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Carvone Hypoglycemic and Hypolipidemic effects by regulation of key proteins involved in fatty acid beta-oxidation in alloxan-induced diabetic rats.

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ABSTRACT : *Essential oils are natural products with various biological characteristics caused by monoterpenes which have a high probability of producing new drugs. Carvone, monoterpenes have hypolipidemic, anti-inflammatory, hypoglycemic and antioxidant effects.* ***Aim:*** *The aim of this research is to show that carvone has medicinal potential in the treatment of diabetes and hyperlipidemia in alloxan-induced diabetic rats, as well as its effect on insulin activity.* ***Material and methods:*** *Four groups of male albino rats were formed, each with eight rats. In Group 1, healthy rats were provided a standard chow diet and were not given any medications. To develop diabetes, the rats in the other three groups were given a single intraperitoneal administration of alloxan (120 mg/kg), followed by a two-week feeding of an atherogenic hypercholesterolemic diet. The diabetic hyperlipidemic control (DHC) group received no treatment, while the other two groups received carvone (50 mg/kg) daily for one month and a combination drug (atorvastatin 10 mg/kg and metformin100 mg/kg) daily for one month, respectively.* ***Results:*** *our findings show a reduction in the levels of blood glucose, cholesterol, triglyceride, LDL, fatty acid desaturase -1(FADS-1), glucose-6-phosphate catalase -1(G6PC-1). In contrast, There was an increase in the level of insulin and Acyle-co-A oxidase-1(ACOX-1) upon administration of carvone. Immunohistochemical investigation and other results agree with biochemical indicators to a great extent.* ***Conclusion****: Carvone has anti-diabetic and anti-hyperlipidemia properties in diabetic hyperlipidemic rats by controlling essential proteins involved in fatty acid beta-oxidation (ACOX-1)*

KEYWORDS *diabetes mellitus, hyperlipidemia, fads-1, g6pc-1, a17cox-1.*

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I. INTRODUCTION

Diabetes is a disease characterized by abnormally high blood glucose levels. Glucose is a sugar that comes from the meals you eat which is the result of a deficiency in the amount and function of insulin, resulting in improper carbohydrate, lipid, and protein metabolism1,2 where Insulin is a hormone that facilitates in the absorption of glucose into your cells, which gives you energy [1]. Prediabetes is a disease that can strike anyone at any time. This means that your blood sugar levels are greater than normal, but not high enough to be diagnosed with diabetes. If you have type 1 diabetes, your body does not generate insulin. If you have type 2 diabetes, which is the most prevalent type, your body does not create or use insulin properly. If there isn't enough insulin, the glucose stays in the body[2].

Glycemic management can usually restore these anomalies in type 1 diabetes mellitus. In type 2 diabetes, however, irregularities often continue long after adequate glycemic management has been achieved, despite the fact that lipid readings improve. In people with diabetes, screening for dyslipidemia is advised [3].

Hyperlipidemia is a lipid metabolic disorder in which total cholesterol, triglycerides, and low-density lipoprotein levels increase while high-density lipoprotein levels decrease One of the most important risk factors for the occurrence and incidence of coronary heart disease is hyperlipidemia. The genesis of atherosclerotic cardiovascular disease is thought to involve hyperlipidemia-related lipid issues. Natural products and their role in maintaining and promoting health and wellness are gaining popularity. The efficacy of dietary plants to lower cholesterol levels has been extensively researched[4]. Lipoprotein abnormalities connected to diabetes mellitus may explain why diabetics are more likely to develop coronary artery disease. Hypertriglyceridemia and low levels of high-density lipoprotein are the most common lipid disorders. Glycemic management can usually restore these anomalies in type 1 diabetes mellitus. In type 2 diabetes, however, irregularities often continue long after adequate glycemic management has been achieved, despite the fact that lipid readings improve. In people with diabetes, screening for dyslipidemia is advised.

Chemical induction using Alloxan is one of the most effective ways to induce experimentally induced diabetis mellitus. Alloxan is a urea derivative that causes pancreatic islet cell necrosis selectively. Furthermore, by altering the dose of alloxan utilized, it has been frequently used to generate experimental diabetes in animals such as rabbits, rats, mice, and dogs with variable degrees of disease severity [5].

Carvone (5-isopropenyl-2-methyl-2-cyclohexenone) is a volatile monocyclic terpenoid found in several essential oils, found in more than 70 plants which is one of the most important ingredients in caraway oil and has been proven to have biological properties [6] .It is available in two enantiomers, each with a distinct biological function. Mint leaves contain the (R)-(+)-carvone, which has a mint odor, while caraway seeds have the (S)-(+)-carvone, which has a caraway odor [7] .The aim of this study is to show how carvone affects diabetic hyperlipidemic rats by measuring various markers such as glucose, insulin, FADS-1, G6PC-1, ACOX-1,and the complete lipid profile.

II. MATERIALS AND METHODS

1.Drugs and chemicals

Powdered cholesterol ,cholic acid and thiouracil were kindly supplied by techno Pharma chem Bahdurarh, Alpha chemical company and Lobachemie (Mumbai, India) respectively. Alloxan monohydrate (98 %) was obtained from Sigma-Aldrich. Atorvastatin was generously provided by pharmaceuticals MUP, Egypt. Atorvastatin was purchased by pharmaceuticals MUP, Egypt. Metformin was provided from a pharmacy (Cadila, Healthcare Ltd, Ahmedabad, India) .

2. Animals

Thirty- two male albino Wistar rats weighing between 150-180 grams were purchased from a major animal house (Zagazig University, Egypt) and adapted for one week under typical environmental conditions, with a free amount of normal water and rodent chow.

3. Ethical consideration

Guidelines for the care of experimental animals published by the National Institutes of Health (NIH), and the entire experimental protocol was carried out. It was approved by Zagazig University's Ethical Committee (ZU-IACUCL / 1/F/16/2019).

4.Experimental design

Before receiving a single intraperitoneal injection of prepared alloxan (120 mg/kg b.w.) dissolved in saline rats were starved for 16 hours [8]. After 6 hour from injection the drinking water was enriched with a 5% glucose solution for 24 hours to overcome the hypoglycemic shock. After three days, using a diagnostic ACC-check test strip (Roch. diagnostics, Monheim, Germany), a blood sample from the cut tip of the tail can be used to confirm the development of diabetes. Rats with blood glucose levels above 150 mg/dl were considered as diabetic rats. The diabetic rats were selected and fed with dietary supplement CCT (normal diet supplied with 4 percent cholesterol, 1 percent cholic acid, and 0.5 percent thiouracil) for two weeks [9]. We investigated a lipid profile to ensure that the rats had hyperlipidemia now we have diabetic hyperlipidemic control rats (DHC). Rats were subdivided into three groups (8 animals /group) along with normal control rats.

This study included the following groups, which were treated as follows:
Group (1) normal rats feed A normal diet, referred to as anegative control group.
Group(2) Diabetic hyperlipidemic control group (DHC), with no treatment and referred to positive group .
Group(3) Diabetic hyperlipidemic rats were given carvone (50 mg/kg b.w) intragastrically dissolved in 1ml corn oil every morning for one month , [10]. This refered to carvone group
Group (4) Diabetic hyperlipidemic rats were given a combination drugs of atorvastatin (10 mg/kg b.w.) dissolved in tween 80[11] and metformin (100 mg/kg b.w.) dissolved in salin for one month [12] .This refered to drug combination group.

5. Blood sampling

At the end of the experiment, rats were fasted, and samples of blood were collected in serum tubes for determination of glucose, lipid profile, FADS-1, and G6PC-1.The other part of blood was collected on the EDTA tube for determination of insulin.

6.Tissue collection

After the blood was obtained, the rats were decapitated, and the pancreas was removed and dried using filter paper before being rinsed with standard normal saline. For immunohistochemistry investigation, the pancreas was kept in paraffin.

7.Biochemical analysis

Coloremetically, the glucose, triglyceride (TAG) and total cholesterol (TC) levels in the blood were assessed using a kit provided by the researchers (Biomed Company,Egypt). HDL-c levels were tested coloremetrically using a kit from (Spin react Company, Girona, Spain)[13]. The Friedewald formula was used to calculate LDL-c values [14].To determine plasma insulin, an ELISA kit (Ls.Bioscience,in co,Germany) was utilized (Ls.Bioscience ,in co,German) [15]. Fatty acid desaturase 1 (FADS-1) were determined by ELISA kit (Uscn Life science inc) , Acyl-coenzyme A oxidase 1(ACOX-1) and glucose -6-phosphate catalase (G6PC-1) were determined by ELISA kit (My Biosource, USA) [16].

8.Immunohistochemical examination of anti-insulin proteins

Serial sections of paraffin blocks of pancreatic tissue, cut at a thickness of 4 m, were immunostained. The tissue portions were deparaffinized and rehydrated in graded ethanol after being deparaffinized in xylene. To prevent nonspecific peroxidase reactions, deparaffinized tissue sections were treated with hydrogen peroxide for 10 minutes.In citrate buffer 0.01 M (pH 6.0), microwave antigen retrieval took 20 minutes. The slides were washed in PBS and then incubated with rabbit polyclonal anti-insulin antibody for 60 minutes at room temperature (GTX111314, dilution 1:100 GeneTex, USA). The DakoEnVision ™ kit was used to visualize the primary antibody binding site (Dako, Copenhagen, Denmark). The peroxidase reaction could be seen after 15 minutes of incubation in diaminobenzidine (DAB). The sections were counterstained with Mayer's hematoxylin.

10.Statistical analysis

SPSS version 23 was used to check, enter, and analyze data for data processing (SPSS Inc., Chicago, IL, USA). The following statistical methodologies were used to analyze the findings of this inquiry. The data were reported as a number and a percentage for qualitative variables, and the mean + standard deviation (SD) for quantitative variables. When comparing two groups, Student's t-tests were employed, and when comparing more than two groups, Oneway analysis of variance (ANOVA) followed by Dunnett's post hoc tests were used. If the P value is less than 0.05, the results are significant.

III. RESULTS And DISSCTION

1. Extraction of carvone

Carvone is an essential oil that is extracted from caraway seeds, carvone extract was a gift from (Lab of Dr.Atef Amer). Using HNMR, the carvone was also discovered to be the key extracted portion .

2.The effect of carvone and combination drugs on blood glucose levels, insulin and (G6PC-1) levels

DHC rats demonstrated a substantial increase in level of blood glucose ($P \leq 0.001$) compared with negative control rats. In contrast to the DHC group, the treated groups carvone and drug combination (atorvastatin& metformin) demonstrated a significant reduction in level of blood glucose ($P \leq 0.001$) (DHC) rats Fig 1A. In analogy comparison with the negative control (Diabetic hyperlipidemic control rats (DHC)), the positive counterparts showed a decrease in serum insulin Fig 1B. Positive control (Diabetic hyperlipidemic control rats (DHC) has shown a significant increase in serum level of G6PC-1($P \leq 0.001$) relative to the negative control. Carvone and combination (atorvastatin& metformin) treated groups revealed a considerable drop in serum level of G6PC-1 ($P \leq 0.001$) in contrast with the positive group Fig 1C.(see Table 1)

Table 1 . The effect of carvone and combination drugs on blood glucose levels and insulin and G6PC-1levels

Groups / Parametres	Negative control	positive control(DHC)	Carvone treated group	Atorvastatin & Metformin-treated group (combination group)
Fasting blood glucose level (mg/dl) mean ± SD (Range)	93.1±5.6** (88-102)	208.7±20.3 (185-250)	98.5±12.2** (72-111)	94.1±18.5** (71-120)
Insulin level (ng/ml) mean ± SD (Range)	8.32±0.58** (7.6-9.1)	4.12±0.66 (3.5-5.01)	6.97±0.49** (5.8-7.4)	6.72±0.14** (6.5-6.9)
G6PC-1(u/l) mean ± SD (Range)	18.41±2.74** (13.6-20.5)	39.5±5.9 (28.7-47.3)	22.7±2.13** (19.8-25.6)	24.2±3.27** (22.1-30.2)

Table 1 .In this table, there was a statistically significant difference between the 1st group (Negative control) and the 2nd group (positive control(DHC)). Also, there was a statistically notable difference between the 2nd group (positive control (DHC) and treatment groups (3rd group carvone treated group and4th group Atorvastatin & metformin-treated group . Values are expressed in Mean ± SD,* represents Statistically significant difference ($p \leq 0.050$)

, **represents Statistically highly significant difference ($P \leq 0.001$) compared to positive control group.

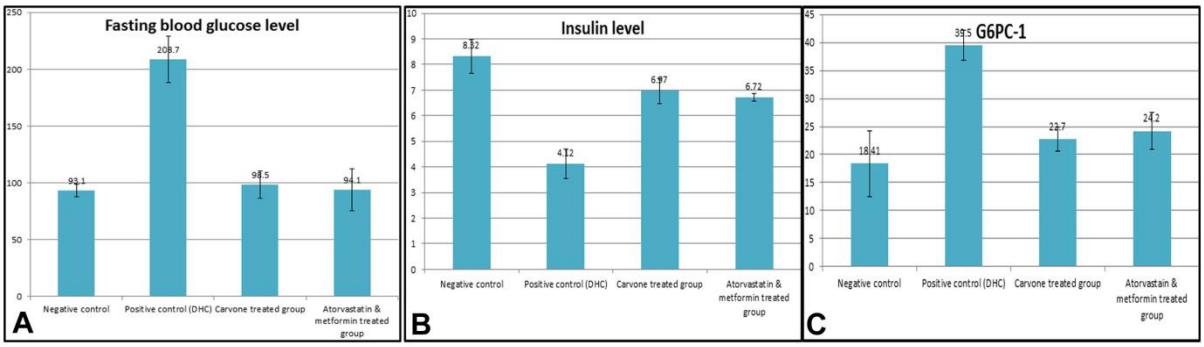

Fig 1 . chart for(A): Fasting blood glucose level, (B): Insulin level ,(C): G6PC-1 level.

3.The effect of carvone and combination drugs on lipid profile levels, (ACOX-1) and (FADS-1) levels

Our results demonstrated a substantial increase in profile (Cholesterol, Triglycride, and LDL) and a decrease in HDL($P \leq 0.001$) in compared with normal group . In comparison to the DHC group, the treated groups carvone and drug combination (atorvastatin& metformin) had significantly lower levels of (cholesterol, triglycride, and LDL) and significantly higher levels of HDL($P \leq 0.001$)Fig 2 A,B,C,D.

In analogy comparison with the negative control (Diabetic hyperlipidemic control rats (DHC)), the positive counterparts showed a decrease in serum ACOX-1 ($P \leq 0.001$). When compared to the positive group, serum levels of ACOX-1 was significantly increase ($P \leq 0.001$) in the carvone and combination (atorvastatin& metformin) treated groups Fig 2E. Positive control (Diabetic hyperlipidemic control rats (DHC) has shown a significant increase in serum level of FADS-1 ($P \leq 0.001$) relative to the negative control. Carvone and combination (atorvastatin & metformin) treated groups revealed a considerable drop in serum level of FADS-1 ($P \leq 0.001$) in contrast with the positive group Fig 2F .(see Table 2)

Table 2 .The effect of carvone and combination drug on lipid profile ,insulin and Acyle-co-A oxidase -1(ACOX-1) levels and FADS_1

Groups / parameters	Negative control	positive control(DHC)	Carvone treated group	Atorvastatin & metformin-treated group (combination group)
Cholesterol level (mg /dl) mean ± SD (Range)	54.1±3.1** (49-58)	95.3±6.3 (85-102)	67±8.5** (49-75)	64.9±9.7** (78-94)
Triglycerides level(mg /dl) mean ± SD (Range)	53.5±3.2** (50-59)	165.7±18.6 (92-292)	70.3±11.8** (55-86)	52.5±8.6** (35-62)
HDL level (mg /dl) mean ± SD (Range)	60.4±3.8** (56-69)	31.9±1.3 (30-34)	39.7±4.7** (30-45)	42.0±4.4** (36-49)
LDL level (mg /dl) mean ± SD (Range)	13.33±3.79** (9 – 16)	60.07±11.46 (54.6 – 75)	34.73±5.46* (31 – 41)	29±3.61** (25 – 32)
ACox_1(ng/ml) mean ± SD (Range)	10.44±0.11** (10.3-10.6)	4.09±0.07 (4.01-4.2)	7.81±0.2** (7.5-8.03)	7.83±0.45** (6.7-8.02)
FADS_1(pg/ml) mean ± SD (Range)	49.48±4.89** (39.2-53.6)	120.69±2.6 (116.9-125.3)	66.3±3.3** (63.2-73.4)	61.4±8.6** (57.1-82.4)

Table 2 .In this table, there was a statistically significant difference between the 1st group (Negative control) and the 2nd group (positive control(DHC)). Also, there was a statistically notable difference between the 2nd group (positive control (DHC) and treatment groups (3rd group carvone treated group and4th group Atorvastatin &

metformin-treated group . Values are expressed in Mean ± SD,* represents Statistically significant difference ($p \leq 0.050$)
, **represents Statistically highly significant difference ($P \leq 0.001$) compared to positive control group .

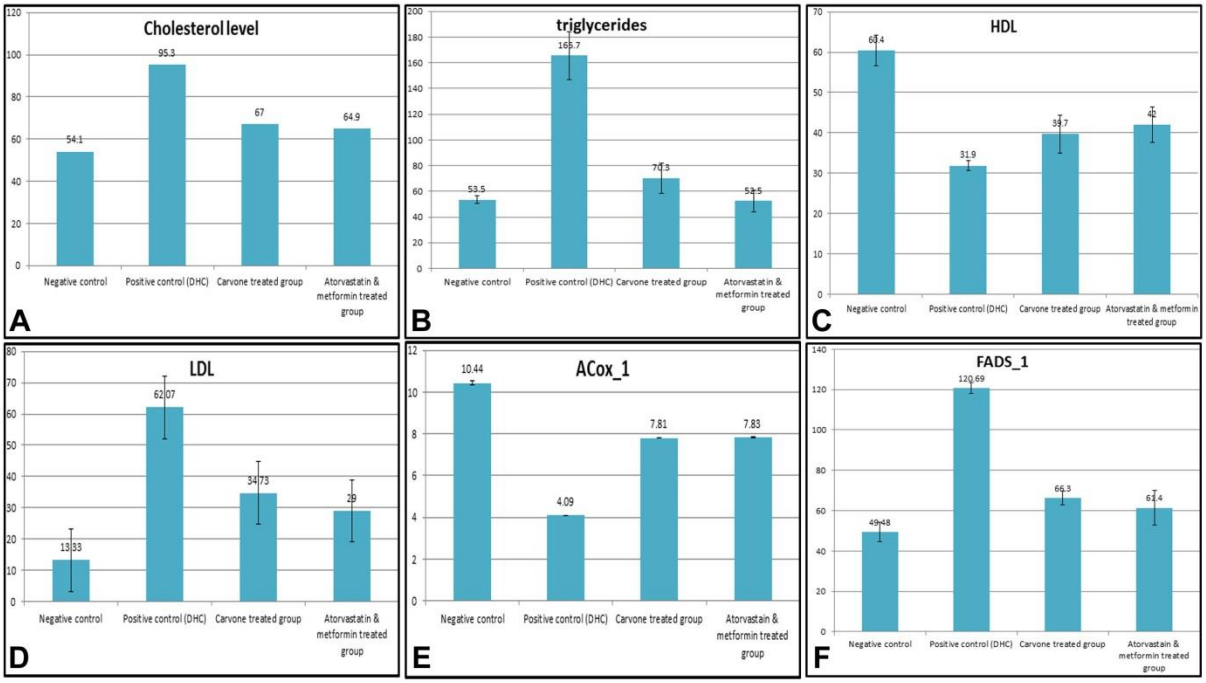

Fig 2 . chart for (A): Cholesterol level, (B): Triglycerides level, (C): HDL level, (D): LDL level among the studied group , (E): ACox_1 level, (F): FADS_1 level among the studied group

4.Immunohistochemical Examination

The immunomicrographs of pancreatic tissues in normal and diabetic hyperlipidemic rats (DHC) were represented in (Fig.3). The normal group exhibited typical appearance of islets of Langerhans with positive insulin staining of beta cells (Fig.3A). Conversely, pancreatic sections from DHC group exposed a less immunoreactive beta cells and a reduction in the size of islets (Fig.3B). Compared to the DHC group, pancreatic sections from carvone group (Fig.3C) and combination (atorvastatin and metformin) group (Fig.3D) displayed a significant enhancment in islet appearance, as well as strong expression of insulin-secreting cells to anti-insulin antibody. These results encourage the potential effect of carvone group along with the combination (atorvastatin and metformin) group in the treatment of diabetes.

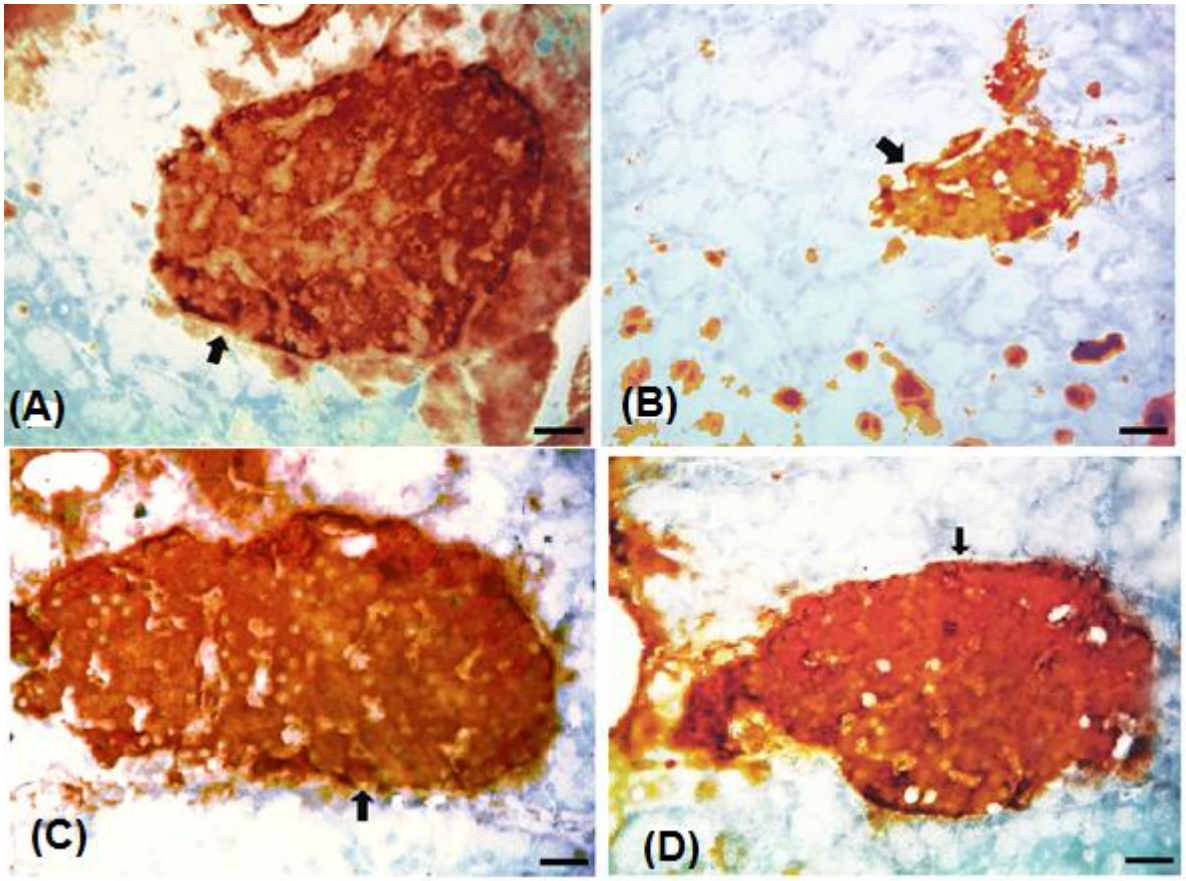

Fig3. Photomicrographs of pancreas sections in all tested groups stained immunohistochemically with anti-insulin antibody. There is a strong positive expression (arrows) in all β-cells of islets of Langerhans in the control group (a) and almost all β-cells of diabetic groups treated with: (c) Carvone (50 mg/kg) in group III and (d) Combination drugs [Atorvastatin (10 mg/kg) + Metformin (100 mg/kg)] in group IV. Notice the low reactivity (arrow) of anti-insulin antibody in group II (b) that represented diabetic hyperlipidemic control rats (DHC). (Anti-Insulin, 400x magnification, scale bar = 100µm).

DISCUSSION

Diabetes mellitus (DM) is a chronic endocrine and metabolic disease characterized by high blood sugar levels and vascular complications. and characterized by insulin insufficiency, insulin insensitivity, or both (micro and macro)[17].

Diabetes is a metabolic condition marked by persistent hyperglycemia, which can lead to major complications such heart disease, neuropathy, renal failure, and retinopathy. Several anti-diabetic medications have been produced, however their therapeutic impact is insufficient. As a result, natural compounds with multi-targeting properties are receiving more attention in the hopes of aiding in the treatment of polygenetic diseases such as diabetes[18]. In the top ten most frequent chronic illnesses, hyperlipidemia is only second to hypertension. It's possible that the disease is primarily due to hereditary factors, but it's more likely that it's an acquired ailment.

Hyperlipidemia is described by most primary care providers as higher fasting total cholesterol levels that may or may not be linked with elevated TG levels. Lipids, on the other hand, are not soluble in plasma and are instead carried as lipoprotein particles. As a result, hyperlipidemia classifications are also based on abnormalities [19].

Many natural products have been utilized to manage blood glucose levels in diabetic patients all around the world. There are various reviews that summaries the most often used natural products for diabetes management [20].

Carvone is monoterpenoid that is cyclic and unsaturated. Many essential oils contain ketones, which can be found in nature. Spearmint, dill, caraway, and angelica are just a few examples [21]. In this work, we looked at the effects of carvone in diabetic hyperlipidemic rats that were given alloxan to induce diabetes and CCT diet to induce hyperlipidemia, and then compared them to standard anti-diabetic (metformin) and anti-hyperlipidemic (atorvastatin) drugs.

We used intraperitoneal rote of alloxan in this study because it is a widely used method for hyperglycemia induction of diabetes because it has the same structure as glucose and causes a large reduction in insulin by destroying B-cells in the islet of Langerhans, resulting in hyperglycemia induction.

After one week of accumulation, rats were given a single dose of freshly prepared alloxan, and after three days, levels of blood were checked;. Diabetic rats were defined as those with a blood glucose level of more than 150. Rats were then fed an atherogenic diet (CCT diet), which consisted of a rat show diet supplemented with (1 percent cholic acid, 4 percent cholesterol, and 0.5 percent thiourasil) for two weeks to induce hyperlipidemia [9, 22].We measure lipid profile , glucose and insulin, to see if carvone has an effect.

Glucose toxicity is a well-known phenomenon that has been linked to the decreased insulin secretion in animal models of diabetes. A substantial body of evidence has developed in humans with type II (non-insulin-dependent) diabetes, demonstrating that a prolonged physiological increase in plasma glucose concentration leads to gradual impairment in insulin. The exact biochemical mechanism(s) causing the hyperglycemia-induced deficiency in insulin secretion is unknown, however it could be linked to a phosphoinositide metabolism problem[23]. The glucose-insulin system is part of a larger human complex system in which interactions between cells are important.The system's overall behaviour is determined by the components. The negative feedback controller is the insulin secretion system. operating between the pancreatic -cells and the level of glucose in the blood When a person eats a snack, for example, the body secretes By raising the rate of sugar consumption or initiating the storage process, more insulin is released into the bloodstream, lowering the glucose level in the blood When there is a low level of glucose in the blood, on the other hand, When the body quits secreting insulin, the metabolic system changes from absorptive to post-absorptive mode [24] .

In the current study, we used diabetic hyperlipidemic control rats (DHC) after inducing diabetes with alloxan and hyperlipidemia with the CCT diet. We found that increasing glucose levels and decreasing insulin resulted in fat mobilization of adipose tissue, resulting in an increased lipid profile. Following a one-month intervention with carvone, metformin, and atorvastatin, we saw a decrease in blood glucose levels. We also observed a decrease in lipid profile[25] in another study that showed that carvone has hypolipidaemic properties which come in the same line of our result .

Plasma cholesterol levels and atherosclerosis have an undeniable causal link. The endothelium plays a major role in atherogenesis, inducing inflammation and the deposition of oxidised LDL in the intima of the arterial wall, which facilitates monocyte recruitment and foam cell production. The endothelium, in most cases, operates as a selective barrier between blood and tissues, with enhanced permeability at arterial branch points/curvatures. The buildup of LDL in the sub endothelial matrix is the first and most atherogenic event. When the amount of LDL in

the bloodstream is high and HDL (a lipid that removes excess cholesterol from the periphery tissues for storage/degradation in the liver) is low, the conditions for this occurrence are ideal[26].

In our research, we looked at the effects of carvone on hyperlipidemia and found that it reduced the levels of (cholesterol, triglycerides,and LDL) while increasing HDL .an another study [27] showed that D-carvone has anti-inflammatory and protective properties in mice with acute lung damage caused by lipopolysaccharide (LPS) also have anti hyperlipidemic effect and this agree with our result.

By generating glycogen and activating gluconeogenesis, the liver maintains euglycemia. Gluconeogenesis is the generation of glucose from an endogenous carbon source, and it is important in disorders like diabetes mellitus. Fasting hyperglycemia is thought to be caused primarily by gluconeogenesis dysregulation where gluconeogenic enzymes pck1 and G6PC are regulated by glucagon, insulin, thyroid hormone, and glucocorticoids. G6PC (glucose-6-phosphate catalase) is the final enzyme in gluconeogenesis, with the highest expression levels in the liver, kidney, and pancreatic beta cells Glucagon secreted by the pancreas' alpha cells interacts to the glucagon receptor during fasting, activating protein kinase (PKA). The PKA phosphorylates cAMP-response element-binding protein and incites gluconeogenic enzyme expression (PCK1 converts oxaloacetate to phosphoenolpyruvate) and (G6PC converts glucose 6 phosphates to glucose). Diet-induced obesity increases the gluconeogenesis rate in the liver. The present findings revealed that as the level of G6PC, a gluconeogenic enzyme, decreases, the transformation of glucose 6 phosphates to glucose declines, resulting in hypoglycemia in carvone and combination drugs in comparison to positive control group . It has been reported that in primary mouse hepatocytes, glycerol triggers G6pc and is the preferred substrate for gluconeogenesis, which is consistent with recent findings [10, 28] .

Hepatic insulin signaling abnormalities are linked to mitochondrial fatty acid oxidation dysregulation. The process by which fatty acids in the form of Acyl-coA are broken down in the mitochondria to provide energy is known as beta-oxidation. It's also the liver's main fatty acid catabolism pathway[29]. Accelerating fatty acid oxidation aids in removing excess hepatic lipid and the restoration of insulin sensitivity[30] . It has been found that treating rats with carvone, and a combination of atorvastatin and metformin leads to improving lipid profile and decreasing triglyceride, LDL, and cholesterol levels in comparison to positive control group.

The flavoenzyme ACOX-1 (Acyle-co-A oxidase -1) catalyzes the initial and rate-determining reaction of classical peroxisomal fatty acid oxidation using straight-chain fatty Acyl-co-A. This product is further decomposed by catalase, which acts as a substrate by donating electrons to oxygen molecules, resulting in hydrogen peroxide [31].This has a significant impact on liver lipid metabolism,where ACOX-1 expression is reduced in diabetic hyperlipidemic patients, but it is increased in carvone and combination therapy (metformin+ atorvastatin) to maintain lipid homeostasis. Parallel to the current findings, it has been reported that phellinus linteus mycelia extract (PLE) has a hypoglycemic and hypolipidemic effect on diabetic rats by increasing the expression of ACOX1 and low-density lipoprotein receptor in the liver, as well as inhibiting the expression of critical hepatic gluconeogenesis enzymes (g6pase, FBPase) and thus lowering hepatic glucose production[32] .

FADS-1 (fatty acid desaturase-1), a protein encoded by the FADS-1 gene family, regulates unsaturated fatty acids, our findings revealed that the level of FADS-1 increased in the diabetic hyperlipidemic group but decreased in the carvone and combination groups (metformin + atorvastatin). As the active form of AMPK (AMP-activated protein kinase) exhibits an anti-lipogenic effect, the activation of AMPK was expected to suppress FADS genes, resulting in a decrease in LDL-C levels. The decline in LDL-C in the metformin-treated group could be due to the drug's effect of potentially activating AMPK to suppress FADS genes [33].

In Immunohistochemical our findings revealed that the pancreatic rats revealed a decrease in the number of immunoreactive beta cells and a reduction in the size of islets stained with anti-insulin, implying that the β-cells in the DHC group were dysfunctional in positive group but in carvone and combination (atorvastatin and

metformin) groups enhanced islet appearance and increased insulin-secreting cells, as well as in parallel with [34] who found a decreased number of insulin immunoreactive cells in the pancreatic islets of diabetic rats.

IV. CONCLUSION

In alloxan-induced diabetic rats, extracted carvone possesses hypoglycemic and hypolipidemic effects, according to this study. Carvone decreases blood glucose levels and increases insulin in diabetic hyperlipidemic rats, according to the findings given here. Carvone also aided in the maintenance of lipid and lipoprotein profile homoeostasis

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