ANTI-DIABETIC EFFECT OF ARTEMISIA ANNUA (KAYSOM) IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Background and Objective: Diabetes is the most common endocrine disorder affecting millions of people worldwide. Nowadays, herbal drugs are gaining popularity in the treatment of diabetes and its complications. The current study was aimed at evaluating the significance of supplementation of Artemisia annua (Kaysom) extract in reducing the metabolic abnormalities accompanied with alloxan-induced diabetes in male albino rats.

Material and Methods: Thirty male albino rats were divided equally into three groups including control, diabetic and diabetic treated with Kaysom extract . A single dose of alloxan(120 mg/kg body weight) was used to induce diabetes in rats. Diabetic rats were administered Kaysom extract orally twice daily for 30 days. At the end of the experimental period, level of serum fasting insulin and glucose in addition to serum lipids profile such as total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL), and very low density lipoproteins(VLDL); serum proteins including total proteins, albumin and globulin; renal markers (creatinine, urea and uric acid) and activity of certain enzymes such as aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and gamma-glutamyltransferase (GGT) was determined for all groups. In. addition, estimation of % change of body weight, values of homeostasis model assessment of insulin resistance (HOMA-IR) and ratios of albumin:globulin (A:G), TC/HDL, LDL/HDL (risk ratios 1 and2) were calculated for each group.

Results: Diabetic rats showed a marked decline (p<0.01) in the level of; serum insulin, body weight (4.98 %),total proteins, albumin, globulin and HDL accompanied with marked elevation (p<0.01) in level of; fasting blood glucose, HOMA_IR, ASAT, ALAT ,GGT, urea, creatinine, uric acid, serum TC, TG, LDL, VLDL and ratios of TC/HDL and LDL/HDL (risk factors) as compared to the corresponding of controls. Supplementation of diabetic rats with Kaysom extract significantly ameliorated most of the estimated biochemical parameters.

Conclusion: These results demonstrate that Kaysom extract may be of advantage in inhibiting hyperglycemia and ameliorating metabolic abnormalities induced by diabetes.

Key words: Artemisia annua (Kaysom), Diabetes, lipids, hyperglycemia, insulin resistance.

INTRODUCTION

Diabetes mellitus (DM) is highly recognized as the most common metabolic and endocrine disorder worldwide. It is linked to disturbances in carbohydrate, fat, and protein metabolism[1]. It is especially important because the global prevalence of diabetes is projected to escalate relentlessly. At least 250 million individuals worldwide suffer from diabetes and this number will be doubled by 2030. Increases in complications will undeniably follow increasing diabetes incidence rates.[2] More than 80% of diabetes deaths take place in low- and middle-income countries[3]. Traditional medicine practices, are considered responsible for an impartial role in primary health care despite modern medicine accessibility[4],where vegetables, culinary herbs, and medicinal plants are among the main choices in the management of diabetes [4,5].

According to the World Health Organization (WHO) report, around four billion people (80% of the world´s population) use herbal medicine[6], with eleven different bioclimatic regions and around 7,500 different plant species. Artemisia Annua is considered as an important medicinal plant species with high content of essential oils and flavonoids, and is thoroughly studied[7].The plant is overexploited by collection for folk medicinal uses, it has the

Arabic common name Qaysom and which grows in the limestone wadis of the north eastern desert, Red See regions and Sinai Oases[8].The major active constituent of Artemisia annua, isartemisinin. Derivatives of this compound include arteether, artemether, artemotil, artenimol, artesunate, and dihydroartemisinin, which, along with artemisin, are currently being used to treat drug-resistant and non-drug resistant malaria. The aerial parts of Artemisia annua contain 0.01-0.8 % of artemisinin per dry weight. Other constituents of Artemisia annua include deoxyartemisinin, artemisinic acid, arteannuin-B, stigmasterol, friedelin, friedelan-3 beta-ol, artemetin, and quercetagetin (6,7) -tetramethyl ether.[9,10]

Several effects such as anti-inflammatory[11], Antioxidativeeffects[12], antihypertensive and anti-hyperlipidemia[13], and antitumoral[14]have been reported for Artemisia. It is widely used in traditional medicine for gastrointestinal disorders, and there are some reports of its effects on urinary tract such as antispasmotic[15]. Neither acute nor subchronic toxocity were noticed in mice with ethanolic extracts of Artemisia annua[16]. In Middle East people use A. annua extract to treat diabetic states. Therefore, The current study was designed to evaluate the efficacy of Artemisia annua (Kaysom) extract in reducing blood glucose level as well as the metabolic abnormalities accompanied with alloxan-induced diabetes in male albino rats.

MATERIAL AND METHODS
Experimental animals
Thirty male albino rats (Rattusnorvegicus) weighing approximately 100-110gwere obtained from Schistosoma Biological Supply Program (SBSP) Theodor Bilharz Research Institute. They were housed in clear plastic cages (2 animals/cage) with wood chips as bedding and given a standard pellet rodent diet, in addition of water adlibitum. The rats were maintained under standard laboratory conditions at $25\pm2^{o}C$, relative humidity $50\pm15\%$ and normal photoperiod (12h light/dark cycle).
Induction of diabetes

The overnight fasted animals were rendered diabetes by a single intraperitoneal injection of alloxan (120 mg/kg body weight) with freshly prepared physiological saline. Alloxan was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Diabetic state of animals was monitored for its stability for seven successive days after alloxan treatment. On day 8 of alloxan injection, only animals with fasting blood glucose levels ≥300 mg/dl were selected as diabetic rats for the current experiment. The control rats were injected with physiological saline alone as placebo.
Preparation of aqueous extract of Artemisia annua (Kaysom):
plant was grinded ,the aqueous extract of Artemisia annua was prepared by boiling 2g of Artemisia annua with 200ml water for 5 min , left cool to room temperature then filtered .The extract was stored in refrigerator(it was daily prepared).The Diabetic rats was treated intragastrically with Artemisia annua twice daily at 8 am and 8 pm for 30days (28.5mg/kg twice /day).
Experimental protocol
The current study was performed in accordance of the International Guidelines regarding animal experiment. Three experimental groups, ten rats for each, were used as follows:
* Group I (Control group): Non-diabetic control rats .
* Group II (Diabetic group): Diabetic rats.
* Group III (Diabetic group + Kaysom): Diabetic rats supplemented with kaysom (28. 5mg/kg twice /day).

Collection of blood and estimation of biochemical parameters
One month after treatment, blood samples were collected from overnight fasted rats in centrifuge tubes by cardiac puncture under mild ether anesthesia. Sera were separated by centrifugation at 4000 rpm for 10 min at 4°C and immediately stored at -20°C for further analysis of biochemical parameters.
Serum glucose was estimated according to the method of Trinder[17]. Serum insulin level was measured by an enzyme immunoassay kit (SPI-Bio société de pharmacologieetd'lmmunoloie-

Bio, France); while values of HOMA-IR were calculated using the following equation:HOMA-IR= fasting serum glucose (mg/dl) x fasting serum insulin (μU/ml)/405.

The activities of ASAT and ALAT were assayed [18]and the activity of serum GGT.[19]Serum albumin and total proteins were measured.[20]Globulin was calculated by subtracting albumin from total proteins[21]. Levels of serum urea, creatinine and uric acid were estimated [22,23]. Serum total cholesterol[24]; triglycerides[25]and HDL[26]were estimated colorimetrically using high quality kits according to manufacturer's protocol; while VLDL was calculated as triglyceride/5andLDL
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was calculated applying the **Friedwald's equation** [27].
Friedewald's equation: LDL (mg/dl) = TC-[HDL + TG/5]. VLDL = TG/5
Risk ratio 1 = TC / HDL Risk ratio 2 = LDL / HDL

STATISTICAL ANALYSIS

The results were expressed as Mean ± SE of 10 rats per group and the statistical significance was evaluated by one way analysis of variance (ANOVA) followed by Duncan post Hoc test using the SPSS/17.0 software. Values were considered statistically significant at $P< 0.05$

RESULTS

Table 1: Effect of Artemisia annua (kaysom) administration to diabetic rats on levels of serum Insulin, glucose and HOMA-IR

	Insulin (μ IU/ml)	Glucose (mg/dl)	HOMA-IR
Control	41.36±0.45[c]	87.44±0.77[a]	8.03±0.23[a]
Diabetic	24.54±0.36[a]	292.20±0.84[c]	15.93±0.40[b]
Diab. + kaysom	37.72±0.77[b]	93.42±0.51[b]	7.83±0.33[a]

Each value represents Mean ±S.E. (n=10 rats).
Values with different superscripts differ from each other significantly (P<0.01).
A significant decrease in the levels of serum insulin accompanied with marked significant elevation in the level of blood glucose were recorded in diabetic rats when compared to the control rats .Marked recovery (P<0.01) in insulin and glucose levels was recorded in diabetic animals post consumption of kaysom extract for one month. HOMA_IR values were significantly higher (P<0.05) in diabetic rats when compared to the corresponding controls, while treatment of diabetic rats with kaysom extract returned HOMA_IR values to approximate normal value.

Table 2: Effect of Artemisia annua (kaysom) administration to diabetic rats on % change of body weight
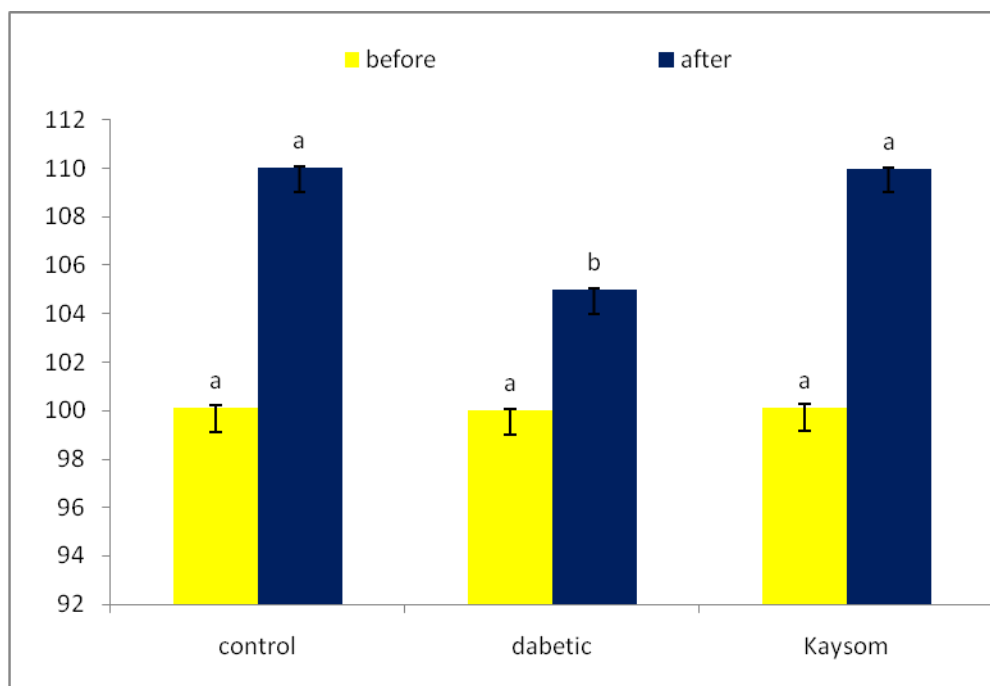
	% Change of BW
Control	9.92± 7.41[b]
diabetic	4.98± 3.54[a]
Diab. + kaysom	9.92± 5.90[b]

Each value represents Mean ±S.E. (n=10 rats).
Values with different superscripts differ from each other significantly (P<0.01).

There was a significant decrease in body weight change (4.98 %) after one month of diabetic induction when compared to that of control rats (9.92 %) . Marked normalization of body weight (9.92 %)was recorded in diabetic animals post consumption of kaysom extract for one month .

Figure 1: Effect of Artemisia annua (kaysom) administration to diabetic rats on body weight change

Each value represents Mean ±S.E. (n=10 rats).

Values with different superscripts differ from each other significantly (P<0.01).

There was a significant decrease in body weight after one month of induction of diabetes when compared to the control rats .Marked return to normal body weight (P<0.01) was recorded in diabetic animals post consumption of kaysom extract for one month.

Table 3: Effect of Artemisia annua (kaysom) administration to diabetic rats on liver function tests

	ALAT (IU/L)	ASAT (IU/L)	GGT (IU/L)	T proteins (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Control	22.46 ± 0.55^a	37.02 ± 0.59^b	2.28 ± 0.11^a	7.40 ± 0.08^b	4.24 ± 0.07^b	3.16 ± 0.05^b	1.34 ± 0.05^b
Diabetic	33.52 ± 0.60^c	53.24 ± 1.07^c	9.66 ± 0.08^c	5.30 ± 0.11^a	3.12 ± 0.05^a	2.18 ± 0.06^a	1.42 ± 0.03^b
Diab. + Kaysom	27.06 ± 0.57^b	34.18 ± 0.93^a	5.34 ± 0.09^b	8.12 ± 0.05^c	4.46 ± 0.06^c	3.66 ± 0.04^c	1.22 ± 0.02^a

Each value represents Mean ±S.E. (n=10 rats).

Values with different superscripts differ from each other significantly (P<0.01).

Diabetic rats showed a significant elevation in the activities of these enzymes, ASAT, ALAT and GGT as compared with the corresponding control group. Treatment of diabetic rats daily with Kaysom significantly abolished the disturbances occurred in the activities of these enzymes. Parameters of serum protein profile (total proteins, albumin, globulin and A/G ratio) were evaluated in control, diabetic and kaysom-treated animals. Diabetic animals showed significant marked decline in serum total proteins,

albumin and globulin relative to the corresponding controls. Treatment of diabetic rats with kaysom extract resulted in modulation of the measured serum protein profile parameters to the normal.

Table 4: Effect of Artemisia annua (kaysom) administration to diabetic rats on serum lipid profiles

	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Risk 1	Risk 11
Control	141.08±0.37[a]	133.10±0.77[a]	47.88±0.48[a]	66.56±0.47 [a]	26.62±0.16[a]	3.00±0.00[a]	1.38±0.02[a]
Diabetic	231.52±0.52[c]	283.96±0.86[c]	38.08±0.38[c]	136.40±0.59[c]	56.80±0.16[c]	6.08±0.03[c]	3.58±0.03[c]
Diab. + Kaysom	194.50±0.27[b]	148.14±0.37[b]	45.84±0,24[b]	118.96±0.24[b]	29.64±0.07[b]	4.22±0.03[b]	2.60±0.00[b]

Each value represents Mean ±S.E. (n=10 rats).
Values with different superscripts differ from each other significantly (P<0.01).
Diabetic animals showed a marked significant elevation in TC, TG, LDL, VLDL and ratios of TC/HDL (risk1) and LDL/HDL (risk11) accompanied with marked decline in HDL relative to the corresponding controls. Treatment of diabetic rats with kaysom extract improved the sera lipids profile as showed by the significant reduction in the values of TC, TG, LDL, VLDL and ratios of TC/HDL and LDL/HDL associated with marked elevation of HDL.

Table 5: Effect of Artemisia annua (kaysom) administration to diabetic rats on renal function tests

	Urea(mg/dl)	Uric acid(mg/dl)	Creatinine(mg/dl)
Control	32.46± 0.54[a]	3.26± 0.10[b]	0.91± 0.005[a]
diabetic	63.44± 0.58[c]	7.24± 0.09[c]	1.66± 0.08[b]
Diab. + kaysom	37.40 ± 0.95[b]	2.08± 0.05[a]	0.91± 0.006[a]

Each value represents Mean ±S.E. (n=10 rats).
Values with different superscripts differ from each other significantly (P<0.01).
The recorded renal markers elevated significantly in the sera of diabetic rats compared to those of control rats for urea, uric acid and creatinine, Treatment of diabetic rats with Kaysom extract ameliorated these parameters towards normal values.

DISCUSSION
In recent years, much attention has been focused on using natural products as an alternative therapy for treatment of many diseases including diabetes mellitus. In the present study, we aimed to evaluate the efficacy of Kaysom (a plant which is a component used in the folk medicine in Sinai to treat diabetes). We Examined the effect of its water extract in reducing glucose level and ameliorate the metabolic abnormalities accompanied with alloxan-induced diabetes in male albino rats.
The reduction in serum insulin level and elevation of glucose level recorded in the alloxan-treated rats was attributed to the hyposecretion of insulin by pancreatic β-cells. Alloxan selectively destroys the pancreatic insulin secreting β-cells and induces hyperglycemia[28].These results are consistent with previous findings recorded by Sivaraj[29].
HOMA-IR has proved to be a robust tool for assessment of insulin resistance [30,31].In the current study, diabetic rats showed high values of HOMA_IR. This finding is in accordance with that of Rossetti[32] who confirmed that high glucose concentrations cause the development of insulin resistance in peripheral tissues owing to impairment of both insulin secretion and insulin sensitivity. The biochemical basis for insulin resistance induced by hyperglycemia may be

attributed to modifications in structure of insulin receptors and the glucose transport system, resulting in impaired signal transmission [33].

Traditional antidiabetic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities, or as simple dietary adjuncts to existing therapies . Where, plants are rich sources of antidiabetic, antihyperlipidemic and antioxidant agents such as flavonoids, gallotannins, amino acids, and other related polyphenols[34] .

Kaysom extract exhibited significant anti-hyperglycemic and anti-hypoinsulinemia activities in diabetic animals as compared to untreated diabetic rats. In the same context, Mandour[8] showed significant decrease in blood glucose level occurred in animals receiving 28.5mg/kg twice /day of A. annua extract. This may be due to stimulation of the secretion of insulin by β cells, inhibition of α cells of the pancreatic islets, or by enhancing insulin activity.[52]

Kaysom extract is abundant with many flavonoids such as afroside, cirsimartin, chrysoplenol and cirsiliol.

There is evidence that hyperglycemia results in the generation of reactive oxygen species, leading to oxidative stress in various tissues, including vascular system. An important link between oxidative stress, inflammatory response and insulin activity is now well established. The ability of antioxidants to protect against the deleterious effects of hyperglycemia and also to improve glucose metabolism and intake must be considered as leads of choice in diabetes treatment. In addition to their antioxidative activity, many flavonoids were demonstrated to act on biological targets involved in type 2 diabetes mellitus such as: α-glycosidase, glucose cotransporter or aldose reductase. In this context, flavonoids behaving as antioxidants were studied as potential drugs by acting as biological targets involved in diabetes mellitus. Researchers observed that blood and brain levels of sugars affixed to proteins known as advanced glycation end-products-or AGEs-were reduced in fisetin-treated compared to untreated Akita mice. These decreases were accompanied by increased activity of the enzyme glyoxalase 1, which promotes removal of toxic AGE precursors.[53]

In addition, a novel hormone called betatrophin found to be secreted by liver and adipose tissues; especially after the improvement of liver function tests that obsereved in diabetic rats post administration of Kaysom extract as mentioned below . This hormone prompts beta cells in the pancreas to multiply and increase in size and produce more insulin[35].It seems also that this extract might help the body use insulin more efficiently.The anti-hypoinsulinemic effect of essential oils components of kaysom extract(camphor,germacreneD,artemisia ketone,1,8-cineole) may be attributed to its protective effect against hepatocyte damage through inhibited the LPS - elicited expression of the proinflammatory mediators IL-1b, TNFa, COX-2 and iNOS[36]. These hepatocytes produce more betatrophin enhancing insulin production by beta cells of pancreas, hence and enhancing body weight .This was in harmony with the present results where treatment with kaysom extract, its flvonoids components such as (afroside, cirsimartin, chrysoplenol and cirsiliol) ameliorated the functionof the liver and the reduction of body weight by inhibition its proinflammatory mediators and protection of hepatocytes. Also, essential oils components such as (linalool,carvacrol, eugenol, artemisia ketone, palustrol, sabinene hydrate, α-terpineol and santolina alcohol camphor, germacreneD, artemisia ketone,1,8-cineole) was shown to have a modulatory effect on the values of HOMA_IR, which may be attributed to enhanced peripheral uptake of glucose and clarify its hypoglycemic effect in addition to the increase in serum insulin level.

Activities of ALAT, ASAT and GGT serve as markers of hepatocyte injury Pari[37]and Kim [38].The elevation in the activities of these enzymes in diabetic rats reflects a state of hepatocyte injury. This lesion may be attributed to the insulin resistance that induces excess synthesis of free fatty acids. The excess in free fatty acids is known to be directly toxic to hepatocytes [39]. Flavonoids Which are active constituents of Kaysom extract, have a potent antioxidant action attenuating the oxidative stress induced by free radicals[40].This may explain the reduction in activity of transaminases and GGT in diabetic rats post

administration of Kaysom extract for 30days. These observations are in analogy to the results obtained by Chandramohan[41] on treatment of alloxan-induced diabetic rats with 3-hydroxymethyl xylitol, the active compound isolated from Caseariaesculenta root, for 45 days.

An overall significant reduction in serum total protein, albumin and globulin in diabetic animals consequents with slight non-significant elevation in A/G ratio were observed in the present study. This corroborates earlier reports recorded by Sivajothi[42] and Chandramohan[41]. Insulin generally has an anabolic effect on protein metabolism as it stimulates protein synthesis and retards protein degradation [43].Reduction of serum total protein in diabetic animals may be due to the hypoinsulinemia induced by treatment of animal with alloxan. Hypoinsulinemia increases the rate of protein catabolism and might have induced a direct adverse effect on the synthesis and secretion of albumin and globulin. In the current study, the elevated levels of serum total proteins, albumin and globulin in diabetic rats treated with Kaysom extract may be related to the recovery of serum insulin levels.Very close results were obtained by Chandramohan[41]and Sivajothi[42] on treatment of diabetic rats with 3-hydroxymethyl xylitol and ethanolic extract of Phyllanthusrheedii, respectively.

The current study revealed high prevalence of hypercholesterolemia, hypertriglyceridemia, high LDL and low HDL levels in diabetic rats which are well known as risk factors for cardiovascular diseases and affect patients with diabetes [44].A study by Kurup[45]showed that, the changes in TC/HDL and LDL/HDL ratios were better predictors of coronary heart disease than the changes in LDL alone. In the present investigation, diabetic animals showed marked elevation in the ratios of TC/HDL and LDL/HDL that increases the risk of coronary heart disease. The current observations agree with those of earlier results obtained by many investigators [46,42,47].Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver [48,49]. This stimulates the hepatic triglyceride synthesis leading to hypertriglyceridemia as well as over production of LDL and VLDL by the liver [48].Results of the present work showed that, Kaysom extract significantly ameliorated sera lipid profiles by reducing the values of TC, TG, LDL, VLDL and ratios of TC/HDL and LDL/HDL and elevating HDL levels. This indicates that, Kaysom extract has a potential role in preventing formation of atherosclerosis and coronary heart disease in diabetic rats. A 1% reduction in serum cholesterol concentration results in a 2% reduction in the prevalence of coronary artery diseases[50]. Kaysom extract contains biofunctional components, such as afroside, cirsimartin, chrysoplenol and cirsiliol, which may play as a regulatory lipid agent and have anti-atherosclerotic effect[36].

Removal of metabolite wastes such as urea, uric acid and creatinine by the kidneys maintains optimum chemical composition of body fluids. In the current study, the increased levels of creatinine, urea and uric acid are evident of kidney dysfunction in diabetic rats. Renal dysfunction indicated by elevation of renal markers in diabetic rats has been proved through previous studies [46,41].Elevation of the renal markers may be due to metabolic disturbance in diabetic animals reflected in high activities of xanthine oxidase, lipid peroxidation, and increased triacylglycerol and cholesterol levels [51]. Treatment of diabetic rats with Kaysom extract reversed these parameters towards normalcy which could be due to decreased metabolic disturbances of other pathways such as protein and nucleic acid metabolism as evidenced by improved glycaemic control. These findings are in consistence with the results obtained by Jarald[46] on treatment of diabetic rats with aqueous extract of the plant Cynodondactylon. Also, Chandramohan[41] obtained similar results after treatment of diabetic rats with 3-hydroxymethyl xylitolfor 45 days.

In conclusion, the results of the present study suggest that Kaysom extract play an important role in inhibiting hyperglycemia and ameliorating metabolic abnormalities induced by diabetes through its antioxidant advantage. Also, it's safe on some vital organs as liver and kidney. However toxicological studies must be

done before justification of its safety for diabetic patients.

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