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The role of *lepidium sativum* seed and *boswellia carterii* polar extracts in ameliorating blood glucose, lipid profile, iron status, immune system and cardiac and hepatic pathology in rat model of diabesity

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Abstract

Diabesity, a combined diabetes and obesity, is a worldwide illness in the 21 century. The aim of the present research was to study blood glucose, lipid profile, iron status, immune system and cardiac and hepatic pathology in diabesity rat model and the potential protection by neutraceuticals. Dietary obesity and diabetes were simultaneously induced in rats as model of diabesity. Three nutraceuticals were tested (*Lepidium sativum* seed ethanol and water extracts and *Boswellia carterii* water extract). Biochemical analysis of blood and histopathological examination of liver and heart were carried out. Phenolic and flavonoidal contents were determined in *Lepidium sativum* seed and *Boswellia carterii.* Results showed iron deficiency, dyslipidemia and significantly elevated interleukin 6, blood glucose and malondialdehyde, in addition to alteration in the histopathology of liver and heart in the diabesity control (DOC) group compared to the normal control. Treatment with the different nutraceuticals produced variable improvement compared to the DOC group. *Lepidium sativum* seed showed higher phenolic and flavonoidal contents than *Boswellia carterii* ($p \leq 0.05$). *Lepidium sativum* ethanol extract was superior in improving dyslipidemia, inflammation and oxidative stress while *Boswellia carterii* water extract was the most anti-diabetic. *Lepidium sativum* seed water extract showed the best improvement in iron status.

Keywords: High fat-high sucrose diet; obesity; diabetes; diabesity; iron status; plasma lipids; interleukin-6; malondialdehyde; liver pathology; heart pathology.

Introduction

Diabesity, a chronic disease represented by combined obesity and diabetes, is a worldwide pandemic. The combined pathogenic changes in obesity and diabetes and their complications are considered a real public health problem that requires deep investigations for eradication.

The role of immune system in pathogenesis of chronic diseases is important to be explored. On the other hand an alteration in immune system might be a result of such chronic diseases [1]. Different theories of immune alterations were assumed [2] including elevated inflammatory cytokines and reactive oxygen species. Also, iron deficiency anemia has been reported during chronic diseases that may be ascribed to elevated cytokines [3]. Therefore an interrelationship between iron status and immunity during chronic diseases has been proposed [4-6]. So, it is worthy to search the interrelationship of immunity, iron status and disease biomarkers in diabesity. On the other hand, diabesity with high glucose tolerance, dyslipidemia and pro-inflammatory condition could be considered as a severe image of metabolic syndrome with a predictable involvement of hepatic and cardiac pathology [7,8].

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Nutraceuticals, which are bioactive constituents extracted from plant foods, are considered of wide biological and medical activity together with high safety margin that may have protective effect towards complications of diabesity. *Boswellia carterii* and *Lepidium sativum* are plant foods of considerable rich sources of medically active compounds that have diverse health benefits. *Lepidium sativum,* known as garden cress, was reported to possess antioxidant, hypoglycemic, anti-inflammatory and hepato-protective effects. It is also reported as being an immunity booster in traditional medicine [9]. *Lepidium sativum* is called hab elrashad in Egypt and used as condiment and nutritious seed and in traditional medicine for different purposes including bone strengthen, anti-anemic, diuretic, analgesic, antipyretic, hypo-cholesterolemic and anti-hypertensive. Oleogum resin of *Boswellia carterii* is obtained by incision into the bark of the Birdwood and the exudate is left to dry on the stem and collected thereafter and it is called frankincense which means al luban in Arabic and also named olibanum while it is called as al-liban el-dakar in Egypt. The oleo-gum resin mainly contains groups of boswellic acids, pentacyclic triterpenes, in addition to phenolic compounds [10]. The oleo-gum resin possesses a wide range of biological activities including anti-inflammatory, anti-diabetic, anti-hyperlipidemic, antimicrobial and antifungal [11]. Boswellic acids obtained by polar extract from *Boswellia carterii* possess *in-vitro* immune-modulatory and anti-inflammatory activity, they inhibit interlukin-2 and gamma interferon while promotes interlukin-10 and interlukin-4 [12,13].

The Objective of the present research was to study the changes in lipid profile, immune system with related cytokines and oxidative stress, iron status, and cardiac and hepatic pathology in rat model of diabetes with dietary obesity (diabesity model). The aim included studying the amelioration of the aforementioned changes on the administration of *Lepidium sativum* seed and *Boswellia carterii* polar extracts. The flvonoidal and phenolic contents of both plants were assessed as bioactive constituents.

Experimental

Materials

-Palm oil (Commercial name: Hanim), was obtained from Arma company, Tenth of Ramadan City, Egypt to be used in diet preparation.

-*Lepidium sativum* Linn. (family: Brassicaceae) seed: Garden cress seed named in Egypt as hab elrashad was obtained from herbal retail, local market, Giza, Egypt to be used as source of nutraceuticals.

- *Boswellia carterii* (family: Burseraceae) oleogum resin: Frankincense, called al-liban el-dakar in Egypt, was purchased from herbal retail, local market, Giza, Egypt and was used for preparation of nutraceutical represented by the water extract.

-Streptozotocin (STZ) was obtained from Sigma Aldrich Company, USA for induction of diabetes. All other chemicals used in the study were of high analytical grade.

Animals

Male Sprague-Dawley rats of body weight ranging from 150 to 160 g were purchased from the Animal House of National Research Centre. Rats were kept individually in stainless steel cages at ambient temperature 25°C ± 2, with 12h light/dark cycle. Food and water were supplied *ad-libitum*. Rats were allowed to acclimatize for 7 days in their cages before starting the experiment. Handling and care of animals were carried out according to the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt, and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Approval No. of the study was 19-175.

Methods

Preparation of the extracts (The nutraceuticals)

Lepidium sativum water extract was prepared by grinding the seeds then maceration for 48 hrs in warm distilled water (40 C^o) with continuous shaking followed by filtration using wide porous tissue cloth with squeezing the cloth containing the residue. The maceration and filtration was repeated 3 times till complete extraction. The obtained combined water extract was freeze dried.

Lepidium sativum ethanol extract was prepared by extracting known weight of dried ground seeds by maceration for 48 hrs in absolute ethanol warmed in water bath (40 C^o) with intermittent shaking and filtered. The process was repeated 3 times and the obtained filtrates (ethanol extracts) were combined. The ethanol was evaporated from the extract at temperature not exceeding 40ºC under reduced pressure.

Frankincense water extract was prepared by maceration in warm distilled water (40 C^o) for 48 hrs followed by filtration. This process was repeated three times till complete extraction. The combined filtrate was freeze dried.

Preparation of nutraceuticals to be administered to rats

The extracts were prepared in distilled water individually to be suitable for dosing rats. The control groups and subgroups were given only distilled water as the vehicle.

Determination of flavonoidal and phenolic contents of garden cress seed and frankincense

Total phenolics were assessed colorimetric in garden cress seed and frankincense oleo-resin and water extract adopting Folin Ciocalteu reagent [14] using gallic acid as a standard. The absorbance was measured at 765 nm by using UVPC spectrophotometer. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g dry plant material. The total flavonoids were assayed according to the colorimetric method described previously [15] using quercetin as a standard. The results were expressed as mg quercetin equivalent (QE) /g dry plant material. Both flavonoidal and phenolic contents were assessed in triplicate.

Preparation of diets

Two diets were prepared, a balanced and a high fat-high sucrose (HFS) diet as could be seen in table 1. The high fat-high sucrose diet was used to induce obesity which is similar to previously prepared diet [16] with some modifications.

Table 1. Composition of diets (g/100g):

Ingredients \ Type of diet	Balanced diet	High fat-high sucrose diet
Casein	12	12
Sunflower oil	10	-
Sheep tallow	-	11.7
Palm oil	-	23.3
Starch	68.5	6
Sucrose	-	37.5
Wheat bran	5	5
Vitamin mixture	1	1
Salt mixture	3.5	3.5

Design of animal experiment

Rats were divided into two groups the first consists of 8 rats and the second include 44 rats. The first group was fed on balanced diet and served as the normal control (NC) while the second group was subdivided into 4 subgroups (1, 2, 3 and 4), each of 11 rats and fed on HFS diet for 10 days to start inducing moderate obesity (overweight). At the start of the experiment, the NC group and the four subgroups were organized to have matched mean initial body weights (IBW). After feeding period of 10 days, oral glucose tolerance (OGT) curves were established utilizing the 4 sub-groups that fed on HFS diet. After an overnight fast; sub-group 1 served as overweight control (OC), the other 3 subgroups were the test sub-groups treated by 500 mg/kg rat body weight from *Lepidium sativum* water extract (LW_1), *Lepidium sativum* ethanol extract (LE_1) and frankincense water extract (FW_1), respectively versus the NC group. All the subgroups and the NC group received orally 1g glucose/kg rat body weight [17] after an hour of dosing the extracts or the vehicle. Blood samples were withdrawn from the tail of rats. Blood glucose was determined in the fating state and after blood glucose by ½, 1, 2, 3 and 4 hrs using glucometer (glucoDr®). The results of OGT curves were tabulated. The rats were continued feeding their diets (the diets received before OGT) for 2 days. Then rats of the second group (four subgroups) were treated by streptozotocin (STZ). STZ was dissolved in freshly prepared 0.1 M cold citrate buffer (pH 4.5) and diabetes was induced through intra-peritoneal injection of STZ (40 mg/kg) to the overnight fasted animals [18]. The NC group was injected intra-peritoneal by the same volume of the vehicle (citrate buffer). The diabetic obese rats (4 subgroups) that having blood glucose $\geq$ 180 mg/dl after 2 hrs of glucose intake were continued feeding on HFS diet. The first subgroup served as diabesity control (DOC). The other 3 subgroups served as test subgroups that were treated daily with 500 mg extract orally/kg rat body weight from *Lepidium sativum* water extract (LW_2), *Lepidium sativum* ethanol extract (LE_2) and frankincense water extract (FW_2), respectively (with the same sequence used in OGT). The NC group that fed on balanced diet from the start of the experiment continued on feeding the balanced diet till the end of the experiment. The NC group and the DOC subgroup were treated by daily oral doses of the vehicle (distilled water). The experiment continued for 3 weeks. At the end of the experiment rats were fasted and blood samples were drawn from anaesthetized rats and received in heparinized test tubes for determination of glucose using glucometer (glucoDr®) and hemoglobin. Plasma was separated by centrifugation at 3000 rpm for further biochemical analysis. Rats were dissected; livers and hearts were kept in 10% formalin for histopathological examination [19]. Rat body weight and food intake were monitored throughout the whole experiment. Body weight gains and total food intakes of different groups and subgroups were calculated after the first 10 days and at the end of the experiment.

Assessed biochemical parameters:

Plasma total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were determined by colorimetric methods [20-23]. Plasma malondialdehyde (MDA), iron, total iron binding capacity (TIBC), alanine transaminase activity and aspartate transaminase activity were assessed

adopting the previous methods [24-27]. Blood hemoglobin was determined as described previously [28]. Plasma soluble transferrin receptors1 (sTfRs) and Interleukin 6 (IL-6) were assayed using ELISA Kits (Elabscience Biotechnology Inc. USA). TC/HDL-C and LDL-C/HDL-C were calculated for denoting cardiovascular risk. Transferrin saturation% was computed by the following formula (plasma iron/TIBC) X 100.

Statistical analysis

Data were expressed as means ± SE. Data were analyzed by one-way ANOVA followed by the Tukey multiple comparison tests using the SPSS statistical program, version 21 [29]. Differences were considered significant at $p \leq 0.05$.

Results and Discussion

Diabesity is a term indicating coexistence of diabetes and obesity with or without dyslipidemia which is a global pandemic of the developing world in the 21 century. Diabesity forms a subset of metabolic syndrome which is accused for cardiovascular diseases risk and liver dysfunction [30,31] and considered real health problems required a radical solution. Dysfunctional immune system might be either a cause or a result of diabesity [1,2]. Iron status is another issue that requires deep investigation especially during obesity and diabetes and how it may be interrelated to the immune system and the hyperglycemia in such chronic diseases [3-6]. The present study is a trial to investigate the aforementioned arguments and to unravel the opacity of such interrelated changes. It was also of importance to study the potential health benefits of nutraceuticals prepared from *Lepidium sativum* and *Boswellia carterii* to alleviate such pathogenic state during diabesity. A rat model of combined obesity and diabetes was used to achieve such goals.

High fat-high sucrose diet similar to that reported previously [16] was used to induce obesity in rats. Table 2 showed that when rats were fed on a balanced diet for 10 days their final body weight showed 39% increase while the group fed on HFS diet demonstrated 50% increase compared to the IBW. Body weight gain of rats fed on HFS diet showed 28% increase from that of the NC group. These results reflect induction of overweight after 10 days of consuming HFS diet. The prevalence of obesity worldwide might be mainly attributed to the increased consumption of energy from highly saturated fats and sugar with reduced physical activity. It was reported that high sucrose consumption (30%) produced significant increase in rat body mass index [32]. Long term feeding 30% sucrose causes hyperglycemia, body weight increase and glucose intolerance [33].

The results of studying oral glucose tolerance of the NC group and the rats of the different subgroups fed on HFS diet for 10 days (the OC subgroup and the test subgroups) are shown in Table 3. It can be noticed that the OC subgroup showed significant increase in fasting blood glucose levels and after 1/2 h, 1h, 2h and 4 hours from glucose ingestion compared to the NC group. This result is similar to the previous work [33,34] that fed rats on high sucrose diet. The significant increase in glucose tolerance on feeding HFS diet in the present study might be related to development of insulin resistance. The different treatments showed significant reduction in blood glucose after 1/2 h, 1h, 2h and 4 hours from ingestion of glucose compared to the OC subgroup except for the level after 1/2 h in case of LE_1 subgroup that showed insignificant change. Insignificant changes in blood glucose were noticed among the test subgroups after 1/2 h, 1h, 2h and 4 hours from glucose treatment except for the LW_1 subgroups after 4hrs that showed significant reduction from that of the LE_1 and FW_1 subgroups. The different blood glucose levels of the test subgroups on different time intervals matched the normal levels except for LE_1 after an hour and LW_1 after 4hrs that shows significant higher and lower levels, respectively. The reduction in blood glucose after treatment with the different extracts might be ascribed to induction of insulin sensitivity and/or reducing intestinal glucose absorption that might be attributed to the bioactive constituents in the extracts represented by flavonoids and phenolic compounds as shown from the present study.

Table 2 showed that at the end of the experiment and after induction of diabetes by streptozotocin, the DOC subgroup fed on HFS diet and the test subgroups demonstrated significant reduction in final body weight and body weight gain compared to the NC group. The LW_2 and FW_2 subgroups showed significant reductions in final body weight and body weight gain compared to the DOC and the LE_2 subgroups. It could be noticed that induction of diabetes prevents the increase in body weight by HFS diet in the present study but certainly would continue produced the pathogenic changes related to HFS diet that simulate the changes in obese subjects or those with metabolic syndrome. Unexplained weight loss in diabetics might be due to that the insufficient insulin could prevent the uptake of glucose from blood into the cell to be used as energy therefore the body starts burning fat and muscle proteins to get energy causing a reduction in overall body weight. In type 2 diabetes there are both insulin resistance and gradual loss of

the capability of pancreatic β-cell to secrete insulin. Insulin resistance certainly leads to greater demand for the hormone and the pancreas cannot keep up with the need [35]. Obese are also insulin resistant. So, combined obesity with diabetes may aggravate insulin resistance resulting in sever weight loss as manifested in the present study. The unexplained weight loss of diabetic has been reported to be a risk factor for diabetic complications [36] like dyslipidemia, cardiovascular diseases and liver dysfunction as could be seen from the subsequent results of the present study.

Table 4, showed significant elevated TC, TG, LDL-C, T-C/HDL-C, LDL-C and LDL-C/HDL-C and significant reduction of HDL-C in the DOC subgroup compared to the NC group pointing to dyslipidemia and risk for cardiovascular diseases which coincided with the result of heart pathology in the present study, these results agreed with previous works [16,37,38]. The elevated lipids in diabetics might be linked to increased mobilization from peripheral fat depot [39]. As was expected, blood glucose of the DOC subgroup showed significant increase compared to the NC group (Table 4). It was reported that overweight subjects were more susceptible to diabetes and its complications; this might be supported by the elevated glucose tolerance of the OC subgroup. Faulty in nutritional intake including over consumption of high saturated fat and sucrose with concomitant lack in exercise might be among the important risk factors for obesity and diabetes. These risk factors collectively lead to insulin resistance, hyperinsulinemia, atherogenic dyslipidemia along with vascular inflammation; such changes in diabesity predisposed cardiovascular diseases [30,40]. All the test subgroups showed significant improvement of the aforementioned lipid parameters compared to the DOC subgroup. The LE_2 subgroup demonstrated the least significant level of plasma TG, LDL-C and LDL-C/HDL-C with simultaneously the highest level of HDL-C showing superiority on the other test subgroups. Also, the test subgroups showed significant reduction in blood glucose compared to the DOC subgroup. The least significant level of blood glucose among the test groups belonged to the FW_2 subgroup that matched the NC group. The LE_2 subgroup showed significant decrease in blood glucose compared to the LW_2 subgroup and significant increase compared to the FW_2 subgroup.

Table 2. Nutritional parameters before and after streptozotocin injection

Before streptozotocin injection								After streptozotocin injection		
Parameters / Types of group and sub-groups	IBW (g)	FBW (g) after 10 days of feeding high fat diet	BW Gain (g)	% increase in FBW relative to IBW		% increase in BW gain from NC	TFI (g)	Parameters / Types of group and sub-groups	FBW (g) at the end of the experiment	BW (g) gain at the end of the experiment
NC	163.5^{a} ± 6.82	227.6^{c} ± 14.56	64.13^{d} ± 10.93	39%		-	464.0^{a} ± 10.98	NC	276.5^{a} ± 11.84	113^{a} ± 14.73
Sub-group1	163.1^{a} ± 8.10	250^{b} ± 11.80	86.89^{b} ± 6.15	53%	50%	28%	378.6^{bd} ± 12.57	DOC	250.3^{b} ± 22.47	87.2^{b} ± 5.67
Sub-group 2	163.4^{a} ± 6.48	246.9^{b} ± 14.51	83.44^{c} ± 12.90	51%			403.8^{cd} ± 19.84	LW_2	220.4^{c} ± 8.67	57^{c} ± 2.67
Sub-group 3	163.6^{a} ± 6.31	227.1^{c} ± 11.33	63.56^{d} ± 11.10	39%			395.1^{cd} ± 15.67	LE_2	249.4^{b} ± 14.51	85.8^{b} ± 6.06
Sub-group 4	163.9^{a} ± 6.76	255.6^{a} ± 14.18	91.67^{a} ± 10.90	56%			384.8^{bd} ± 12.73	FW_2	219.9^{c} ± 18.60	56.00^{c} ± 1.00

In each column, same letters mean non-significant difference while different letters mean significant difference at $p< 0.05$. Data are expressed as mean values ± standard error of the mean.

IBW: Initial body weight, FBW: Final body weight, BW: Body weight, TFI: Total food intake, NC: Normal control group, DOC: Diabesity control subgroup, LW_2: The subgroup treated with water extract of Lepidium, LE_2: The subgroup treated with ethanol extract of Lepidium, FW_2: The subgroup treated with water extract of frankincense.

Table 3. Blood glucose response (Glucose Tolerance Curve) of rats fed on HFS diet for 10 days (OC), the NC group and the groups given different treatments.

BG / Groups /subgroups	Fasting blood glucose	Blood glucose after half an hour.	Blood glucose after an hour.	Blood glucose after two hours.	Blood glucose after four hours.
NC	62.67 ^{a}± 3.60	125.33 ^{b}± 18.04	98.17 ^{a}± 2.98	83.67 ^{a}± 4.88	77.00 ^{a}± 2.69
OC	70.44 ^{b}± 1.83	159.63 ^{c}± 13.85	131.37 ^{c}± 7.65	121.33 ^{b}± 9.32	99.59^{c}± 5.60
LW_1	67.17 ^{ab}± 3.60	124.17 ^{b}± 4.22	104.00 ^{ab}± 1.69	98.00 ^{a}± 7.35	61.00 ^{b}± 2.96
LE_1	66.60 ^{ab}± 3.50	137.80 ^{bc}± 4.53	114.80 ^{b}± 4.32	85.20 ^{a}± 4.40	74.20 ^{a}± 1.40
FW_1	68.33 ^{ab}± 3.17	112.17 ^{b}± 7.10	103.33 ^{ab}± 5.38	90.50 ^{a}± 6.10	72.83^{a}± 3.36

In each column, same letters mean non-significant difference while different letters mean significant difference at $p< 0.05$. Data are expressed as mean values ± standard error of the mean.

NC: Normal control, OC: Overweight control, LW_1: The subgroup treated with water extract of Lepidium, LE_1: The subgroup treated with ethanol extract of Lepidium, FW_1: The subgroup treated with water extract of frankincense, BG: Blood glucose.

Table 4. Fasting blood glucose and plasma lipids of different experimental groups.

Parameters / Groups /subgroups	Fasting blood glucose mg/dl	TC (mg/dl)	TG (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	TC/ HDL-C	LDL-C /HDL-C
NC	58.56^{d}± 1.37	85.14^{c}±0.45	88.55^{c}±1.97	20.08^{b}±1.06	47.74^{b}±0.90	1.79^{c}±0.04	0.42^{b}±0.03
DOC	92.44^{a}± 1.16	147.59^{a}±1.19	127.03^{a}±3.07	81.46^{a}±5.67	35.71^{c}±1.09	4.15^{b}±0.13	2.29^{a}±0.18
LW_2	75.14^{b}± 0.70	87.44^{bc}±2.43	95.40^{c}±2.50	8.52^{c}±0.47	46.16^{b}±1.30	1.90^{c}±0.04	0.18^{c}±0.01
LE_2	66.20^{c}± 0.73	94.28^{b}±3.14	64.92^{d}±1.74	6.35^{d}±0.55	57.94^{a}±1.29	1.627^{c}±0.55	0.11^{d}±0.01
FW_2	59.46^{d}± 1.09	91.64^{bc}±2.82	109.98^{b}±3.02	21.79^{b}±2.79	47.86^{b}±0.84	1.92^{c}±0.08	0.46^{b}±0.06

In each column, same letters mean non-significant difference while different letters mean significant difference at $p< 0.05$. Data are expressed as mean values ± standard error of the mean.

NC: Normal control, DOC: Diabesity control subgroup, LW_2: The subgroup treated with water extract of Lepidium, LE_2: The subgroup treated with ethanol extract of Lepidium, FW_2: The subgroup treated with water extract of frankincense, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, TC: total cholesterol, TG: Triglycerides.

In Table 5, MDA was significantly high in the DOC subgroup compared to the NC subgroup reflecting high oxidative stress that progressed to inflammation as could be seen from the significantly elevated IL-6 (Table, 5). The study of Mahmoud et al [41] showed significant increase in IL-6 in high fat/ streptozotocin induced diabetic rats which is similar to the present result. It was reported that during overweight a state of inflammation is continually present [38]. Alteration in immune system in diabesity model of the present study might be reflected in the elevated MDA (induced by free radicals that lead to lipid peroxidation) and IL-6 inflammatory cytokine. Invasion of antigen or tissue damage in the body normally stimulates the immune system to release inflammatory cytokine and free radicals to combat the antigen or the tissue damage however detrimental consequences may occur resulting in continual release of such elements leading to pathologic condition as in chronic diseases such as diabesity. The levels of MDA and IL-6 were significantly reduced by all test subgroups with variable degrees pointing to reduction of oxidative stress and inflammation. The LE_2 and LW_2 subgroups showed significant reduction of IL-6 and MDA compared to FW_2. LE_2 showed the least values of MDA and IL-6 reflecting superiority as antioxidant and anti-inflammatory in the present model. These results denoting the anti-inflammatory, antioxidant and/or immune-modulatory effects of the different extracts used in the present study which might be attributed to the presence of bioactive constituents discussed in the subsequent results (Table 7) and the cited previous researches.

ALT and AST activities showed significant increase in the DOC subgroup compared to the NC group (Table 5), indicating hepatic dysfunction. Liver dysfunction might be induced due to development of metabolic syndrome by feeding HFS diet [7] with concomitant induction of diabetes (as could be seen from histopathology results Fig. 1). Also, Erukainure et al. [37] reported elevated ALT and AST activities in diabetic rats. The elevations of the activities of AST and ALT in diabetic rats have been attributed to the release of these enzymes from the damaged

hepatic cells into blood stream [42]. The different test subgroups showed complete regression of ALT and AST activities to normal levels denoting hepato-protective effect of the tested extracts.

Iron status (Table 6) showed insignificant change concerning hemoglobin when the DOC subgroup was compared with the NC group while plasma iron and %transferrin were significantly reduced along with significant elevation of TIBC and sTfRs. Although there was no change in hemoglobin level however the reduced serum iron and % transferrin with simultaneous increase in TIBC and sTfRs may indicate iron deficiency. High level of sTfRs was reported during iron deficiency [43,44] which was proportional to tissue depletion of iron. The sTfRs are efficient marker in diagnosis of iron deficiency even during inflammatory condition because they are not acute phase reactant [45]. During elevated iron demand due to iron deficiency, elevated erythropoiesis, or megaloplastic anemia; the sTfRs are elevated. Patients suffer inflammatory conditions often have reduced plasma iron and transferrin with elevated ferritin consequently these usual indicators are unreliable for detecting iron deficiency in such conditions, however sTfRs are more suitable during inflammation [46,47]. In patients that suffer chronic inflammation like in subjects with diabesity, ferritin is not a good marker for iron deficiency because it is an acute phase reactant that elevated during inflammation so it may give wrong result.

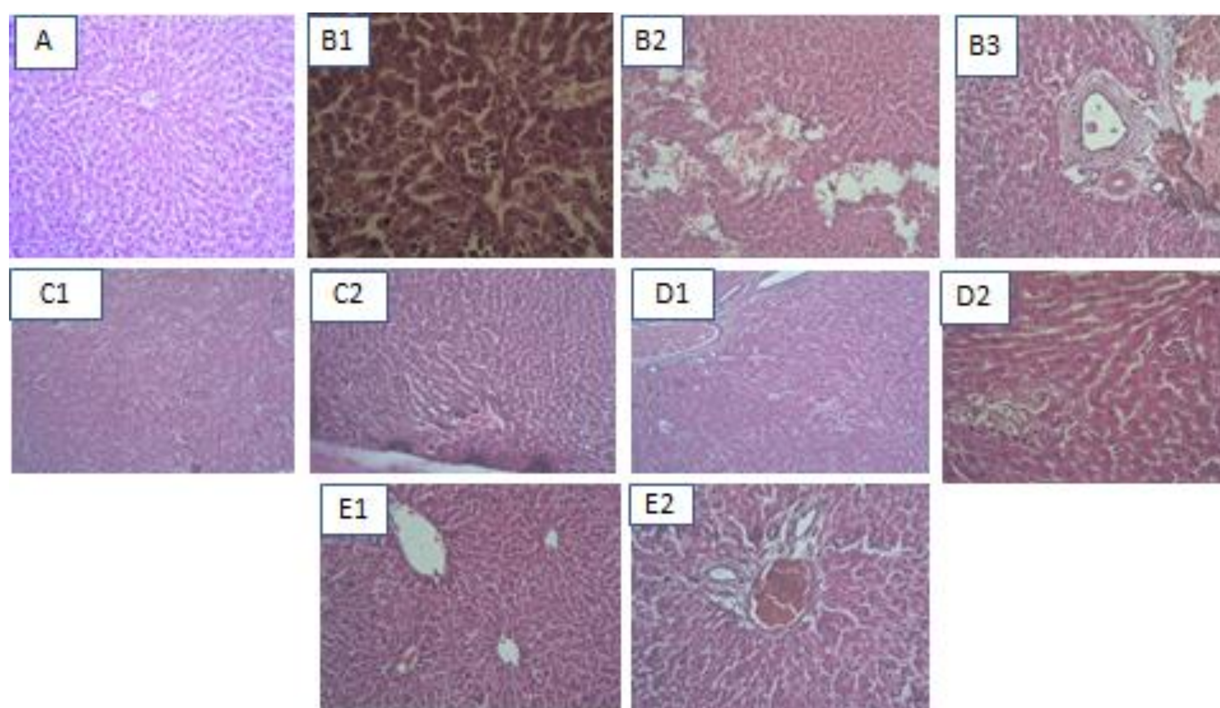

Fig 1: Histopathological changes of liver of different experimental groups

A: Normal control shows normal appearance; **B1-B3** (DOC sub-group), **B1**: Demonstrated vacular degeneration of hepatocytes, binucleated nuclei in the hepatocytes and focal inflammatory cells aggregations (H&EX400), **B2**: Liver of rat showed severely dilated hepatic sinusoids with red blood cells (H&EX200), **B3**: The liver revealed fibrous connective tissue proliferations with portal area, newly formed bile ductules and disorganization of hepatocytes(H&EX200); **C1&C2**(Rats given water extract of *Lepidium sativum*), **C1**: Liver demonstrated moderate vacuolar degeneration of hepatocytes (H&EX200), **C2**: Liver showed dilated sinusoids with red blood cells (H&EX200); **D1&D2**((Rats given ethanol extract of *Lepidium sativum),* **D1:** The liver revealed focal vacuolar degeneration of hepatocytes, fibrous connective tissue proliferation in the portal area and newly formed bile ductules (H&EX200), **D2**: The liver showed focal vacuolar degeneration and inflammatory cells infiltrations in dilated sinusoids, **E1&E2** (rats given water extract of *Boswellia carterii*), **E1**: Liver of rat demonstrated moderate necrosis and degeneration of hepatocytes and dilated hepatic central vein (H&EX200). **E2**: Liver of rat showed dilated central vein and moderate fibrous connective tissue proliferation in the portal area (H&EX200).

DOC: Diabesity control

Table 5. Plasma IL-6, MDA, and the activities of AST and ALT of different experimental groups

Parameters / Groups /subgroups	IL-6 pg/ml	MDA (nmol/ml)	AST (U/L)	ALT (U/L)
NC	65.12^{b}±6.83	5.69^{b}±0.17	60.00^{bc}±3.09	46.67^{b}±1.05
DOC	110.14^{a} ±25.92	8.24^{a}±0.11	83.40^{a}±2.80	76.00^{a}±2.00
LW_2	62.06^{b} ±11.14	4.98^{c}±0.29	58.00^{c}±2.45	42.62^{bc}±3.43
LE_2	45.83^{b} ±2.06	4.82^{c}±0.26	61.57^{bc}±3.01	41.14^{c}±1.40
FW_2	72.18^{ab}±12.89	5.96^{b}±0.14	69.00^{b}±5.29	45.33^{bc}±3.73

In each column, same letters mean non-significant difference while different letters mean significant difference at $p< 0.05$. Data are expressed as mean values ± standard error of the mean.
NC: Normal control, DOC: Diabesity control, LW_2: The subgroup treated with water extract of Lepidium, LE_2: The subgroup treated with ethanol extract of Lepidium, FW_2: The subgroup treated with water extract of frankincense, IL-6: Interleukin-6, MDA: Malondialdehyde, ALT: Alanine transaminase, AST: Aspartate transaminase.

Table 6. Blood hemoglobin and plasma iron, TIBC, % transferrin, soluble transferrin receptors 1 of different experimental groups.

Parameters / Groups /subgroups	Hemoglobin (g/dL)	Iron (µg/dl)	TIBC (µg/dl)	% Transferrin	Soluble Transferrin Receptors1 ng/ml
NC	14.51^{a}±0.37	104.63^{c}±3.78	281.67^{c}±3.28	37.71^{b}±1.79	0.64^{b}±0.01
DOC	14.88^{a}±0.76	75.26^{d}±2.33	341.43^{b}±6.75	22.12^{c}±0.96	0.93^{a}±0.04
LW_2	15.64^{a}±1.03	159.88^{a}±8.51	346.61^{b}±40.12	49.89^{a}±6.69	0.80^{ab} ±0.13
LE_2	14.65^{a}±1.48	139.13^{b}±6.57	474.71^{a}±4.16	29.36^{bc}±1.59	0.80^{ab}±0.07
FW_2	17.33^{a}±1.10	133.12^{b}±8.34	389.11^{b}±23.97	34.88^{b}±2.93	0.76^{ab}±0.09

In each column, same letters mean non-significant difference while different letters mean significant difference at $p< 0.05$. Data are expressed as mean values ± standard error of the mean.
NC: Normal control, DOC: Diabesity control, LW_2: The subgroup treated with water extract of Lepidium, LE_2: The subgroup treated with ethanol extract of Lepidium, FW_2: The subgroup treated with water extract of frankincense, TIBC: Total iron binding capacity.

Table 7. Total phenolic and flavonoids of *Lepidium sativum* seed and *Boswellia carterii*

Parameters / Type of plant or extract	Phenolic content (mg GAE/g dry sample)	Flavonoids content (mg QE/g dry sample)
***Boswellia carterii* oleo-resin**	7.32^{c}± 0.13	0.17^{c}± 0.004
Water extract of *Boswellia carterii*	10.55^{b}± 0.20	0.96^{b}± 0.07
***Lepidium sativum* seed**	23.68^{a}± 0.33	3.17^{a}± 0.15

Data is expressed as mean ± S.E. Means with different superscripted letters in the same column are significantly different.
GAE: Gallic acid equivalent, QE: Quercetin equivalent

Abdominal obesity has been reported to have high serum hepcidin and reduced iron availability owing to increased inflammatory factors [48]. The increased level of hepcidin negatively affects iron absorption and may lead to iron deficiency [49]. Serum iron and transferrin saturation decreased significantly with the increase in waist circumference [50]. Hypoferritinemia without anemia might be due to disorder in iron metabolism [51]. Iron deficiency may occur prior to developing iron deficiency anemia. Overtime however iron deficiency always lead to anemia. So, it might be expected that elongation of the experimental period of the present study might possibly lead to iron deficiency anemia. A notable decrease in blood hemoglobin was reported in high fat/ streptozotocin induced diabetic rats of an experimental period of 6 weeks which is longer than the present study [41]. Another study [37] showed also reduction in hemoglobin in alloxan diabetic rats. It was reported that hemoglobin was reduced with concomitant elevation in liver and bone iron in streptozotocin induced diabetic rats and that elevation of intracellular iron leads to anemia [52]. Iron deficiency can impair glucose homeostasis in animals

and human and may negatively affect glycemic control and predispose to more complications. On the other hand diabetes and its complications are associated with iron deficiency which if corrected might facilitate diabetes control and prevent or minimize the occurrence of complications [53].

No improvement in TIBC and soluble transferrin receptors were noticed in all test subgroups while plasma iron and %transferrin saturation were significantly improved compared to the DOC subgroup except for the LE_2 subgroup in case of %transferrin saturation that only showed insignificant improvement.

Parameters representing the immune system assessed in the present study were IL-6 and MDA. Significant elevations in IL-6 an MDA were noticed in the DOC subgroup compared to the NC group. The chronic inflammatory response has its origin in the links existing between the adipose tissue and the immune system. Obesity has been reported to alter the immune function. In addition, evidence has arisen that an altered immune function contributes to the pathogenesis of obesity. There is a positive feedback loop between local inflammation in adipose tissue and altered immune response in overweight subjects, both contributing to the development of related metabolic complications. The overweight-associated increase in the production of leptin (pro-inflammatory) and the reduction in adiponectin (anti-inflammatory) seem to affect the activation of immune cells. Non-essential fatty acids can induce inflammation through various mechanisms (such as modulation of adipokine production or activation of Toll-like receptors). Also; nutrient excess and adipocyte expansion trigger endoplasmic reticulum stress; and hypoxia occurring in hypertrophied adipose tissue stimulates the expression of inflammatory genes and activates immune cells [54]. The expression of liver hepcidin is increased by inflammation in chronic diseases [55] like diabesity. The pro-inflammatory cytokine IL-6 is thought to be central to this mechanism [56]. Once released, hipcidin is bound to ferroportin and inhibits iron release from the iron stores and hinders dietary iron absorption [57, 58] that certainly leads to iron deficiency as the case in the present study. Hepcidin mRNA expression in liver was significantly elevated in STZ-induced diabetic rats [59] that blocks the intestinal absorption of iron and the release of iron from its deposits. In the same study hemoglobin reduction with elevated inflammatory cytokines including IL-6 were demonstrated.

Figure 1&2 showed histopathological changes in liver and heart of different experimental groups. As could be seen, the model of diabesity used in the present study demonstrated harmful effect in the liver and the heart. Inflammation, fibrosis and degeneration of hepatocytes together with bi-nucleated nuclei, severely dilated hepatic sinusoids with red blood cells, newly formed bile ductules and disorganization of hepatocytes were noticed in the DOC subgroup. These pathological changes were reflected in the elevated ALT and AST that noticed in the present study pointing to liver dysfunction. The heart of the DOC subgroup showed inflammatory cells infiltration in the pericardium, severe necrosis and degeneration of the cardiac muscles and intramuscular hemorrhages. The heart of the DOC subgroup also demonstrated perivascular edema and thickened blood vessel wall, congested blood vessel and severe necrosis and degeneration of cardiac muscles, together with inflammatory cells infiltrations inside blood vessel (vasculitis). The heart pathological changes of the DOC subgroup go in line with the significantly elevated TC/HDL-C and LDL-C/HDL-C that indicate highly cardiovascular disease risks. The high level of IL-6 and MDA might be among the causative factor of hepatic and cardiac pathological changes in the DOC sub group. The test subgroups showed variable improving effect in both liver and heart histopathology.

Plant foods that are rich in phytochemicals might serve as sources of nutraceuticals and ingredients of functional foods that might work as complementary to therapy for diabsity. Among the extracts used as nutraceuticals in the present study are those prepared from *Boswellia carterii* and *Lepidium sativum*. Table 7, illustrated the phenolic and flavonoidal contents of *Boswellia carterii* oleo-resin and *Lepidium sativum* seed. *Lepidium sativum* seed showed higher phenolic and flavonoidal contents than *Boswellia carterii* oleo-resin ($p \leq 0.05$). Also, flavonoidal and phenolic contents of *Lepidium sativum* seed showed significant high level compared to *Boswellia carterii* water extract. The polar extract of Boswellia prepared in the present study is the water extract which could be more or less simulate the ethanol extract reported previously to contain volatile oil and resin [60]. The same study demonstrated that the main content of resin is boswellic acids while the volatile oils contain antioxidant compounds. Boswellic acids are natural corticosteroids of anti-inflammatory and immuno-modulatory effects [12,61,62]. Drinking water containing *Boswellia carterii* was demonstrated previously to improve immunity and elevate hemoglobin level in broiler chickens [13]. Antidiabetic and anti-hyperlipidemic, effects of *Boswellia carterii* were reported previously [11]. The results of the aforementioned studies agreed with the present results. The presence of phenolic and flavonoidal compounds in *Boswellia carterii* as could be seen from the present result may impart both antioxidant and anti-inflammatory activities to the extract.

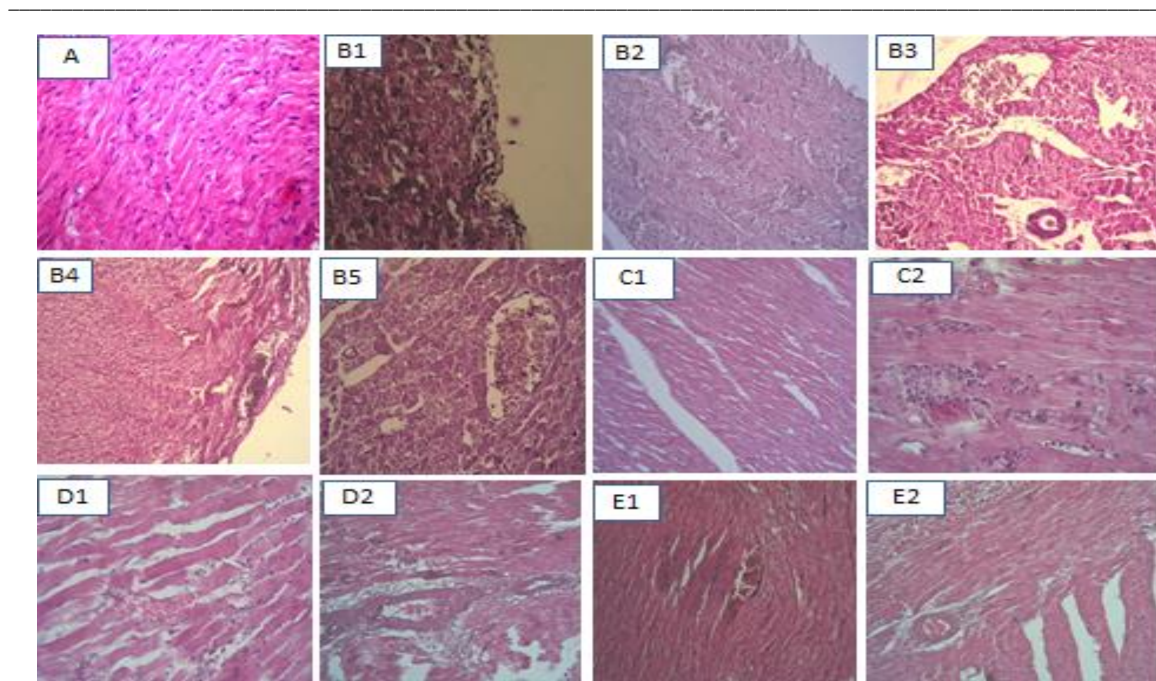

Fig 2: Histopathological changes of heart of different experimental groups

A: Normal control shows normal histological structure (H&EX400); **B1-B5** (DOC subgroup), **B1**: The heart showed inflammatory cells infiltration in the pericardium (H&EX400), **B2**: Heart demonstrated severe necrosis and degeneration of the cardiac muscles and intermuscular hemorrhages (H&EX400). **B3**: Heart revealed intermuscular hemorrhage, perivascular edema and thickened blood vessel wall (H&EX200). **B4**: Heart showed congested blood vessel and severe necrosis and degeneration of cardiac muscles (H&EX200), **B5**: Heart demonstrated inflammatory cells infiltrations inside blood vessel (vasculitis) (H&EX400); **C1&C2** (Rats given water extract of *Lepidium sativum*), **C1**: Heart showed necrosis and degeneration of the heart muscle and intermuscular hemorrhage (H&EX400), C2: Heart demonstrated severe necrosis and degeneration of the myocardial muscles and focal inflammatory cells aggregations (H&EX400); **D1&D2** (Rats given ethanol extract of *Lepidium sativum)*, **D1**: Heart showed necrosis and degeneration of the cardiac muscles and intermuscular hemorrhage of edema(H&EX200), **D2**: Heart showed destruction of the endothelial lining the blood vessel and perivascular edema(H&EX200), **E1&E2** (Rats given water extract of *Boswellia carterii*), E1: Heart demonstrated moderate necrosis and degeneration of the myocardial muscle and intermuscular hemorrhage (H&EX200), E2: Heart showed congested blood vessels, perivascular edema and necrosis and degeneration of the myocardial muscle (H&EX200).

DOC: Diabesity control

Lepidium sativum was demonstrated by Chatoui et al to possess antioxidant activity due to presence of phenolic compounds in both water and ethanol extract [63]. Also the results of the present study showed the presence of phenolic and flavonoidal compounds in *Lepidium sativum* which support the work of Chatoui et al. It was also reported that the polar constituents in the seed of *Lepidium sativum* were represented by alkaloids such as lepidine, glucotropaeolin, N, N′-dibenzyl urea, N, N′-dibenzyl thiourea, sinapic acid and its choline ester (sinapine) in addition to bioactive flavonoids such as 5-4-dihydroxy-7, 8, 3, 5 tetramethoxyflavone, and 5-3-dihydroxy-6, 7, 4’ trimethoxyflavone [64]. The health benefits of the water and ethanol extracts of *Lepidium sativum* seen in the present study might be ascribed to the presence of the aforementioned compounds. A previous study illustrated that the *Lepidium sativum* total alkaloids at a dose of 250 mg/Kg improved blood glucose, TC, TG. LDL-C, HDL-C in alloxan induced diabetic rats which were attributed to the reduction in oxidative stress. The mechanism of their effect as hypoglycemic agents might be attributed to potentiation of pancreatic secretion of insulin from the remaining β-cells [65]. These results are similar to the present results on treating rats with diabesity with either the water or ethanol extract of *Lepidium sativum.* Ethanol extract of *Lepidium sativum* was reported to have free radical scavenging and antioxidant activities pointing to the presence of potential antioxidant compounds of high activity [66]. Also, it is worth mentioning that ethanol extract of *Lepidium sativum* was reported by Raish et al to possess hepato-protective effect through improving

hepatic injuries via inhibiting oxidative stress, inflammation and apoptosis in liver. These were manifested by improving liver function represented by ALT, AST and alkaline phosphatase with reduction of MDA and restoration of antioxidant enzymes [67]. The results of the present study are similar to the work of Raish et al. In support to the present study; *in-vitro* assay of methanol extract of *Lepidium sativum* seeds demonstrated anti-inflammatory effect through preventing protein denaturation [68].

Attenuating the inflammatory biomarker (IL-6) by the studied extracts could inhibit hipcidin release with consequent improvement of iron status as demonstrated in the present study. Also, the improved iron status on administration of *Lepidium sativum* seed extracts in the present study might also be attributed to the presence of folate and iron as 80 µg and 1.3 mg per 100g dry seed, respectively according to USDA data base and which might be extracted in such polar extracts. Human study showed that supplementation of *Lepidium sativum* seeds are used to treat iron insufficiency [69]. The presence of flavonoids in the tested extracts in the present study might be partially responsible for the anti-diabetic potentials along with the anti-inflammatory activities as reported previously in high fat/ streptozotocin induced diabetic rats [41,70].

Conclusion

Although hemoglobin showed insignificant change in obese rats with associated diabetes and dyslipidemia (diabesity); the plasma iron, %Transferrin were significantly reduced along with significant elevation of TIBC and soluble transferrin receptors which reflect iron deficiency. Immune system represented by IL-6 and MDA showed significant increases of both parameters denoting alteration in immune system that induced inflammation and free radicals. Thereby alterations in immune system with reduced iron status and dyslipidemia are interrelated criteria in diabesity rat model. *Boswellia carterii* oleoresin water extract and *Lepidium sativum* seed water and ethanol extracts exert protection to diabesity associated iron deficiency in rats. This could be due to reducing lipid peroxidation and attenuating the inflammatory biomarker IL-6. In addition, the study showed that the extracts are capable of improving dyslipidemia, cardiovascular risks and histopathological changes of liver and heart in the diabesity rat model thus might be prescribed as adjunct nutraceuticals to dietary regimen and remedy for diabesity. The antioxidant, anti-inflammatory, imunomodulatory and hepato- and cardio-protective effects of the extracts might be attributed to their phenolic and flavonoidal contents determined in the present study as well as other previously reported bioactive ingredients. *Lepidium sativum* seed ethanol extract was superior in improving dyslipidemia, inflammation and oxidative stress while *Boswellia carterii* water extract was the most efficient as anti-hyperglycemic. *Lepidium sativum* seed water extract showed the best improvement in iron status.

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