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Histological Effect of Curcumin on Aortic Changes "Induced By Obesity" in the Male Albino Rats (Light and Electron Microscopic Study)

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ABSTRACT:

Background: Obesity can incidence ischemic heart disease and stroke (the leading causes of mortality worldwide). Curcumin can protect against atherosclerosis and platelet aggregation. The aim of this work was to study the light and electron microscopic changes induced by obesity in aorta of albino rats and the possible role of curcumin in their correction.

Material and methods: Eighty male albino rats were divided into four groups: **Control group:** fed normal laboratory diet for one month. **Obese group:** received one intra-peritoneal injection of Triton (250 mg/kg) to induce obesity and fed normal laboratory diet for one month. **Recovery group:** similar to obese group but left for another month. **Treated group:** similar to obese group but treated with oral curcumin (50 mg/kg/day) for another month. Finally, body weight and serum lipid profile were measured. Aortic samples were taken for LM and EM study. LM slides were photographed and analyzed, while ultra-thin sections were examined by TEM. For statistical analysis: means, standard errors and P-values were calculated (for body weight, serum lipid profile, atherosclerotic indices, thickness of aortic tunica intima and number of vascular smooth muscle cells "VSMCs" in aortic tunica media).

Results: Body weight, serum lipid profile and atherosclerotic indices showed significant increase in obese group then significant improvement in treated group. LM study revealed significant increase in both intimal thickness and VSMCs number of obese group then insignificant improvement in recovery group and significant improvement in treated group. EM study of obese group revealed thickened intima, endothelial "hook like projection", wide sub-endothelial space, ruptured internal elastic lamina "IEL" and increased interstitial fibers. Recovery group revealed slight improvement in intimal thickness, interstitial fibers but ruptured IEL still present. Treated group revealed marked improvement in the form of smooth endothelial surface, narrow sub-endothelial space and continuous IEL, however, few interstitial fibers still present.

Keywords: Obesity, Triton, Curcumin, Aorta, Cardiovascular, Albino rats.

INTRODUCTION:

Obesity is one of the main causes for incidence of cardiovascular disease [(1)]. A ten kg higher body weight (specially, when the obesity is of abdominal distribution) is associated with a 3.0 mm Hg higher systolic and 2.3 mm Hg higher diastolic blood pressure. These increases translate into an estimated 12% increased risk rate for ischemic heart disease and 24% increased risk rate for stroke [(2)]. Cardiovascular disease, especially ischemic heart disease and stroke, remain the leading cause of mortality worldwide and a major contributor to disability and rising costs of healthcare. In 2010 alone, cardiovascular disease was a primary cause of 15.6 million global deaths [(3)].

Nowadays, there is an increased interest for using natural dietary products to manage obesity and related health problems due to their safety, efficacy and cost [(4)]. One of these compounds is curcumin which is derived from the rhizomatous herb, turmeric (curcuma longa). Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antiviral and antibacterial activities [(5)]. It also protect against atherosclerosis and platelet aggregation.

The aim of this work was to study the light and electron microscopic changes in the aorta induced by obesity in the male albino rats (injected intra-peritoneally by Triton WR 1339 "250 mg/kg" to induce hyperlipidemia and obesity more than that of cholesterol and oil supplemented diets). The work aimed also to evaluate the hypolipidemic and histologic effect of curcumin extract "50 mg/kg/day" to correct previous changes.

MATERIAL AND METHODS:

All experimental procedures were carried out according to principles and guidelines of Ethics Committee of Faculty of Science, Al-Azhar University, Cairo, Egypt, and according to "Guide for care and use of laboratory animals" issued by US National Institute of Health for use and welfare of experimental animals [(6)].

In this study, eighty male albino rats of Wistar strain, weighing 120 ± 5 grams and ranging from 4-5 weeks in age were obtained from the National Institute for vaccine and anti-serum "VACSERA", Cairo, Egypt and housed in 8 stainless steel cages measuring 120 X 60 X 60 cm (10 rats / cage) in a well-ventilated animal house at the Faculty of Science, Al-Azhar University, Cairo, Egypt. Animals were fed standard laboratory diet with plenty of water to ensure normal growth. All animals were housed for 1 week prior to our study for acclimatization with the current conditions. The weight of animals was recorded every week.

Triton WR 1339 (Tyloxpole)® was purchased from sigma-Aldrich USA in the form of vial 50 ml containing 50 grams of triton. Triton was dissolved in a normal saline to make a solution at a concentration of 100 mg/ml (1 gram of triton/9 ml of normal saline). Triton was given by one intra-peritoneal injection at a dose of 250 mg/kg (i.e. 0.25 ml of the prepared solution/100 grams body weight) according to ***Walaa and Saad*** [(7)].

Curcumin was purchased from Sabinsa corporation USA in the form of curcumin C3 complex® capsule containing curcuma longa root standardized extract 1000 mg with purity >95%. One capsule was evacuated and mixed with distilled water to make curcumin suspension at a concentration of 2 mg/ml (one capsule, 1000 mg of curcumin/500 ml of distilled water). Curcumin was given orally by oro-gastric tube at a dose of 50 mg/kg (2.5 ml of the prepared suspension/100 grams body weight) according to ***Yuanyuan et al.*** [(8)], who revealed that curcumin is safe at this dose.

The eighty male albino rats were divided into four groups (20 rats for each group) as the followings:

1. Group I (Control group): fed normal laboratory diet for 1 month then sacrificed.
2. Group II (Obese group): tacked one intra-peritoneal injection of Triton (250 mg/kg) to induce obesity and fed normal laboratory diet for 1 month then sacrificed.
3. Group IV (Recovery group): similar to group II but left for another 1 month (for spontaneous recovery) then sacrificed.
4. Group III (Treated group): similar to group II but treated with oral curcumin (50 mg/kg/day) by oro-gastric tube for another 1 month then sacrificed.

Collection of blood samples and determination of serum lipid profile: After overnight fasting, blood samples were drawn under diethyl ether anesthesia from the retro-orbital plexus of veins of the rats before sacrificing by decapitation. This was occurred after 1 month from the beginning of the experiment for control and obese groups and after 2 month from the beginning of the experiment for recovery and treated groups. Blood samples were collected without anticoagulant in clean dry Wassermann tubes and left in slope position to clot at room temperature. The tubes were centrifuged at 3000 rpm for 5 minutes and the non-haemolysed serum was carefully separated and transferred into clean dry epindorffs which kept frozen at -20 ^{0}C until used for biochemical analysis.

Serum level of total cholesterol (TC) and triacylglycerol (TG) were measured by commercial kits (Vitro Scient Company Egypt). However, serum level of low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (vLDL-c) and high density lipoprotein cholesterol (HDL-c) were measured by commercial kit (Spectrum Company Egypt).

Preparation of tissues for histological EM studies: After anesthesia, blood sampling and decapitation; abdomen and chest ware opened longitudinally, then while the heart was still beating a small incision in the right atrium was done using scalpel and a 12 gauge canola was inserted in the right ventricle to infuse a solution of 2.5% glutraldehyde in a normal saline containing one ampule of heparin directly in the heart. This infuses the rat tissue with the fixative and avoids any destruction or change in the ultra-structure of the cells. Immediately after perfusion of the fixative, aorta was excised and cute into a very small circles about 1mm thick in a dish containing 2.5% glutraldehyde solution, then putted in a small plastic pipes filled with 2.5% glutraldehyde.

According to ***Mark et al.*** [(9)], tissue was transferred to osmium tetroxide that reacts strongly with membrane lipids and with proteins. Osmium was a heavy enough metal to confer some electron density to the membranes and other cellular structures to which it binds. Sample was then embedded by infiltrating it with resins that can be polymerized into a hard plastic suitable for thin sectioning. After imbedding tissue was transferred in to a tissue block (capsule) that was ready for cutting and staining for EM examination.

Semi-thin sections (0.5-1.0 µm) ware stained by toluidine blue and used as a guideline to the area of interest and further trimming (Reagents: 1% toluidine blue and 2% borate in distilled water). Ultrathin sections, 40-100 nm (typically 60 nm for TEM) ware spread on 200 or 300 mesh copper grids and stained with uranyl acetate followed by lead citrate to improve contrast. This was easily done by floating the grids (while sections face down) on droplets of stain, followed by water rinses. A Jem.1010 Transmition electron microscope (Germany) was used for EM examination.

Preparation of tissues for histological LM studies: The remaining part of aorta was washed by normal saline to remove the blood and then fixed in 10% formol saline for 24 hours. The fixed tissues were washed in running tap water to remove the fixative from them, dehydration was done gradually in ascending grades of alcohol by putting the tissues in 50% alcohol then in 70% alcohol and finally in 100% alcohol. Clearing of the tissues was done to remove alcohol and to allow the fixed tissues to be miscible with xylol.

The cleared fixed tissues were put in melted soft paraffin in an oven at 50 ^{0}C for 24 hour then transferred into melted hard paraffin in an oven at 55 ^{0}C for one hour. Specimens were then transferred to casts filled with melted hard paraffin. The casts with their contents were cooled in ice until the paraffin was completely solidified forming blocks of hard paraffin with the tissues in its centers. Each block of hard paraffin was cut into thin sections (5 micrometer) by rotatory microtome.

Paraffin sections representing all groups were then placed on clean glass slides smeared with albumin glycerin, allowing few drops of albumin glycerin to flow beneath them. The slides were warmed on a hot plate then leaved for several hours in the incubator to dry [(10)]. The slides were then stained by Haematoxylin and eosin stain, Masson trichrome stain and Mallory trichrome stain.

Morphological study:

The stained sections (by haematoxylin and eosin stain, Masson trichrome stain and Mallory trichrome stain) were photographed at the image analysis unit of Al-Azhar Faculty of Medicine in Cairo, Egypt. The images were then analyzed by Optimas (version 6.21.19, Media Cybernetics, 1998); after histological LM examination, two parameters were measured in aorta, thickness of tunica intima and number of smooth muscle cells in tunica media.

1. Thickness of tunica intima: Measuring of the thickness of the aortic tunica intima was done from aortae of four animals in each group, for each animal, thickness was measured from four point (0, 90, 180 and 270 degree) at the aorta from the first elastic lamellae to the surface of the endothelium, the average thickness for each animal then calculated. Lastly the means and standard error for each group was calculated and one way analysis of variance (ANOVA) test was done to compare between the four groups.
2. Number of smooth muscle cells in tunica media: Counting the number of smooth muscle cells in aortic tunica media was done at four point (0, 90, 180 and 270 degree) in the aortae of four animals in each group, the average number of smooth muscle cells in the aortic tunica media for each animal then calculated. Finally the means and standard error for each group was calculated and one way analysis of variance (ANOVA) test was done to compare between the four groups.

Statistical analysis: Using Excel 2010, mean values, standard errors and P-values were calculated in all groups for the following parameters:

- Body weight.
- Serum level of lipid profile (total cholesterol, triglyceride, vLDL-c, LDL-c and HDL-c).
- Atherosclerotic indices (Castelli's risk index "CRI", atherogenic coefficient "AC" and atherogenic index of plasma "AIP").
- Thickness of aortic tunica intima and number of vascular smooth muscle cells (VSMCs) in aortic tunica media.

To determine impact of obesity on lipid profile and atherosclerotic indices variables, ANOVA (one-way analysis of variance) was used, with Tukey post-hoc test for multiple comparisons. Intimal thickness and media smooth muscle cell variables either raw or transformed have violated the assumptions necessary to run the analysis of variance (normality of data and homogeneity of variances). Therefore, non-parametric alternative Kruskal-Wallis test was used, accompanied by pairwise comparison using Mann Whitney U test with Bonferroni correction. SPSS software (version 22; IBM Inc., Chicago, Illinois, USA) was used for analyses. The possibility of 0.05 or less was considered significant.

RESULTS:

A. GENERAL RESULTS:

1. Behavior and appetite: After Triton WR 1339 injection; rats were calm, easy to handle without injuries or deaths. In the first month, appetite was increased in the obese group more than in the control group; this was indicated by increased amount of food needed for each group (about 20 grams/ rat/ day for the obese group and 14 grams/ rat/ day for the control group).

However, in the second month, appetite was lowered in the treated group (about 13 grams/ rat/ day) more than in the recovery group (about 17 grams/ rat/ day). The rat's stool in the treated group was greasy in comparison with that of the recovery group. No changes occurred in the skin or hair.

2. Body weight: At the first month, the obese group increased in weight (up to 70 grams per week) while other groups showed lower rate of weight gain (about 30 grams per week). After one month, both of treated and recovery groups, started to lose weight but it was faster in the treated group (about 35 grams per week) than in the recovery group (about 10 grams per week).

After one month, we found a significant increase in the body weight of the obese group when compared to the control group. However, at the end of the experiment the treated group showed a significant reduction in their body weight when compared with the recovery group, (table 1 and chart 1).

Table (1): Comparison between mean values, standard errors and P-values of "body weight" in various groups

Groups	Body weight (grams)
Control	242 ± 04.37 ^{c}
Obese	358 ± 10.70 ^{a}
Recovery	318 ± 08.15 ^{b}
Treated	260 ± 05.81 ^{c}

Means with different superscript vary significantly ($P < 0.05$).
(a) High value - (d) Low value - (b) and (c) In between.

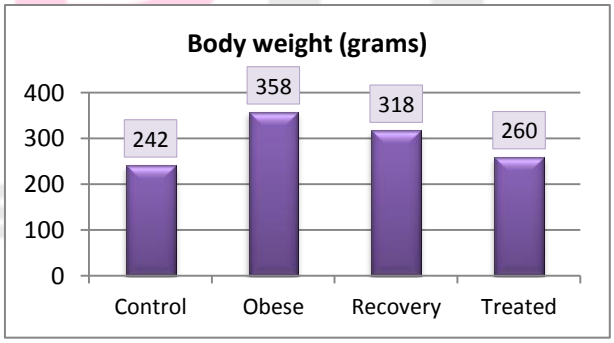


Chart (1): A histogram comparing between mean values of "body weight" in various groups

B. BIOCHEMICAL RESULTS:

Serum lipid profile: at the end of the experiment, the serum lipid profile was measured for each rat. This profile includes serum level of total cholesterol "TC", triglyceride "TG", very low density lipoprotein-cholesterol "vLDL-c", low density lipoprotein-cholesterol "LDL-c" and high density lipoprotein-cholesterol "HDL-c".

The results observed in table (2) and chart (2) cleared that: the serum level of lipid profile (apart from serum level of high density lipoprotein-cholesterol "HDL-c") showed a significant increase in the obese group when compared to other groups. However, they showed a significant reduction in both treated and recovery groups when compared to the obese group (the

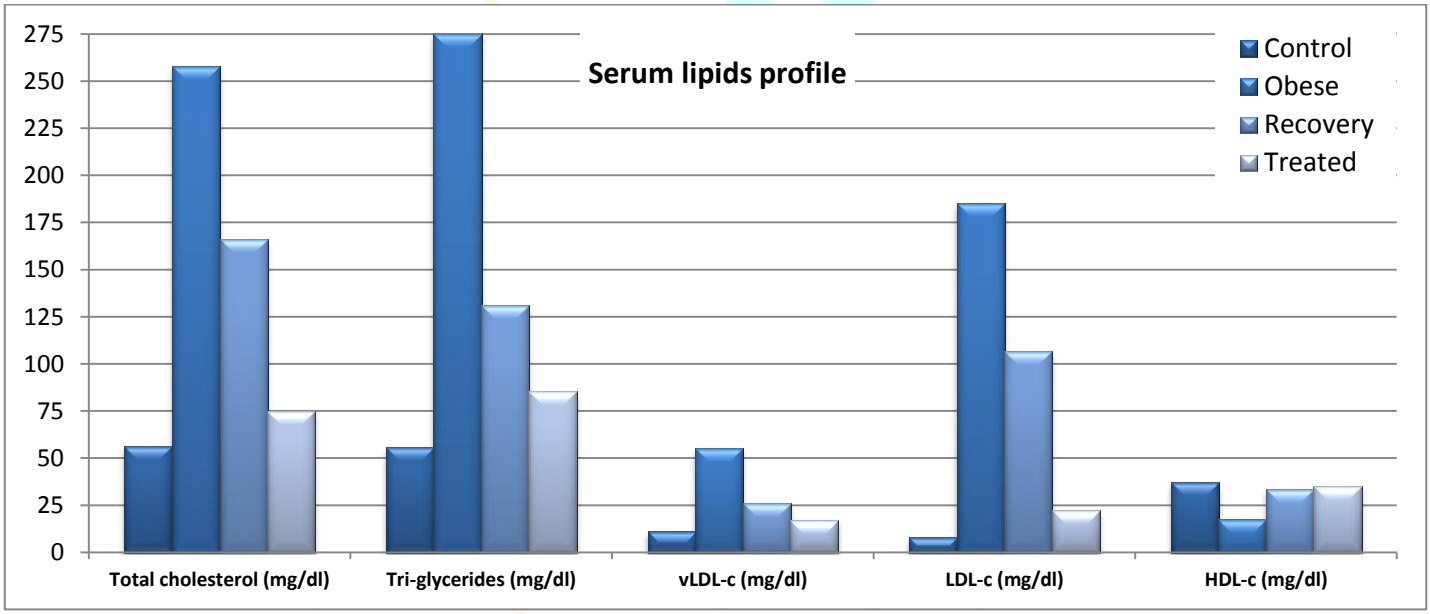
reduction was more significant in the treated group than that of the recovery group). In general, the serum level of these chemicals still higher in all experimental groups when compared to the control group.

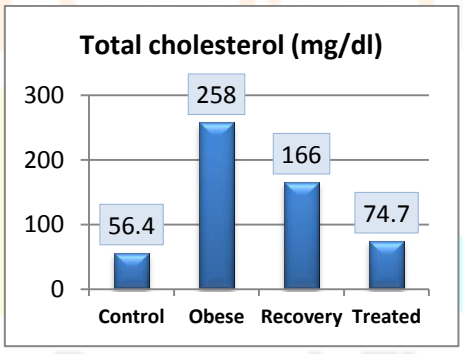
On the other side, the serum level of high density lipoprotein-cholesterol "HDL-c" showed a significant reduction in the obese group when compared to other groups. However, it showed a significant increase in both treated and recovery groups when compared to the obese group (the increase was more in the treated group than that of the recovery group). In general, the serum level of high density lipoprotein-cholesterol "HDL-c" still lower in all experimental groups if compared to the control group.

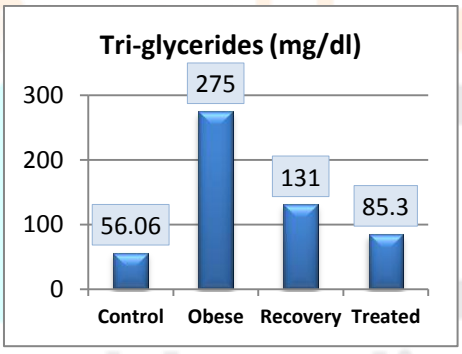
Table (2): Comparison between mean values, standard errors and P-values of "serum lipids profile" in various groups

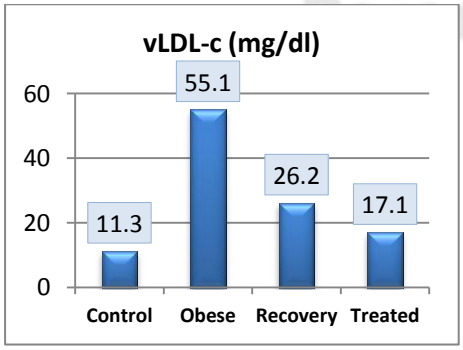
Groups	Total cholesterol (mg/dl)	Tri-glycerides (mg/dl)	vLDL-c (mg/dl)	LDL-c (mg/dl)	HDL-c (mg/dl)
Control	056.40 ± 03.25 ^{c}	056.06 ± 04.61 ^{c}	011.30 ± 00.92 ^{c}	008.26 ± 02.46 ^{c}	036.90 ± 01.61 ^{a}
Obese	258.00 ± 12.30 ^{a}	275.00 ± 12.10 ^{a}	055.10 ± 02.41 ^{a}	185.00 ± 14.30 ^{a}	017.80 ± 00.36 ^{b}
Recovery	166.00 ± 05.25 ^{b}	131.00 ± 14.60 ^{b}	026.20 ± 02.92 ^{b}	107.00 ± 06.58 ^{b}	033.60 ± 02.08 ^{a}
Treated	074.70 ± 04.30 ^{c}	085.30 ± 03.67 ^{c}	017.10 ± 00.73 ^{c}	022.30 ± 03.94 ^{c}	035.30 ± 01.12 ^{a}

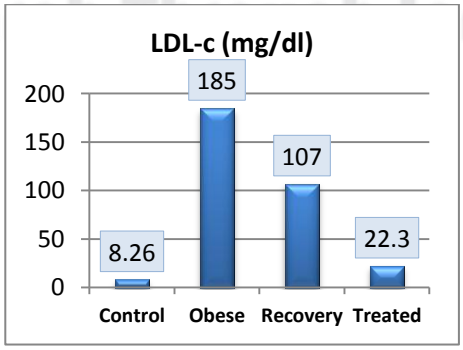
Means with different superscript vary significantly ($P < 0.05$).
(a) High value - (d) Low value - (b) and (c) In between.











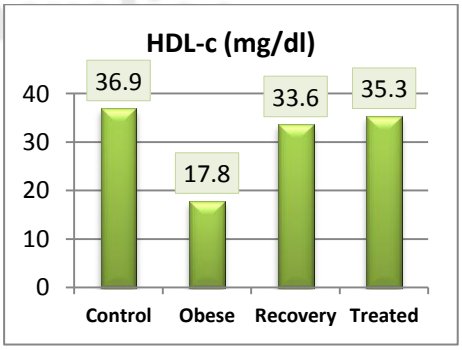


Chart (2): A histograms comparing between mean values of "serum lipid profile" in various groups

Atherosclerotic indices: after measuring the lipid profile (serum level of TC, TG, vLDL-c, LDL-c and HDL-c) for each rat at the end of the experiment, atherosclerotic indices were calculated. They were considered as better markers for atherosclerotic

lesion extent (based on serum lipids). ***World Health Organization*** [(11)] reported that: Castelli's risk index "CRI", atherogenic coefficient "AC" and atherogenic index of plasma "AIP" were more sensitive risk predictors for atherosclerotic cardiovascular disease.

- Castelli's risk index "CRI" is a more powerful coronary risk predictor than total cholesterol "TC", low density lipoprotein-cholesterol "LDL-c" and high density lipoprotein-cholesterol "HDL-c" [(12)].
- Atherogenic coefficient "AC" mirrors the atherogenic tendency that entire lipoprotein fractions were bound to generate [(13)].
- Atherogenic index of plasma "AIP" is a strong marker to predict the risk of atherosclerosis and coronary heart disease [(14)]. It reflects the true relationship between protective and atherogenic lipoprotein and is associated with the size of pre- and anti-atherogenic lipoprotein particle. It has been suggested that the AIP value of under 0.11 was associated with low risk of CVD, the values between 0.11-0.21 and upper than 0.21 are associated with intermediate and increased risks of CVD, respectively [(15)].

The results observed in table (3) and chart (3) cleared that: atherosclerotic indices (Castelli's risk index "CRI", atherogenic coefficient "AC" and atherogenic index of plasma "AIP") showed a significant increase in the obese group when compared to other groups. However, they showed a significant improvement in both treated and recovery groups when compared to the obese group (the reduction was more significant in the treated group than that of the recovery group). In general, atherosclerotic indices were higher in all experimental groups when compared to the control one.

Table (3): Comparison between mean values, standard errors and P-values of Castelli's risk index (CRI), atherogenic coefficient (AC) and atherogenic indices (AIP) in various groups

Groups	Castelli's risk index (CRI)	Atherogenic Coefficient (AC)	Atherogenic index of plasma (AIP)
Control	01.53 ± 0.03 ^{c}	00.53 ± 0.03 ^{c}	00.18 ± 0.05 ^{d}
Obese	14.60 ± 0.79 ^{a}	13.60 ± 0.79 ^{a}	01.19 ± 0.02 ^{a}
Recovery	05.03 ± 0.34 ^{b}	04.03 ± 0.34 ^{b}	00.58 ± 0.05 ^{b}
Treated	02.12 ± 0.14 ^{c}	01.12 ± 0.14 ^{c}	00.38 ± 0.02 ^{c}

Means with different superscript differ significantly ($P < 0.05$).
(a) High value - (d) Low value - (b) and (c) In between.

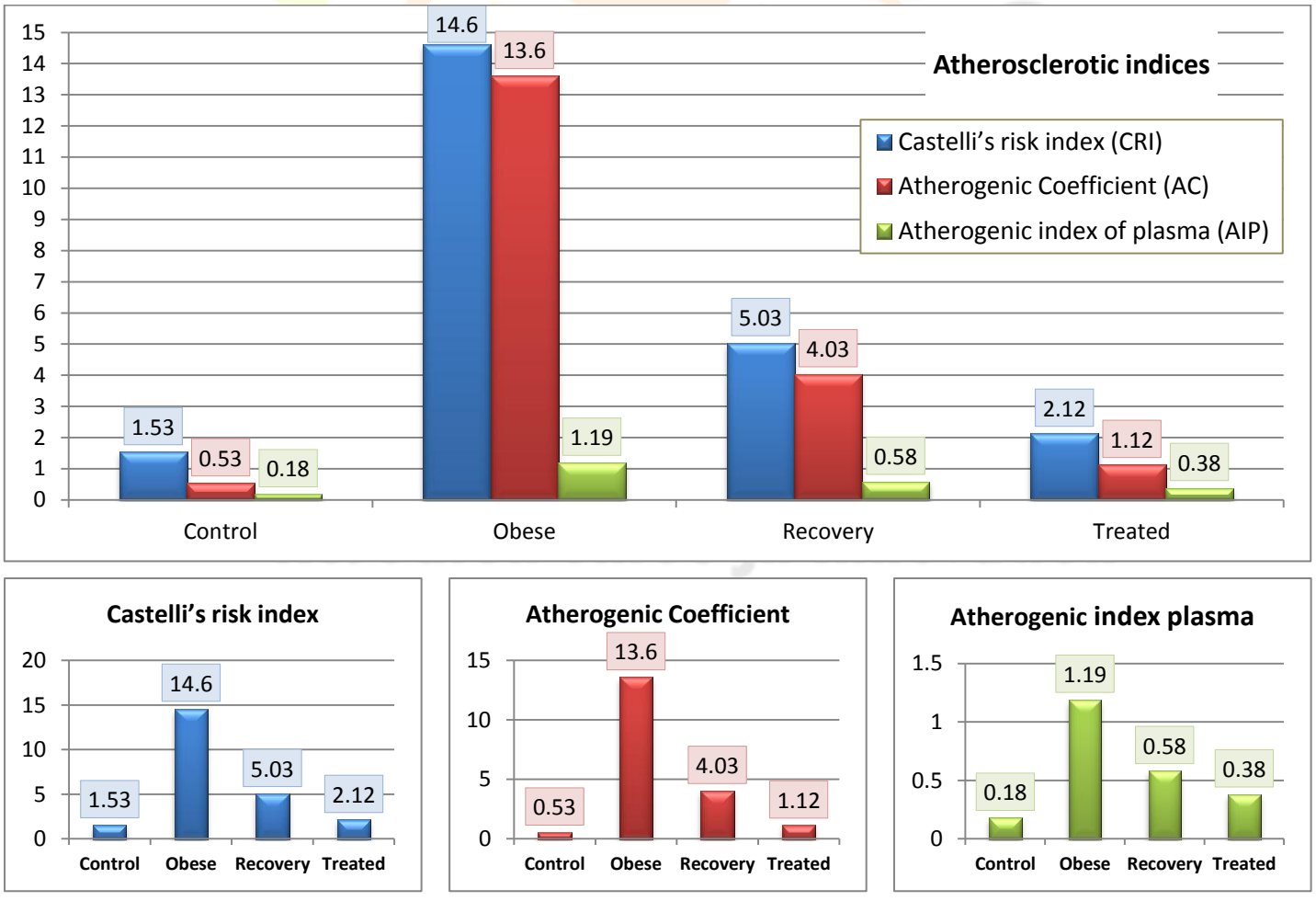


Chart (3): A histogram comparing between mean values of Castelli's risk index (CRI), atherogenic coefficient (AC) and atherogenic index of plasma (AIP) in various groups

C. LIGHT MICROSCOPIC RESULTS:

Control group: histological examination of sections obtained from aortae of the control group and stained by haematoxylin and eosin stain, showed thin endothelium with continuous smooth surface and narrow sub-endothelial space. Tunica media contained regularly arranged wavy elastic lamellae with vascular smooth muscle cells (VSMCs) in-between. Tunica adventitia was devoid from increased perivascular adipose tissue (Figure 1 "A1"). Sections stained by Masson trichrome and Mallory trichrome stains showed normal media containing elastic lamellae and VSMCs in-between (Figure 1 "A2 and A3").

Obese group: histological study of sections obtained from aortae of the obese group and stained by haematoxylin and eosin stain, revealed abnormal changes in the form of thickened endothelium and presence of fatty streak in the media with proliferation of vascular smooth muscle cells (Figure 1 "B1"). Sections stained by Masson trichrome and Mallory trichrome stains showed increased interstitial fibers (Figure 1 "B2 and B3").

Recovery group: histological examination of sections obtained from aortae of the recovery group and stained by haematoxylin and eosin stain, showed slight decrease in the endothelial thickness (that was induced by obesity). Moreover, in comparison with the obese group there was moderate decrease in the amount of both vascular smooth muscle cells and fatty streaks between them (Figure 1 "C1"). Sections stained by Masson trichrome and Mallory trichrome stains showed also slight improvement in the interstitial fibers compared with that of the obese group (Figure 1 "C2 and C3").

Treated group: after treatment with curcumin for 1 month, histological study of sections obtained from aortae of the treated group and stained by haematoxylin and eosin stain, revealed marked decrease in the endothelial thickness (that was induced by obesity). There was also marked improvement in the obesity induced vascular smooth muscle cell proliferation. Fatty streaks disappear from the wall of aortae (Figure 1 "D1"). Sections stained by Masson trichrome and Mallory trichrome stains showed slight improvement in the interstitial fibers between vascular smooth muscle cells (Figure 1 "D2 and D3").

D. STATESTICAL ANALYSIS:

Automated image analysis of our photographed histological sections was done using Optimus software (version 6.2.1, Media Cybernetics, USA) to measure two histological parameters in aortae of each group:

- Thickness of aortic tunica intima.
- Number of vascular smooth muscle cells (VSMCs) in aortic tunica media.

The results (observed in table 4 and chart 4) revealed a significant increase in the thickness of aortic tunica intima of the obese group when compared to the control one. On the other hand, we detected a significant decrease in the treated group and insignificant decrease in the recovery group when compared to the obese group. In general, the thickness of aortic tunica intima still high in all experimental groups (if compared to the control one).

Table (4): Comparison between mean values, standard errors and P-values of "thickness of aortic tunica intima" in various groups

Groups	Thickness of aortic tunica intima (µm)
Control	03.20 ± 0.12 [b]
Obese	10.10 ± 0.94 [a]
Recovery	08.73 ± 0.44 [a]
Treated	03.64 ± 0.16 [b]

Means with different superscript vary significantly ($P < 0.05$).
(a) High value - (d) Low value - (b) and (c) In between.

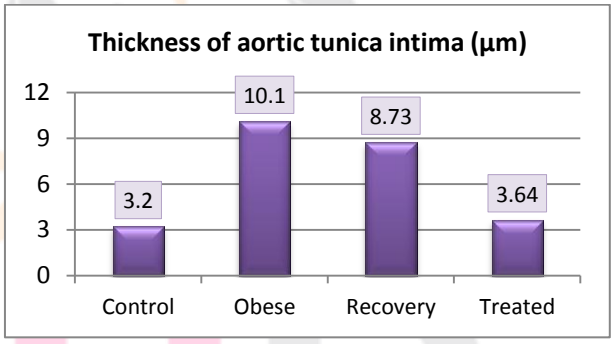


Chart (4): A histogram comparing between mean values of "thickness of aortic tunica intima" in various groups

As regard to the number of vascular smooth muscle cells (VSMCs) in aortic tunica media (table 5 and chart 5), we revealed a significant rise in the obese group when compared to the control group. Moreover, we detected a significant decrease in the treated group and insignificant decrease in the recovery group when compared to the obese group. In general, the number of vascular smooth muscle cells in aortic tunica media still high in all experimental groups (if compared to the control one).

Table (5): Comparison between mean values, standard errors and P-values of "VSMCs in aortic tunica media" in various groups

Groups	VSMCs in aortic tunica media (number)
Control	32.2 ± 4.53 [c]
Obese	51.0 ± 4.42 [a]
Recovery	45.5 ± 2.72 [ab]
Treated	35.0 ± 2.95 [bc]

Means with different superscript vary significantly ($P < 0.05$).
(a) High value - (d) Low value - (b) and (c) In between.

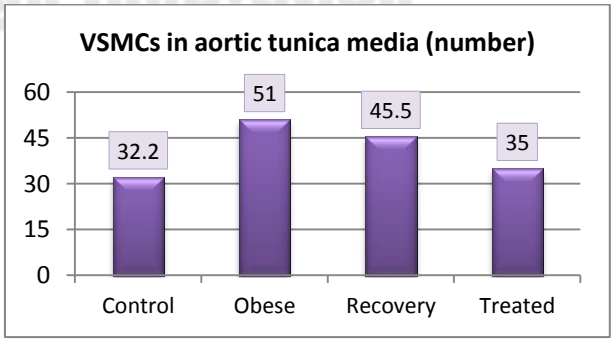


Chart (5): A histogram comparing between mean values of "VSMCs in aortic tunica media" in various groups

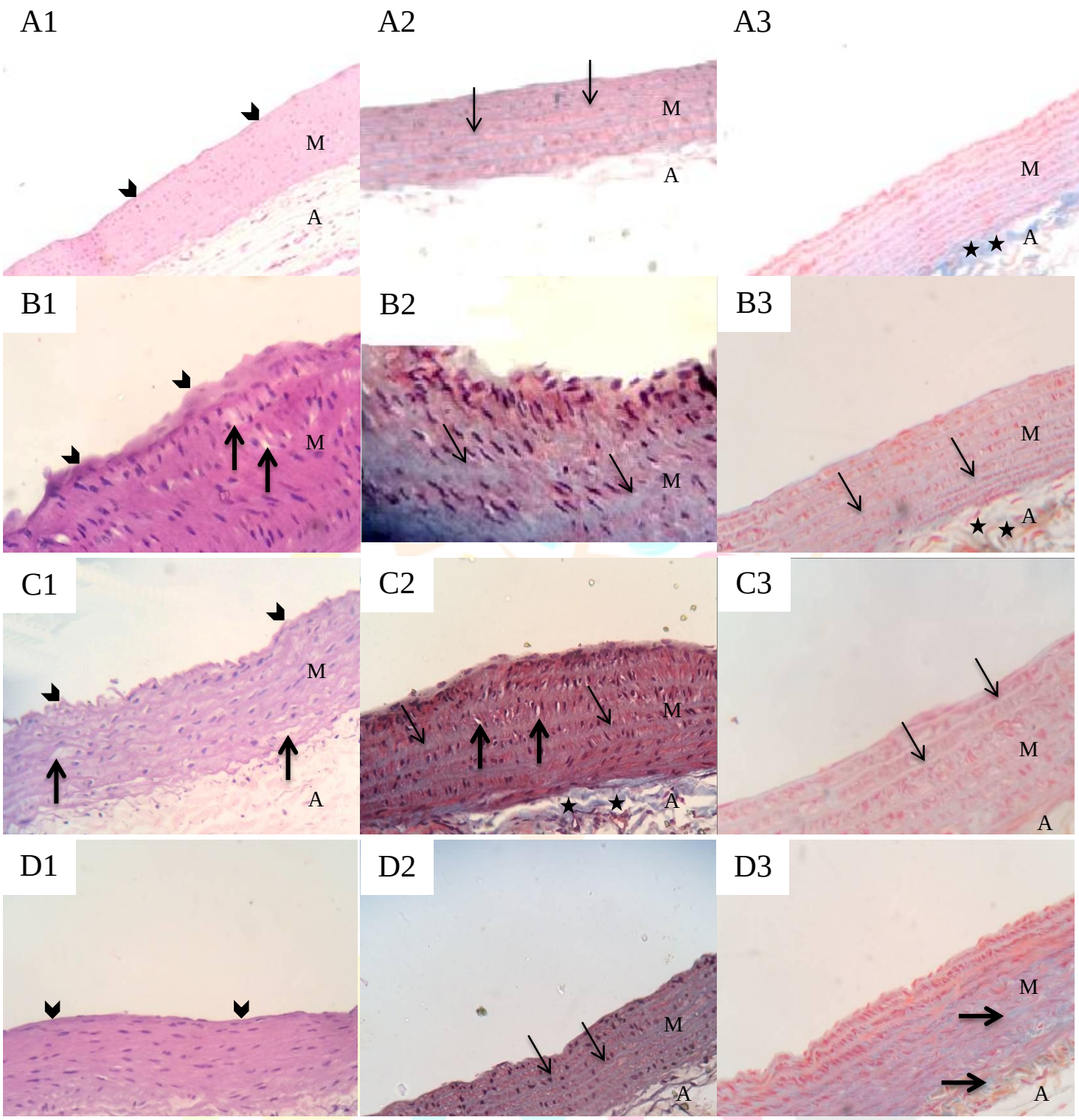


Figure (1): Photomicrographs of aortic sections obtained from various experimental groups and stained by Haematoxylin and eosin stain (Left column "1"), Masson trichrome stain (Middle column "2") and Mallory trichrome stain (Right column "3"). All photomicrographs were magnified as 400x.

(A) Control group: showed normal thin endothelium with continues smooth surface "arrow heads". Tunica media "M" showed regular wavy elastic lamellae "thin arrows" with vascular smooth muscle cells (VSMCs) in-between. Tunica adventitia "A" contained loose connective tissue "stares" without increase in the perivascular adipose tissue.

(B) Obese group: showed thick endothelium "arrow heads". Tunica media "M" showed fatty streaks "thick arrows" and increased interstitial fibers "thin arrows" between proliferated VSMCs. Tunica adventitia "A" showed increased perivascular adipose tissue "stares".

(C) Recovery group: showed mild decrease in the endothelial thickness (induced by obesity) "arrow heads". Some fatty streaks "thick arrows" between VSMCs of tunica media "M" still present and associated with moderate amount of interstitial collagen fibers "thin arrows". Tunica adventitia "A" showed increased perivascular adipose tissue "stares".

(D) Treated group: showed normal thin endothelium with smooth surface "arrow heads" and normal tunica media "M" containing elastic lamellae "thin arrows" with VSMCs in-between. Fatty streaks disappear from the wall of aorta. However, small amount of interstitial collagen fibers still present in tunica media and tunica adventitia "thick arrows".

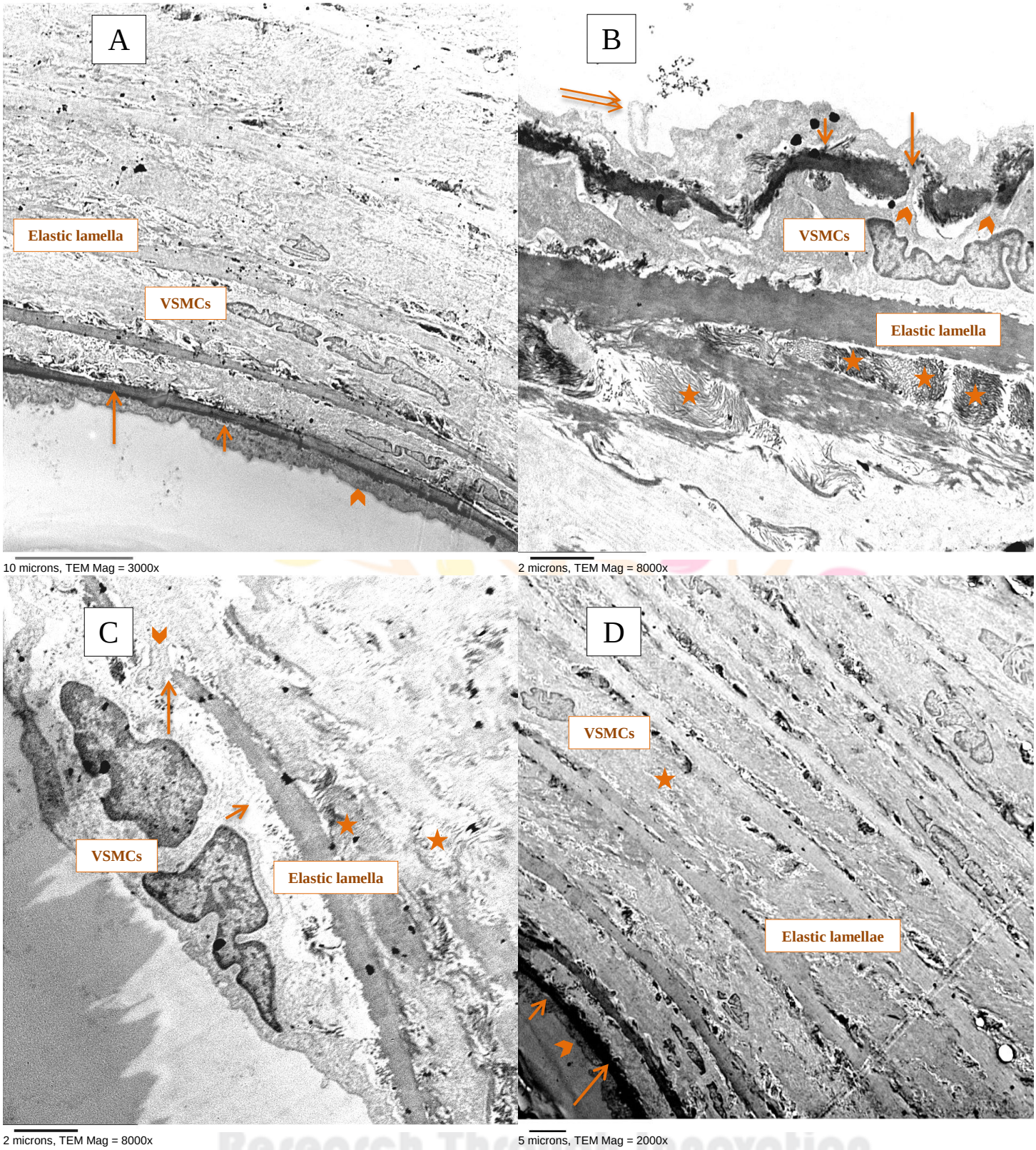


Figure (2): Magnified electron photomicrographs of ultra-thin sections obtained from aortae of various experimental groups.

(A): EM of aorta of the control group showed smooth surface of endothelial cells "arrow head" with narrow sub-endothelial space "short arrow" and intact continuous internal elastic lamina "long arrow". "Elastic lamellae" are normally appeared with vascular smooth muscle cells "VSMCs" in-between (3000x).

(B): EM of aorta of the obese group showed thick tunica intima with "hook like projection" from endothelial cell membrane "double arrow", wide sub-endothelial space "short arrow" and rupture of internal elastic lamina "long arrow" occupied by processes of VSMCs "arrow heads". Interstitial fibers "stars" were excessively deposited between VSMCs (8000x).

(C): EM of aorta of the recovery group showed wide sub-endothelial space "short arrow" and rupture of internal elastic lamina "long arrow" occupied by cytoplasmic process of VSMCs "arrow head". Interstitial fibers "stars" were slightly deposited between VSMCs (8000x).

(D): EM of aorta of the treated group showed thin continues endothelium with smooth surface "arrow head", narrow sub-endothelial space "short arrow", continuous internal elastic lamina "long arrow", regularly arranged "elastic lamellae" and few interstitial fibers "stars" deposited between "VSMCs" (2000x).

E. ELECTRON MICROSCOPIC RESULTS:

1. Control group: EM examination of ultra-thin sections obtained from aortae of the control group revealed continuous endothelial cells with smooth surface and cleared cytoplasm without cholesterol or lipid droplet inclusion. They also revealed narrow sub-endothelial space with intact internal elastic lamina. In tunica media we detected normally arranged elastic lamellae with vascular smooth muscle cells (VSMCs) in-between and few amount of extra-cellular fibers without leucocytic or fatty cellular infiltration (Figure 2A).

2. Obese group: EM examination of ultra-thin sections obtained from aortae of the obese group revealed multiple variations from normal in the form of thickened tunica intima associated with "hook like projection" from endothelial cell membrane into aortic lumen. They also revealed wide sub-endothelial space filled with fibrillar and granular substances. Internal elastic lamina was ruptured with extension of cytoplasmic processes from vascular smooth muscle cells (VSMCs) to reach the sub-endothelial space and even extended in-between endothelial cell to reach the aortic lumen. There was also marked increased interstitial fiber deposition between VSMCs (Figure 2B).

3. Recovery group: EM examination of ultra-thin sections obtained from aortae of the recovery group revealed slight improvement in both of the thickness of tunica intima and the deposition of interstitial fibers between VSMCs. However, rupture of internal elastic lamina still present and associated with sub-intimal cytoplasmic extension of VSMCs (Figure 2C).

4. Treated group: EM examination of ultra-thin sections obtained from the aortae of the treated group revealed great morphological improvement in the form of regain of smooth surface of endothelial cells with clear cytoplasm, narrow sub-endothelial space and continuous internal elastic lamina. However, interstitial fiber deposition still present but markedly deceased when compared to those of the obese group (Figure 2D).

DISCUSSION:

Obesity refers to abnormal or excessive fat accumulation with an increase in the body weight. Changes in dietary pattern, such as increased consumption of high fat diet are considered a primary cause of this problem [(16)].

Increased lipid profile of cholesterol and triglyceride has been suggested to be a major risk factor predisposing obese subjects to develop cardiovascular disorders. Some of cholesterol is used by tissues and other returned to liver but if there is much LDL-c in blood, cholesterol may be deposited in different body tissues [(17)]. On the other hand, HDL-c picks up cholesterol and takes it back to liver for reprocessing or excretion by a pathway called reverse cholesterol transport [(18)]. Consequently, decreased HDL-c is associated with decreased cholesterol removal from extra hepatic tissues and increased risk of developing cardiovascular diseases [(19)].

Atherosclerotic indices reflect the true relationship between protective and atherogenic lipoprotein and are associated with the size of pre- and anti- atherogenic lipoprotein particle [(15)]. Moreover, the atherosclerotic indices are significant and independent predictors for cardiovascular disease risk and might be better than traditional lipid parameters [(20)].

All of the currently available anti-lipidemic therapies have their own inherent shortcomings and disadvantages. Therefore, natural treatments have been investigated as potential therapies for lowering blood lipid levels [(21)].

Growing evidence has suggested that curcumin can be used to treat obesity and obesity-related metabolic diseases because it can correct hyperlipidemia, insulin resistance, hyperglycemia and inflammatory symptoms associated with obesity and metabolic diseases [(22)]. Curcumin is the active component of turmeric. Both curcumin and turmeric are well-tolerated and safe, even at high dose (8000 mg/day) with no apparent toxicity [(23)].

Curcumin can induce a cardio-protective effect, through its lipid-lowering properties [(24)]. Also it can improve obesity-induced cardiac changes, via anti-oxidative stress and anti-inflammatory mechanisms in mice [(25)]. Curcumin supplementation lowers plasma triglycerides and cholesterol concentrations, by reducing the expressions of lipogenic genes [(26)].

Our study was focused on the possible therapeutic effect of the curcumin (which has gained an increased interest in recent years) as a potential treatment for obesity-related complications. In the present study, 60 rats from 80 (120 ± 5 grams and 5 weeks old) were treated with triton for a duration of 1 month and designed to exhibit obesity features characterized by an increase in the body weight and presence of hyperlipidemia.

We found a significant increase in body weight of the obese group when compared to the control one, these results agree with ***Leopoldo et al.*** [(27)] who studied the cardiac changes in a rat model of diet-induced obesity. The increase in body weight was probably due to increased adipose tissue resulted from hyperlipidemia as observed in our results. This conclusion agrees with ***Gocmen et al.*** [(28)] who reported that, the increase in the body weight induced by obesity is mainly due to increase in the adipose tissue.

After curcumin treatment we found a significant reduction of body weight in the treated group when compared to the obese one. These results were agreed with ***Maithilikarpagaselvi et al.*** [(29)], who studied the effect of curcumin on hepatic fat accumulation and hyperlipidemia in high-fructose-fed male rats. Body weight loss without caloric restriction are interesting and is probably due to inhibition of lipid metabolic pathways, detected in this study by improved lipid profile after curcumin treatment, with subsequent decrease in adipose tissues of the body.

The same explanation was suggested by ***Ejaz et al.*** [(30)], who reported a significant weight reduction in the obese C57/BL mice after curcumin treatment, he proposed that it was through inhibition of lipid metabolic pathways regulated by Adenosine monophosphate-activated protein kinase (AMPK) and key transcription proteins involved in adipogenesis which cause an increase in basal metabolism, energy expenditure and weight loss. Moreover, weight reduction induced by curcumin treatment may be due to decreased fat absorption from gastrointestinal tract as observed in this study by increased amount of fat in rats excreta detected by greasy excreta of the treated group compared to the excreta of the recovery group. This explanation was

agreed with ***Funamoto et al.*** [31]. We also found a significant reduction of body weight in the recovery group compared to the obese group. However, this reduction of body weight was less than that of the treated group.

On the other side, ***Sarker et al.*** [32] disagreed with our results. He reported that curcumin treatment has insignificant effect in reducing body weight. This disagreement might be due to the use of genetically modified C57BL/6 mice, instead of albino rat used in our experiment.

Regarding lipid profile in our study, rats given an intra-peritoneal injection of triton WR 1339 showed a significant increase in total cholesterol, triacylglycerol and vLDL-c (up to 4.5 times higher than the control). Moreover, there was a highly significant increase in LDL-c (up to 22 times higher than the control). On the other side obese rats exhibited a significant decrease in HDL-c (up to 2 times lower than the control). These results were in accordance with those reported by ***Ciftci et al.*** [33]. The possible cause for this increase could be explained by ***Kuroda et al.*** [34] who reported that hyperlipidemia induced by Tiriton WR 1339 was due to increase in 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase activity leading to inhibition of lipoprotein lipase responsible for hydrolysis of plasma lipids.

After one month of curcumin treatment there was a significant decrease in serum levels of TC, TG, VLDL-c and LDL-c and a significant increase in serum level of HDL-c compared to the obese group, these results agreed with those of ***Azza et al.*** [16]. The cause of this improvement was explained by ***Ramirez-Tortosa et al.*** [35] who stated that: curcumin decrease the activity of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase enzyme leading to hydrolysis of plasma lipids by lipoprotein lipase and subsequent decrease in serum and liver cholesterol, triglycerides and free fatty acid levels.

On the other side, our result disagreed with ***Sahebkar*** [26] who reported a non-significant lipid-lowering effect of curcumin. However, his meta-analysis had limited sample sizes. Furthermore, most of trials that have reported positive effects of curcumin on blood lipid levels were published subsequent to the above-mentioned meta-analysis [21].

Calculation of atherosclerotic indices of serum lipid levels, in our study, revealed a significant increase of Castelli's risk index "CRI", atherogenic coefficient "AC" and atherogenic index of plasma "AIP" in the obese group if compared to those of the control group. There was a strong relation between the elevation in atherosclerotic indices and the presence of histological changes in aortae. These results agreed with the report of ***World Health Organization*** [11] stated that: atherosclerotic indices were more sensitive risk predictors for atherosclerotic cardiovascular disease.

Our results were also agreed with those of ***Jesús et al.***, ***Michel et al.*** and ***Shabnam et al.*** who found that Castelli's risk index "CRI" is a more powerful coronary risk predictor than total cholesterol "TC", low density lipoprotein cholesterol "LDL-c" and high density lipoprotein cholesterol "HDL-c" [12]. While atherogenic coefficient "AC" mirrors the atherogenic tendency that entire lipoprotein fractions were bound to generate [13]. Moreover, atherogenic index of plasma "AIP" is a strong marker to predict the risk of atherosclerosis and coronary heart disease [14]. It reflects the true relationship between protective and atherogenic lipoprotein and is associated with the size of pre- and anti- atherogenic lipoprotein particle. It has been suggested that value of AIP under 0.11 is associated with low risk of CVD, the values between 0.11-0.21 and upper than 0.21 are associated with intermediate and increased risks of CVD, respectively [15].

After curcumin treatment we detected a significant improvement of the treated group more than that of the recovery group in both atherosclerotic indices and histological changes in aortae. The possible mechanism of this improvement was probably through reduction of serum TC and TG and increasing serum level of HDL-c which in turn prevent fat deposition in aortic wall.

Morphological analysis of aortic histology, in our study, revealed a significant increase in both of the thickness of aortic tunica intima and the number of vascular smooth muscle cells (VSMCs) in aortic tunica media of the obese group when compared to the control one. Furthermore, we detected proliferation of collagen fibers between VSMCs, these findings was constant with ***Feng et al.*** [36].

The abnormal intimal thickness was composed initially of very little amount of extra cellular matrix and few vascular smooth muscle cells, as reported by ***Virmani et al.*** [37]. Later on, extracellular deposition of lipid between VSMCs occur, as explained by ***Yutaka et al.*** [38] who proposed that infiltration of aortic wall by lipid and/or apo-lipoprotein is explained by deposition of lipids from serum in the interstitium. The VSMCs proliferation was probably due to increase in the serum cholesterol as observed in the results of this study, this explanation was agreed with ***Chen et al.*** [39] who reported that the cause of VSMCs in their study was the high level of serum cholesterol.

On the other side, we detected a significant decrease in both the thickness of aortic tunica intima and the number of vascular smooth muscle cells (VSMCs) in aortic tunica media of the treated group when compared to the obese group. While in the recovery group histological study revealed insignificant decrease of these parameters.

The possible mechanism of this improvement was probably through reduction of serum TC and TG and increasing serum level of HDL-c which in turn prevent fat deposition in aortic media. Moreover, another possible mechanism responsible for anti-atherogenic action of curcumin may depend on the induction of hemeoxygenase-1 (HO-1), which is a potent antioxidant and vasculoprotective enzyme. Therefore, induction of HO-1 has been claimed to inhibit development of atherosclerosis in mice, as reported by ***Abraham and Kappas*** [40].

Qin et al. [41] suggested that curcumin inhibits VSMCs proliferation through restoring caveolin-1 expression that leads to the suppression of over activated extracellular signal-regulated kinases signaling and causes cell cycle arrest at G1/S phase.

The electron microscopic changes in aortae of the obese group in our study revealed thickened intima, endothelial "hook like projection", wide sub-endothelial space, ruptured internal elastic lamina and increased interstitial fibers. Recovery group revealed slight improvement in intimal thickness, interstitial fibers but ruptured internal elastic lamina still present. Treated group revealed marked improvement in the form of smooth endothelial surface, narrow sub-endothelial space and continuous internal elastic lamina, however, few interstitial fibers still present.

The possible causes of these ultra-structural changes in our study was probably due to increased intracellular accumulation of lipid inclusion, resulted from increased serum lipids and decreased serum HDL-c (responsible for pick up cholesterol and TG from the peripheral tissue to the liver).

The mechanisms underlying the accumulation of collagen in the obese group were explained also by ***Pugliese et al.*** [(42)], who stated that abnormalities in insulin metabolism and insulin growth factors, such as transforming growth factor beta-1 (which directly promote collagen expression) were related to the excessive collagen content. The increase in interstitial collagen fibres may be also caused by high level of cytokines, endothelin and renin-angiotensin-aldosterone, according to ***Rondinone*** [(43)].

Unger [(44)] has suggested that excessive lipid accumulation expands the intracellular pool of fatty acyl-coenzyme-A beyond the needs of the cell for oxidation, providing a substrate for potentially harmful non-oxidative pathways such the creation of de novo ceramides [(45)] and lipid peroxidation [(46)].

SUMMARY AND CONCLUSION:

Obesity is one of the leading causes of cardiovascular disease. This study was performed to evaluate the effect of oral supplementation of curcumin on the aortic changes induced by obesity in the male albino rats treated with Triton WR 1339 (to induce obesity) and fed normal laboratory diet.

In this study, eighty male adult albino rats were divided into four equal groups of 20 rats each.

- **Group (1):** negative control received normal diet only.
- **Group (2):** positive control fed on normal diet and received Triton WR 1339 (250mg/kg once) by intra-peritoneal rout.
- **Group (3):** as group 2 then left for another one month for spontaneous recovery.
- **Group (4):** as group 2 then received curcumin (50 mg/kg/day) orally for another one month.

At the end of this study rats were weighed, their serum lipid profile and atherosclerotic indices were assessed while histological changes in their aortae were analyzed statistically and studied morphologically by LM and EM. The obtained results revealed that, rats treated with triton WR 1339 tended to exhibit obesity features characterized by an increased body weight, elevated serum lipid profile and atherosclerotic indices; beside features of atherogenic aortic changes. However, curcumin treated group showed a significant reduction in their body weight, serum lipid profile and atherosclerotic indices beside improvement in atherogenic aortic changes. Moreover, recovery group showed a significant improvement in their body weight, serum lipid profile and atherosclerotic indices beside insignificant improvement in atherogenic aortic changes. These results suggest that, curcumin supplementations may have some benefits on cardiovascular diseases in patients suffering from obesity.

RECOMMENDATION:

- Firstly, healthy diet programs should be followed to avoid obesity and obesity related complication.
- Secondly, curcumin additive in diet should be used as it has great benefit in several diseases.
- Tertiary, we recommend use of natural product and plants extract as a drug substitutes because they are effective and safe.

DECLARATIONS:

- Consent for Publication: I verify that the author has agreed to submit the manuscript.
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- Competing interests: None.
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