate adipogenic differentiation on the level of human bone marrow mesenchymal stem cells (hBMSCs) via initiation of Wnt/ β – Catenin signalling pathway with subsequent hang-up of fat synthesis[28].

On the other hand, other researches presented contradictory results which approved that suppression of Wnt4 or Wnt5a expression prevent lipid accumulation and decrease expression of adipogenic genes[29]. Both Wnt 5a and Wnt 5b induces the expression of PPARγ and FABP4, which subsequently induce adipocyte differentiation and inhibit β – Catenin- dependent Wnt signalling at the beginning of adipogenesis[30].

Boron compounds are present in nature in two forms, Orthoboric acid which is hydrogen borate (H_3BO_3) and Borax which is sodium pentaborate pentahydrate NaB[12]. Orthoboric acid is a weak inorganic acid with antiseptic properties. Low concentrations of orthoboric acid doesn′t pose any toxicity. However, like other chemicals it is poisonous if swallowed or inhaled in large quantities.

In the present study, statistical analysis of the mean body weight of the rats revealed that orthoboric acid treated groups, group III (HFD and BA 8weeks) and group IV (HFD and BA 12weeks) showed a significant decrease in the mean body weight of the rats especially in rats of group III in comparison to subgroup IIa and subgroup IIb. The same results have been confirmed before when found that small dosage of oral boric acid cause significant decrease in weight with no harmful effects when experimental rats showed decrease in body weight mean 28.1% while rats of the control group showed no loss of weight but weight gained mean 0.09%[13]. Orthoboric acid has been used as a dietary enhancement for short- term and long-standing loss of weight[14] while cause inhibition of adipogenesis in progenitor cells in *vitro*[16].

This weight losing effect of orthoboric acid can be explained by some researchers who have found that intake of oral boric acid can cause over expression of thermogenic proteins in the adipose tissue and skeletal muscle tissues via uncoupling proteins (UCPs) pathway. Increasing thermogenesis via this pathway leads to faster destruction of lipid and loss of weight[31].

In the current work, examination of group III (HFD and BA 8weeks) and group IV (HFD and BA 12weeks) revealed unilocular adipocytes of variable sizes. Each cell contained a single large unstained vacuole with thin rim of cytoplasm at the periphery. Large number of small sized adipocytes and corrugated cell boundaries could be noticed in group III.

On examination of oil red O stained sections of group III (HFD and BA 8weeks) showed that it was formed of unilocular polygonal adipocytes of variable sizes. Each cell contained a single large lipid droplet occupying whole the cytoplasm. The same results were seen in group IV (HFD + BA 12weeks). However, less optically dense oil red O stained lipid droplets compared to HFD subgroup IIa and IIb could be noticed.

Small size of adipocytes and corrugated cell boundaries could be explained by reduction in lipid content inside the cytoplasm due to inhibitory effect of orthoboric acid on adipogenesis, moreover, the same explanation can be used to demonstrate the cause of low optical density of oil red O stain in these orthoboric acid treated groups III and IV. Some authors have found that Boron treatment suppresses mitotic clonal expansion and adipogenesis-related genes, including PPAR-γ via activation of β-catenin pathways(16).

As regard group III, statistical analysis showed significant decrease in mean body weight, mean size of adipocytes, mean optical density of oil red O compared to group IV. These results have been explained by authors who have found that boron treatment was able to produce

mild destruction of lipid in 3T3-L1 cells signifying that the lipolytic action of boron was not as efficient as its anti-adipogenic action[16].

CONCLUSION AND RECOMMENDATIONS

Orthoboric acid is an ideal alternative drug for treatment of obesity. Orthoboric acid was found to inhibit lipid accumulation in adipocytes via stimulation of Wnt - β Catenin pathway which in turn play an important role in inhibition of adipogenesis process. It is recommended that further studies should consider giving orthoboric acid for the control group and consider also a longer duration of the experiment to assess if any side effects appeared along the period of experiment. Moreover, follow up of body weights is essential for long term drug resistance and recurrence of weight gain.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

الدور المحتمل لحمض أورثوبوريك على تكوين الشحوم في ذكر الجرذ الابيض البالغ دراسة هستولوجيةوهستوكيميائية مناعية

رضا احمد حسن، منى حسين رأفت، محمد عبد الرحمن أحمد مكاوى، سمير نيروز إبراهيم

قسم الهستولوجيا وبيولوجيا الخلية، كلية طب عين شمس، القاهرة، مصر

خلفية: تعد السمنة مشكلة عالمية تؤدي إلى زيادة المرض ومعدل الوفاة. كما يعتبر الاستخدام السريري لبعض الخيارات العلاجية لعلاج السمنة محدود بسبب آثارها الجانبية الشديدة. كما يعد حمض الأورثوبوريك علاج جديد وآمن ومفيد للوقاية من زيادة الوزن التي يسببها النظام الغذائي عالي الدهون.

الهدف: دراسة تأثير حمض الأورثوبوريك على تكون الشحوم في ذكور الجرذان البيضاءالبالغة.

المواد والطرق: اشتملت الدراسة على ثلاثين من ذكور الجرذان البيضاء البالغة. تم تقسيم الجرذان الى أربعة مجموعات رئيسية: المجموعة الأولى: الضابطة. المجموعة الثانية:(مجموعة النظام الغذائى عالى الدهون) والتىتم تقسيمها إلى مجموعتين فرعيتين: المجموعة الفرعية (ا): النظام الغذائى عالى الدهون لمدة ثمانية اسابيع والمجموعة الفرعية (ب): النظام الغذائى عالى الدهون لمدة اثنا عشراسبوعا. المجموعة الثالثة: (النظام الغذائى عالى الدهون وحمض الأورثوبوريك) لمدة ثمانية اسابيع.المجموعة الرابعة: النظام الغذائى عالى الدهون لمدة اثنا عشر أسبوعا ولكن بدءًا من الأسبوع الثامن تم اعطاء الجرذان حمض أورثوبوريك يوميًا لمدة اربعة أسابيع.تم استخدام حمض الأورثوبوريك بجرعة ٢,٥ مجم/جرذ عن طريق الفم عبر أنبوب داخل المعدة. تم تسجيل أوزان الجسم لجميع الجرذان أسبوعيا خلال فترة التجربة.تم جمع عينات الأنسجة الدهنية في نهاية التجربة ومعالجتها بصبغةالهيماتوكسيلين والأيوسين ,الزيت الاحمر وصبغة هيستوكيميائيه مناعية مضادة ل ب-كاتينين. كما تم إجراء التحليل المورفومتري والإحصائي.

النتائج : أظهرت مجموعةالنظام الغذائى عالى الدهون زيادة ذو دلاله ملحوظة في وزن الجسم وحجم الخلايا الدهنية مقارنة بالمجموعة الضابطة ،بينما أظهرت المجموعات المعالجة بحمض الأورثوبوريك انخفاضًا ذو دلاله ملحوظة في وزن الجسم وحجم الخلايا الدهنية مقارنة بمجموعةالنظام الغذائى عالى الدهون. أما فيما يتعلق بتأثير حمض الأورثوبوريك على تكوين الشحوم أظهرت المجموعات المعالجة بحمض الأورثوبوريك تفاعل كيميائي مناعي إيجابي مع ب. كاتينين مع انخفاض لاحق فى تراكم الدهون تم تأكيده بانخفاض الكثافة البصرية لصبغة الزيت الاحمر-و مقارنة بمجموعة النظام الغذائى عالى الدهون.

الاستنتاجات: الجرعة المنخفضة من حمض الأورثوبوريك عن طريق الفم كانت قادرة على خفض وزن الجسم لذلك يمكن اعتباره علاجًا مناسبًا للسمنة نظرًا لدوره الحيوي في تثبيط تكون الشحوم.