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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(2):1213-1223. **OPEN ACCESS**

Antiobesity and cardioprotective impact of *Saccharum officinarum* peels' extract on experimentally induced obese male rats.

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Obesity is a chronic disease with multi risk factors characterized by an accumulation of visceral fat which causes aptitude towards cardiovascular diseases. Biological activity of *Saccharum officinarum* was multiple for many decades. This study aimed to evaluate the effectivity of *Saccharum officinarum* peels' extract (SOPE) on obesity and atherosclerosis in obese rat model.40 male albino adult rats were randomly and equally divided into four groups, Group (I): served as control group, group (II): high fat diet (HFD) induced obese rats (non-treated group), Group (III): obese rats treated with (SOPE) and Group (IV): obese rats treated with xenical. Rats were evaluated for final body weight, abdominal circumference(AC), Body mass index(BMI), leptin, lipid profile, Oxidative stress, cardiac enzymes, systolic & diastolic blood pressure, heart rate and histoarchitectures for cardiac and aorta tissues. The present study revealed that HFD-induced obese rats showed significantly increased in final BW, AC, BMI, leptin, total cholestrole(TC), triglycerides(TG), low density lipoprotein(LDL), very low density lipoprotein(VLDL), lactate dehydrogenase(LDH), cardiac troponin I(CTnI), creatin kinase(CKMP), systolic blood pressure(SBP), diastolic blood pressure(DBP), heart rate(HR) and clear oxidative stress. Also, hemorrhage in cardiac tissues and atherogensis in aorta tissue of their histoarchitecture has been shown. On the other hand consumption of (SOPE) reversed all these manifestations even in absence of caloric restriction. (SOPE) seems to be an effective target in prevention and potential cure for all risks of obesity and atherosclerosis.

Keywords: Obesity, *Saccharum officinarum* peels' extract (SOPE), leptin, oxidative stress, atherosclerosis, Cardiovascular diseases.

INTRODUCTION

Obesity is a chronic disease with multi risk factors characterized by an accumulation of visceral fat which causes aptitude towards cardiovascular diseases. (Yamauchi and Kadowaki,2013). A plethora of mechanisms, including abnormalities in lipid metabolism, insulin resistance, inflammation, endothelial dysfunction, a dipocytokines imbalance, and inflammasome activation have been suggested to underlie the relationship between obesity and atherosclerosis (Lovren et al., 2015). Atherosclerotic vascular lesions of patients with higher BMI values are more frequent and advanced compared to subjects with normal body weight (McGill et al., 2002). Among individuals without CVD, higher BMI has an independent, linear association with subclinical myocardial injury (Chiadi et al., 2014).

Weight increase is positively associated with the probability of uncontrolled hypertension in obese and overweight hypertensives (Peter Sabaka et al., 2017). As known key regulators of body weight and homeostasis of the energy were leptin and its receptors. A decrease in tissue sensitivity to leptin leads to the development of obesity and metabolic disorders, such as insulin resistance and dyslipidemia (Olga Gruzadeva et al., 2019). Increased levels of leptin and resistance of it may organize reactive oxygen species generation, increasing oxidative stress and promoting inflammation (Sava Berger and Vsevolod, 2018). In addition, many evidences have demonstrated that the piovital roles of oxidative stress in the initiation and progression of cardiovascular diseases (Weilue et al., 2019). Oxidative stress occurs in the myocardium and correlates with dysfunction of the left ventricle. Myocardial calcium handling negatively affected by reactive oxygen species (ROS), cause arrhythmia, and contribute to cardiac remodeling by inducing hypertrophic signaling, apoptosis, and necrosis (Thomas Münzel et al., 2017). Changing the lifestyle is still the main way to lose weight. However, this method is always difficult to implement and the weight rebound is common (CGI). Given the dangers of obesity and the shortcomings of western medicine, alternative treatments should be further investigated (Park et al., 2016 and Ikramuddin et al., 2016). Sugar cane with the scientific name of *Saccharum officinarum* (SO), is cultivated worldwide due to the economical and medicinal value of its high yielding products. The sugarcane wax photochemistry (obtained from stalks and the leaves of sugarcane), juice and its products has demonstrated that the presence of various alcohol, fatty acid, higher terpenoids, phytosterols, flavonoids, -O- and -C-glycosides, and phenolic acids. The future prospective of some products of the sugarcane has been discussed, which needs a phytopharmacological studies and has an important potential to be medicinal product has great value (Amandeep Singh et al., 2015). And based on many studies that have confirmed that herbal medicine is effective in the treatment of obesity (Liu et al., 2017) and natural plant products are expected to be potential ingredients for the development of nature-sourced anti-obesity products in weight loss segment due to rising consumer health awareness (Nan-Nong Sun et al., 2016). Therefore, this study is aimed to evaluate the effectivity of *Saccharum officinarum* peels' extract on obesity and atherosclerosis in obese rat model in comparison to orlistat as reference anti-obesity drug.

MATERIALS AND METHODS

Experimental animals:

40 healthy adult male albino rats of initial body weight 150 – 180 gm were included. Rats were randomly and equally divided into 4 groups, group(I) normal group (control) which received standard chew diet for 28 day. Group (II) received only HFD for 28 day without any treatment for induction of obesity. group(III) received HFD induced obesity treated with *Saccharum officinarum* peels' extract had dosed from the 8th day 2ml (200 mg)/every rat daily from SOPE for the 28th day& group (IV) received HFD induced obesity treated with Xenical had dosed from the 8th day 2ml (2.4 mg)/every rat from prepared suspension for the 28th day. The animal handling were in the faculty of medicine at Zagazig University (No P1-1-2018).At the end of the experiment (the 28th day) the final weight, Abdominal circumference and body mass index were dected to determine the extent of effectiveness of (SOPE) as antiobesity compared to Xenical also,the systolic,The diastolic and Heart rate were determined by using Power lab. Afer that,the blood samples were withdrawn from fasted rats for preparation the serum samples. The heart and aorta were immediately removed from each rat in all groups, rinsed with cold normal saline, dried with filter paper, and kept in 10% formaline-Saline at 40°C for the histological examinations.

Chemical agents:

Xenical^{R} (orlistat 120mg) that available in the market was prepared as suspension for orally dose for the the 4th group (Xenical group) by concentration 2.4 mg/rat in this group.

(Wax extraction) and Sample Preparation:

Saccharum officinarum peels' extract was obtained from fresh *Saccharum officinarum* stems using a mechanical ginder and prepared for dosage the 3rd group according to method of (Phukan and Boruah, 1999).

Laboratory analysis:

ECG and Blood pressure:

Rats were anaesthetised with 1.5g/kg urethane given up and then placed in a supine position with spontaneous breathing for the

measurement ECG. The measurements of ECG were recorded as two phases of 30 mins each phase consisting of 20 cycles. Parameters such as RR, PR, QRS and QT intervals were evaluated using the ECG Analyse software.

Systolic and diastolic blood pressure were measured using Kent non- invasive blood pressure instrument (CODA). Animals were anaesthetise with 1.5g/kg urethane and then placed in a restrainer. Their tails were connected to a cuff and volume pressure recorder, which in turn was connected to the CODA software with computer monitoring and acquisition of data. Bp were monitored for 1 hr in 15 cycles.

The obtained serum was stored at -20°C to determine the serum levels of leptin, TC, TG, HDL, LDL.c, VLDL.c, LDH, CK-MP, Troponin I(CTnI), GSH, SOD, CAT, MDA. After the rats were sacrified, the whole heart and aorta were removed from each rat in all groups, rinsed with cold normal saline, dried with filter paper, and kept in 10% formaline-Saline at 40°C for the histological examinations. Serum leptin levels were determined using Rat leptin Elisa kit (DRG INTERNATIONAL INK.,USA) (Catalog No.:EIA4607).serum cholestrole(TC) levels were determined using kit (Biosource Europe S.A.- Rue de l'industtrie ,8-B 1400 Nivelle –Belgium) .Serum Triglycerides levels(TG) were determined using kit (Biosource Europe S.A.- Rue de l'industtrie ,8-C 1150 Nivelle –Belgium).Serum HDL levels were determined using kit (Biosource Europe S.A.- Rue de l'industtrie ,8-A-1340 Nivelle –Belgium).Serum LDL levels were calculated according to(LDL=TC - TG/5 – HDL). Serum VLDL levels were calculated according to (VLDL = TG/5). Serum LDH levels were determined using Commercial kit Catalog Number 279 001, provided by Egyptian Company for Biotechnology. Serum CK-MP levels were determined using Rat CK- MP (creatin kinase MP Isoenzyme) ELISA kit,cataloge No:MPS2515061 G69. Serum Troponin I (CTnI) levels were determined using Commercial kit, cataloge Number SE12034 provided by Sigma-Aldrich Co. Serum (GSH) levels were determined using kits of (Biodiagnostic Company, Dokiki, Giza, Egypt). Serum (SOD) levels were determined using Biodiagnostic kit (Biodiagnosticcompany, Dokki, Giza, Egypt). Serum (CAT) levels were determined using kit of (Biodiagnostic Company, Dokiki, Giza, Egypt). Serum (MDA) were determined using Biodiagnostickit (Biodiagnostic company, Dokki, Giza, Egypt).

Statistical analysis:

Using all statistical analysis were done by a statistical for social science package "SPSS" 14.0 for Microsoft Windows, SPSS Inc. and considered statistically significant at atwo-sided $P<0.05$.Numerical data were expressed as mean ± SD. The levels of markes were analyzed by ANOVA but the Mann-Whitney U-test was used for comparisons between independent groups (Schwartz et al., 2018).

RESULTS

I-Effect of *Saccharum officinarum* peels' extract (SOPE) on various indices of obesity and cardiac enzymes (LDH, CK-MP &CTnI):

The present result in Figure (1)showed a significant increase ($P<0.0001$) in the values of final body weight (bw), abdominal circumference (AC), body mass index (BMI), Leptin levels, cardiac enzymes (LDH, CK-MP&CTnI) levels in the obese (non- treated) rats are (352.30±13.16 g, 19.40±1.17 cm, 0.87±0.038 g/cm^2, 76.38±2.71 pg/ml, 346.43±5.45 U/L, 48.93±1.02 pg/ml& 11.46±2.34 pg/ml) respectively compared with control group. Obese rats treated with SOPE showed a significant decrease ($P<0.0001$) in the values of bw, AC, BMI, Leptin, LDH, CK-MP&CTnI levels which are (240.80±2.78 g, 16.0±0.82 cm, 0.70±0.065 g/cm^2, 31.05±0.69 pg/ml, 160.06±7.57 U/L, 21.80±0.66 pg/ml& 3.48±0.68 pg/ml) respectively versus obese rats in obese group and obese rats treated with xenical(Fig.1).

II- Effect of (SOPE) in increasing levels of high intensity lipoprotein (HDL-c), celation of oxidative stress & rising (anti oxidative) indices in 3rd group:

The present result in Figure (2)showed a significant increase ($P<0.0001$) in the values of TC, TG , LDL, VLDL &MDA levels in the obese (non-treated) rats are (145.22±6.64 mg/dl, 135.16±8.71mg/dl, 103.35±4.98 mg/dl, 27.03±1.74 mg/dl and 12.74±0.63 nmol/ml) respectively but, there was a significant decrease ($P<0.0001$) in the values of [HDL, GSH, CAT&SOD levels] are(14.84±1.08 mg/dl,0.31±0.05 mmol/g, 0.46±0.07 U/g&1.16±0.16 U/g) respectively compared with control group. Obese rats treated with SOPE showed a significant decrease ($P<0.0001$) in the values of TC, TG, LDL, VLDL &MDA levels which are (62.48±5.86 mg/dl, 53.75±4.67 mg/dl, 25.39±2.75 mg/dl , 10.75±0.93 mg/dl &4.78±0.47 nmol/ml) respectively and there was a-significant

increase (P<0.0001) in the values of [HDL,GSH, CAT&SOD] levels are(25.68±2.77 mg/dl, 0.88±0.08 mmol/g, 1.12±0.19 U/g& 3.01±0.35 U/g) respectively versus obese rats in obese group and obese rats treated with xenical(Fig.2).

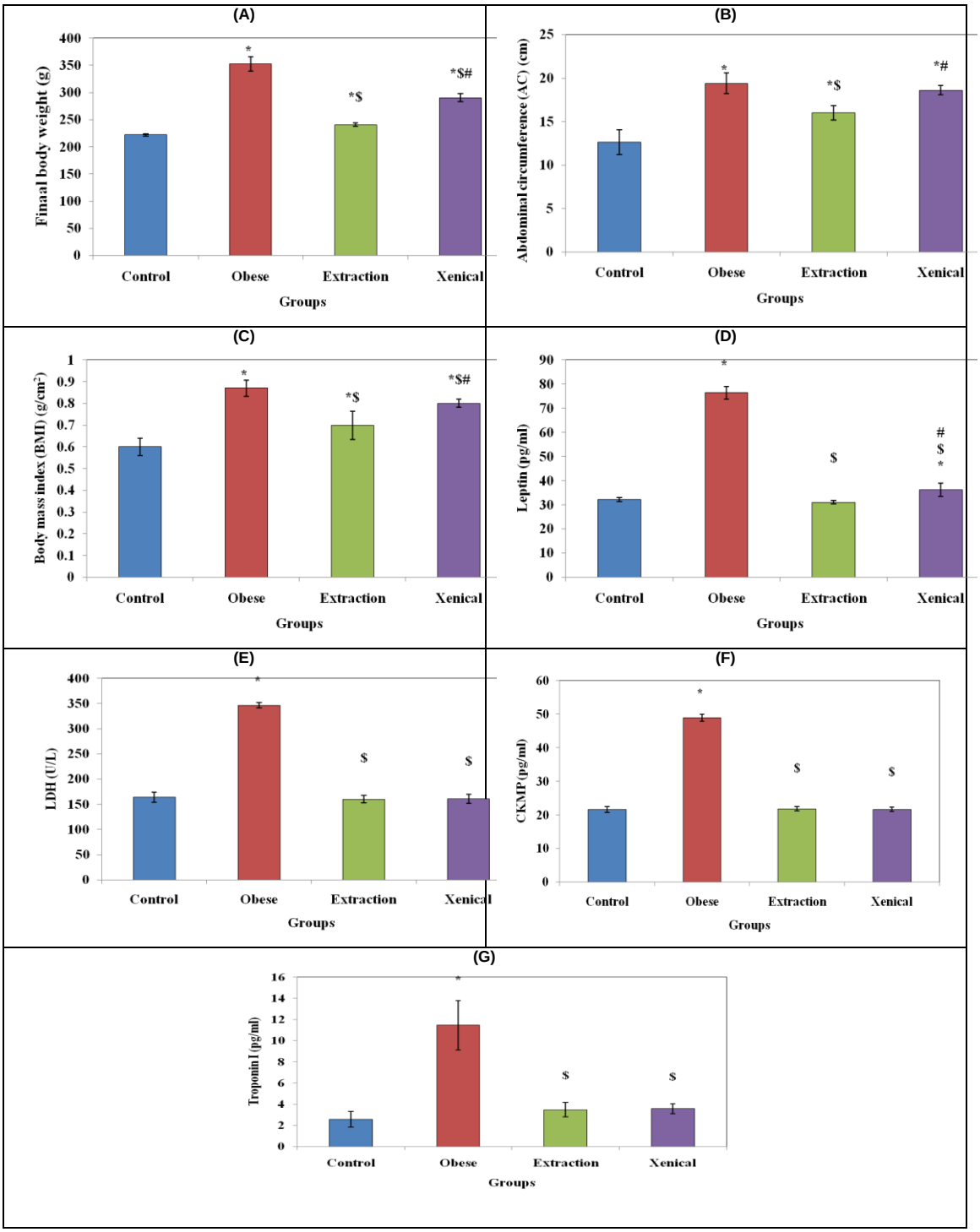


Figure1: showed (A) final body weight, (B) abdominal circumference (AC) , (C) body mass index (BMI), (D) Leptin, (E) lactate dehydrogenase(LDH), (F) creatin kinase(CK-MP)&(G) troponin I(CTnI) in Control ,* = significant *VS* control , $ = significant *VS* Obese , # = significant *VS* Extraction).

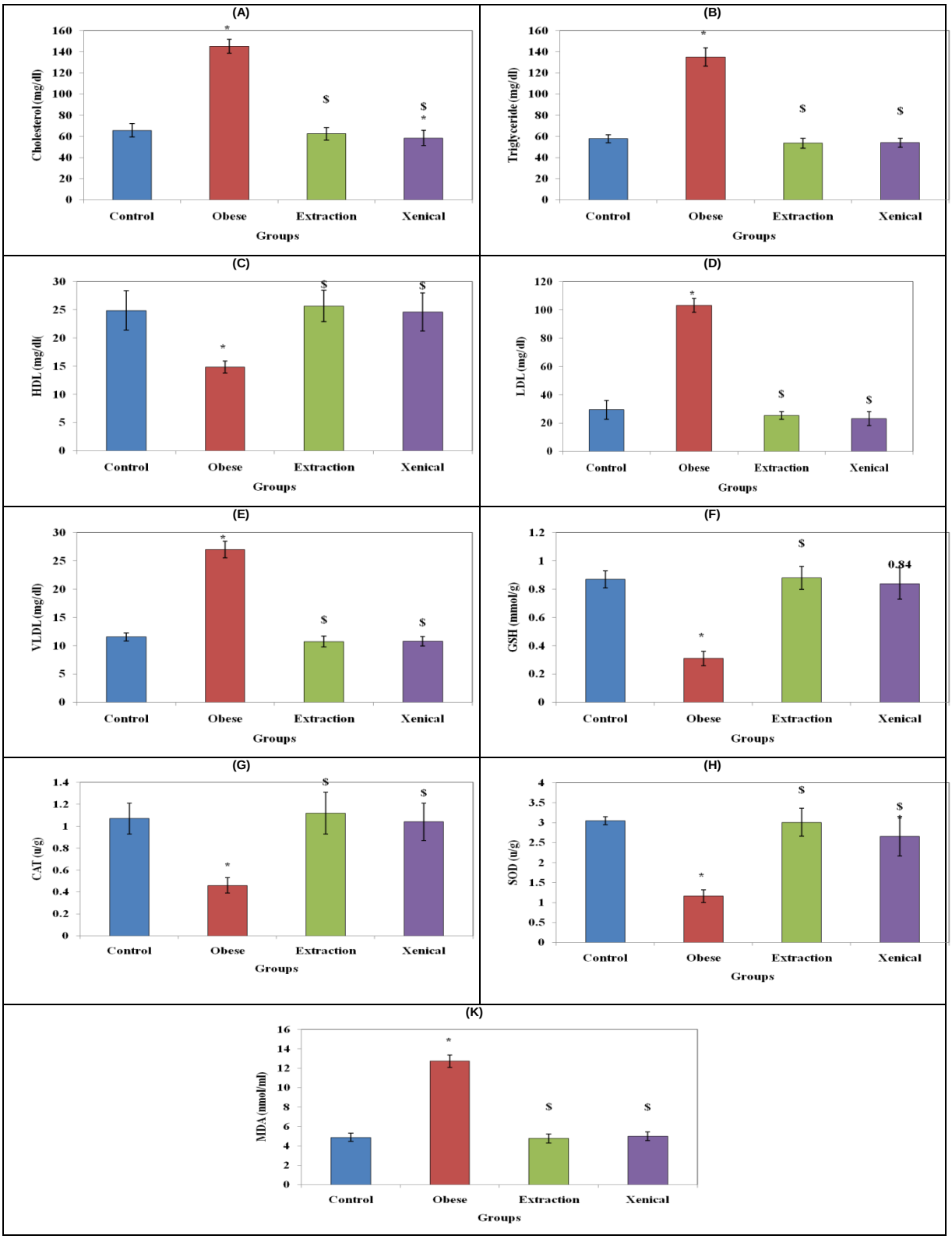


Figure2: showed serum lipid profile & Oxidative stress ; (A)cholestrole, (B)triglycerides, (C) HDL,(D) LDL,(E) VLDL , (F)GSH, (G) CAT, (H) SOD and(K) MDA in Control,* = significant *VS* control, $ = significant *VS* Obese , # = significant *VS* Extraction).

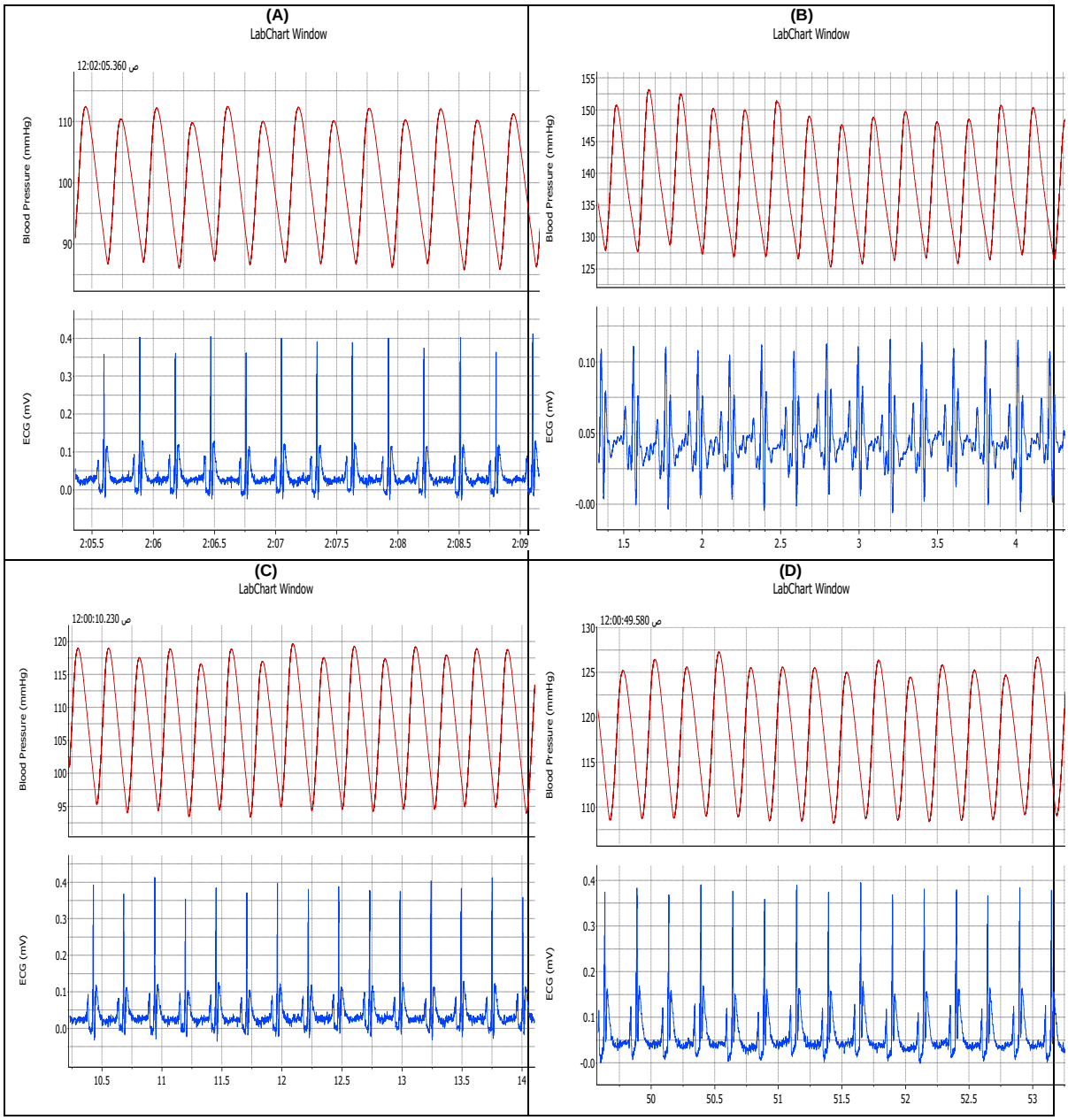


Figure 3: showed readings of charts of blood pressure(SBP & DBP) and Heart rate by using power lab, (A) charts of normal group (control), (B) charts of obese group, (C) charts of extraction group and (D) charts of xenical group.

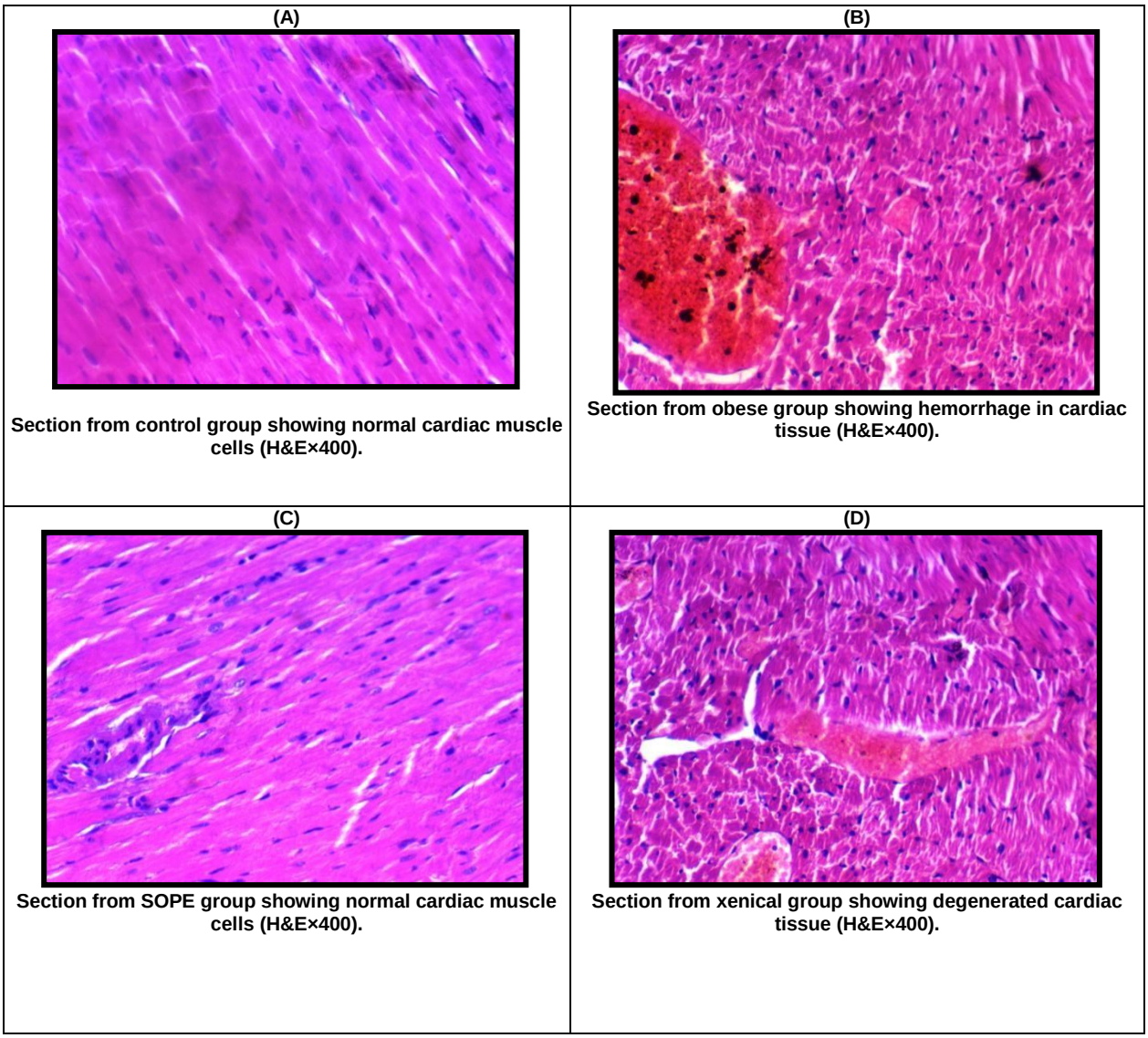


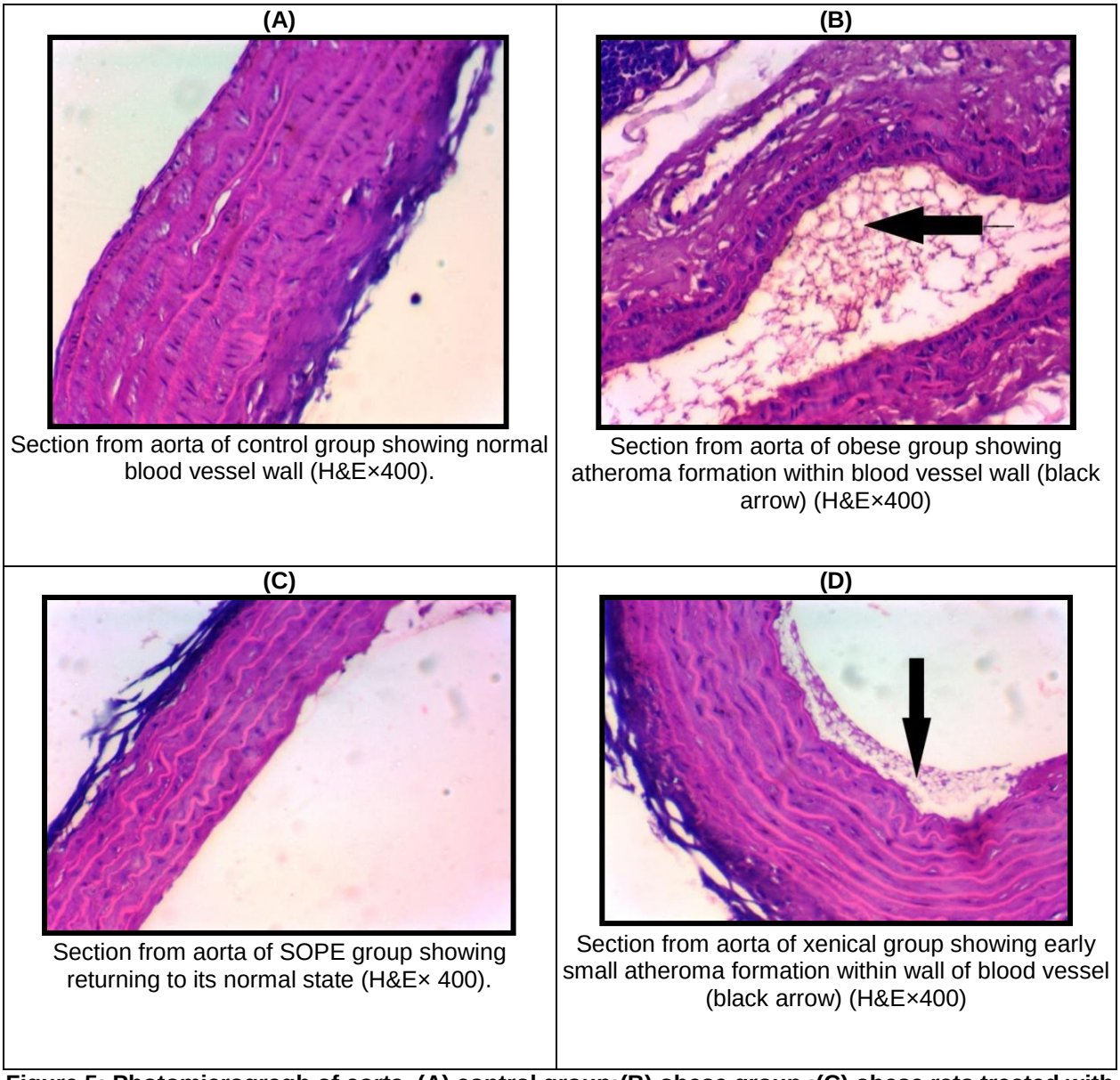
Figure 4: Photomicrogragh of cardiac tissue, (A) control group ;(B) obese group; (C) obese rats treated with SOPE group and (D) obese rats treated with xenical group.

III- Regarding ECG and blood pressure in Figure(3) revealed an increases in the systolic, diastolic blood pressure and heart rate in group II of rats (non treated) (Fig. 3B) compared to control rats which appeared normal heart beats and blood pressure (Fig. 3A). In group III, obese rats treated with SOPE showed decreasing and reback to near normal state of ECG and blood pressure either systolic or diastolic at the end of experiment (Fig. 3C) in comparsion with group II(non treated) and group IV of obese rats treated with xenical which showed a mild increase in the heart rate and a slight increase of diastolic blood pressure (Fig. 3D).

IV-In the present study figure (4) showed histological examination of heart tissues in all groups. Fig (4.A) photomicrograph of normal cardiac tissue formed of longitudinal section of cardiac muscle fibers which separated by thin connective tissue stroma. Fig(4.B)photomicrograph of cardiac tissue of rats group exposed to a high fat diet causing obesity and showing large area of hemorrhage with degenerated cardiac muscle fibers and inflammatory cells aggregation. Fig(4.C) photomicrograph of cardiac tissue of obese rats treated with SOPE showing return of the cadiac muscle fibers to its normal state and absence of hemorrage and inflammatory cells. Fig(4.D)photomicrogragh of cardiac tissue of obese rats group treated with xenical showing

degenerated cardiac muscle fibers and small area of hemorrhage.

V-Histopatological examination of aorta in the presented study Figure(5) was performed in all studied group. Fig(5.A) photomicrograph of normal aorta formed of flat endothelial cell as intima, smooth muscle fiber and elastic tissue as media and connective tissue stroma as adventitia. Fig(5.B) photomicrogragh of aorta of obese rat showing large a theroma(←) formed of foamy histiocytes, lymphocytes and fibrin threads which obliterating the lumen of the aorta. Fig(5.C) photomicrograph of aorta of obese rats treated with SOPE showing complete absence of the a theroma and return of the aortic wall to its normal state. Fig(5.D) photomicrograph of aorta of obese rat treated with xenical showing reduction in the size of the a theroma(↓) and re-opening of the lumen.



Section from aorta of control group showing normal blood vessel wall (H&E×400).

Section from aorta of obese group showing atheroma formation within blood vessel wall (black arrow) (H&E×400)

Section from aorta of SOPE group showing returning to its normal state (H&E× 400).

Section from aorta of xenical group showing early small atheroma formation within wall of blood vessel (black arrow) (H&E×400)

Figure 5: Photomicrogragh of aorta, (A) control group;(B) obese group ;(C) obese rats treated with SOPE group and (D) obese rats treated with xenical group.

DISCUSSION

Based on its medicinal use, we evaluated the potential effect of *Saccharum officinarum* peels' extract (SOPE) on treatment of obese rats with some cardiovascular disorders. In the present study, high fat diet(HFD) induced obese rats was areal reason for occurance of metabolic syndrome, accumulation of visceral fats that is considered one of the most important risk indicator of obesity on cardiac muscle, vascular sclerosis, occurance of oxidative stress, leptin resistance and many of its associated risk factors. Our results are in agreement with many investigators who noted that ,obesity is a major risk factor for atherosclerotic vascular disease and cardiometabolic syndrome and strongly associated with an increase in systemic oxidative stress (Lovren et al., 2015). The extent of fat accumulation in obese humans closely correlates with the markers of oxidative stress. Similarly, the oxidative stress is augmented in plasma and adipose tissue from obese mice. Increased oxidant production, through quenching NO, has been linked to increased atherosclerosis susceptibility (Furukawa et al. 2004). When the plasma cholesterol levels increases , it causes changes of the arterial endothelial permeability leading to the migration of lipids, especially LDL-C particles, into the arterial wall, forming a chronic condition case in which arteries harden through build-up of plaques. Recently, this redox crosstalk concept was extended; in AT-II-induced hypertension, NOX-2 activation triggers Sirt3 S-glutathionylation leading to acetylation of vascular SOD2 and reduced SOD2 activity, all of which resulted in elevated mitochondrial superoxide, diminished endothelial nitric oxide bioavailability, and aggravation of hypertension (Dikalova et al., 2017; Dikalov and Dikalova, 2018). Furthermore, lack of leptin or leptin resistance leads to accumulation of lipids in non-adipose peripheral tissues due to loss of leptin'santisteatotic effects and results in a variety of lipotoxic effects (Unger, 2005). Morever, arole of leptin in the establishment of adequate redox system was recently demonstrated by administration of leptin antagonist during neonatal leptin surge, resulting in a lower activity of several antioxidant enzymes in the spleen and proinflammatory profile of cytokines in leukocytes (Mela et al., 2017). All in all, these informations indicate that leptin plays an effective role in the regulation and development of the redox system and that elevated leptin levels, that is hyperleptinemia, may produce oxidative stress and promote inflammation. Also, in our results, atherosclerosis that one of common results of obesity caused peripheral vascular resistance that lead to high blood pressure in vessels, moreover increased body weight that happened in obese group(non treated group)& induced obese group(treated with xenical) was a burden on cardiac muscle to supply all of the body with blood causing associated heart diseases and finally AMI. All of these criteria reversed by consumption of *Saccharum officinarum* peels' extract. Our results are in agreement with (Noa and Mas, 2005) who found that, Policosanol is a cholesterol-decreasing drug isolated from wax of sugar cane with concomitant antiplatelet effects.

Despite all the therapeutic advances in the field of cardiovascular diseases, cardiology and in particular coronary artery disease, remain the leading cause of the disability worldwide and death there by underlining the importance of acquiring new therapeutic options in this field(Alberto Dominguez-Rodriguez et al., 2011). Morever, the anti obesity drugs prescribed in conventional medicine induce many side effects, including drying of the mouth, insomnia, anorexia, formation of thrombi, constipation and neurological symptoms (Van Der Schoor et al., 2014 and Mead et al., 2016). Therefore it was necessary to have a herbal medication as an alternative natural product that is available and not have side effects as many chemical medications for obesity and atherosclerosis treatment. Hence, the obtained results in treating obese rats with of *Saccharum officinarum* peels' extract (SOPE) revealed a significant decrease in obesity and cardiovascular indices. Our results are in agreement with(Singh et al., 2015) who reported that, the lipophilic compounds are the important components of sugarcane wax have various pharmacological effects like sympathomimetic, antihypercholesterolemic, and antithrombotic activities.

CONCLUSION

Treatment with *saccharum officinarum* peels' extract (SOPE) seems to be an effective target in prevention and potential cure for all risks of obesity and atherosclerosis.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The present study was carried in the animal care

house, faculty of medicine, Zagaig university, Egypt. The authors would like to thank prof.Dr.Kamal El-Kasheshy, professor and head of pathology, pathology department, faculty of medicine, Zagazig university, Egypt for his kind helps in histopathological examinations. And also, our great thanks to Dr.Shereen samy mahmoud, lecture of clinical pharmacology, pharmacology department, faculty of medicine, zagazig university,Egypt for her kind helps in ECG&blood pressure charts readings.

AUTHOR CONTRIBUTIONS

The supervision of prof.Dr. Nabil A. Soliman, Samih I. El Dahmy ,Shalaby A.A designed the experiments and reviewed the manuscript. Riham S.Al-Shahat performed the practical parts of this study. Also, Riham S.Al-Shahat wrote the manuscript with help of Aya Sh. Metwally.All authors read the manuscript and approved the final version.

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