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Anti-hyperglycemic and anti-hyperlipidemic effect of spices *(Thymus vulgaris, Murraya koenigii, Ocimum gratissimum and Piper guineense)* in alloxan-induced diabetic rats

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Abstract

This research was carried out to access the phytochemical and proximate composition of four spices (*Thymus vulgaris, Piper guineense, Murraya koenigii and Ocimum gratissimum)* and to determine the effect of their orally administered aqueous extract on lipid profile in alloxan-induced diabetic rats. The animals were grouped into six of 5 rats each. Groups B to F were induced diabetes by intraperitoneal injection of alloxan monohydrate with a dose of 170mg/kg body weight. Crude aqueous extracts (500mg/kg body weight) of each of the spices were orally administered to the rats. The result of phytochemical analysis showed the presence of flavonoid, alkaloid, saponin and tannin in all the spices and flavonoid was observed to be in highest quantity in all the spices. Proximate analysis revealed the presence of moisture, ash, proteins and carbohydrate. Thymus vulgaris showed very low moisture because it was purchased as a dry product. Fasting blood sugar, total cholesterol, low density lipoproteins (LDL), very low density lipoproteins (VLDL) and triacylglycerol significantly ($p<0.05$) decrease while high density lipoproteins (HDL) showed significant increase ($p<0.05$) compared to diabetic control and only total cholesterol was significantly ($p<0.05$) lowered compared to normal control rats. This study showed that these spices extracts can be used to control diabetes and reduce risk of cardiovascular complications arising from metabolic disease such as diabetes.

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Introduction

Diabetes mellitus is one of the commonest metabolic disorders resulting from abnormal high blood sugar level. However, hyperglycemia, hyperlipidemia and hypercholestemia are some criteria indicating metabolic syndrome and these conditions are closely associated with insulin resistance (Deguchi andMiyazaki, 2010). Another well-known complication of diabetes is hepatic fat accumulation. Fat is stored in the form of triglycerides and hepatic fat accumulation may be a manifestation of increased fat transport to the liver, enhanced hepatic fat synthesis, and decreased oxidation or removal of fat from the liver.

The use of herbs for prevention and/ or treatment of diseases are as old as human kind and so many synthetic drugs are of plant origin. The medicinal values of these plants are usually due to the presence of phytochemical content (Essien *et al*., 2012) and the most important of these phytochemicals include alkaloids, tannins, flavonoids and phenolic compounds. When these compounds are extracted and administered to animals they exert some biochemical and pharmacological actions in them.

Ocimum gratissimum is a culinary herb belonging to Lamiaceae family. It is grown in several regions of the world (Ismail, 2006) and commonly found in many gardens around village huts in Nigeria. It is used in treating nausea, flatulence and dysentery. The essential oil extract contain so many compounds: methylcharvicol, linalol (terpen), caffeic acid, safrole, quarcetin, methylcinnamate, limenene, β-pinene, etc. and are beneficial for the alleviation of mental fatigue, colds, spasm and rhinitis, and as a first aid treatment for wast sting and snake bites (Ismail, 2006).

Piper guineense is a member of the family piperaccae. It is consumed widely in some parts of West Africa especially Nigeria and Ghana on account of nutrient and medicinal properties. *Piper guineense* is consumed by women after child birth to enhance uterine contraction for the expulsion of placenta and other remains from the womb (Ekenem *et al.,* 2010, Udo *et al*., 1999). It is a potent inhibitor of cytochrome P3A4 (Usia *et al*., 2005) and possess anti-inflammatory, anticonvulsant and antibacterial activities. Some of the active compounds are piperine, α-pinene, α-thujene, camphene, β-pinene, myrcene, etc, (Tchoumbougnang *et al*., 2009).

Murraya Koenigii is a tropical to subtropical shrub in the family of rutaceae and it is a native of India. It is commonly grown in gardens around village huts in Nigeria. Medically, *M. koenigii* are used as diuretics, antioxidant, antimicrobial, anti-inflamatory, hepatoprotective, antihypercholestrolemia, antidiabetic (Arulselven *et al*., 2006). It is a potential plant that can cause significant advancement in the age of attainment of puberty with a positive role in follicular development, folliculogenesis and desired effect on development of genital organs in females (Nandini *et al*., 2010). Researcher have shown that *M. koenigii* is made up of α-pinene, sabinene, β-pinene, bornylacetate, tarpine 4-ol, γ-terpinene, α-humalene, 3-carene (Chowdhurry *et al*., 2008; Walde *et al*., 2005; Raina *et al*., 2002).

Thymus vulgaris is a low growing herbaceous plant of the family lamiceae. It is useful as infusion to treat cough, diabetes, cold and chest infections and also digestive upset in syrup form. It is soothing for sore throat as thyme is antiseptic, antibiotic and antifungal. The essential oil can be used as rub for aching joints or rheumatic pain and can also be used in the treatment of athlete's foot. The phytochemical constituents (flavonoids, thymol, euganol, alphatic phenol, saponin, flavones, leteolin) makes this spice a pharmacological agent (Dorman and Dean, 2000). Although, there are reports on hypoglycemic properties of *T. vulgaris* and hypolipidemic effect of *M. koenijii* but, there is no any known work that compared the effect of these species on anti-hyperlipidemic effect in alloxan- induced diabetic rats. Therefore, this work was designed to determine and compare the anti- hyperglycemic and anti-hyperlipidemic effects of all these spices in alloxan-induced diabetic rats.

Materials and methods

Collection and preparation of spices

Fresh leaves of *P. guineense, T. vulgaris, O. gratissium* and *M. koenigii* were purchased from Ogbete Main Market Enugu-Nigeria. The Botanical identification was carried out at the department of Plant Science and Biotechnology, Faculty of Biological and Physical Sciences, Abia State University Uturu. The fresh leaves collected were sorted and all dead matter and unwanted particles were removed. The leaves were air dried for two weeks and grounded into powder using electric blender. The grounded powders were stored in air tight container labeled according to the different species of the spices. A total of 5mg of the grounded powder were weighed out from each container and soaked separately in 10mls of distilled water for 12hrs at room temperature. The mixtures were filtered using Watman (NO 1) filter paper. The filtrates were dried in an incubator at a temperature of 40^{o}C to produce a gel-like extracts which were diluted to 500mg/kg body weight with distilled water.

Animal treatment

Thirty (30) male albino rats weighing between 160-220g were used for the study. The rats were randomly placed into 6 groups (group A-F) of 5 rats each. The animals were acclimatized for 7days before the commencement of the experiment and were all allowed free access to food (animal pellet) and water *ad libitum* throughout the experiment. Groups B to F were induced diabetes by giving 170mg/kg body weight of alloxan monohydrate intraperitoneally. Five days after, fasting blood sugar were determined. The extracts were administered as follows:

Group A: The animals were non-diabetic and were given distilled water (placebo), and served as normal control.

Group B: The animals were diabetic and were given distilled water (placebo), and served as diabetes control.

Group C: The animals were diabetic and were given 500mg/kg body weight per day of *T. vulgaris* aqueous extract.

Group D: The animals were diabetic and were given 500mg/kg body weight per day of *P. guineense* aqueous extract.

Group E: The animals were diabetic and were given 500mg/kg body weight per day of *M. koenigii* aqueous extract.

Group F: The animals were diabetic and were given 500mg/kg body weight per day of *O. gratissium* aqueous extract.

Approval for animal studies was obtained from the animal ethic committee of Faculty of Applied Natural Sciences Enugu State University of Science and Technology Enugu-Nigeria.

Collection and analysis of blood sample

The animals were sacrificed after three months by anaesthetizing in a jar containing cotton wool soaked in chloroform. The blood samples were collected through cardiac puncture into plain tubes, allowed to clot and spun in Angular centrifuge (Techmel and Techmel, Engl. Model 80-2) at 3000rpm for 5min to obtain sera. The separated serum samples were stored in refrigerator at -4^{o}C until the next day for the analysis.

Glucose oxidase enzymatic method was used to determine fasting blood sugar (Trinder, 1969). Total cholesterol was determined by enzymatic method while colorimetric method was used to determined high density lipoprotein–cholesterol (HDL) and triacyglycerol (Ellefson and Caraway, 2009). Very low density lipoprotein (VLDL) and low density lipoproteins (LDL) were carried out by calculation method (Ellefson and Caraway, 2009).

Statistical analysis

Values were represented as mean ± S.D. Data was analyzed with analysis of variance (ANOVA) and students T test. Group means were also compared using Duncan's multiple range tests at $P<0.05$.

Result

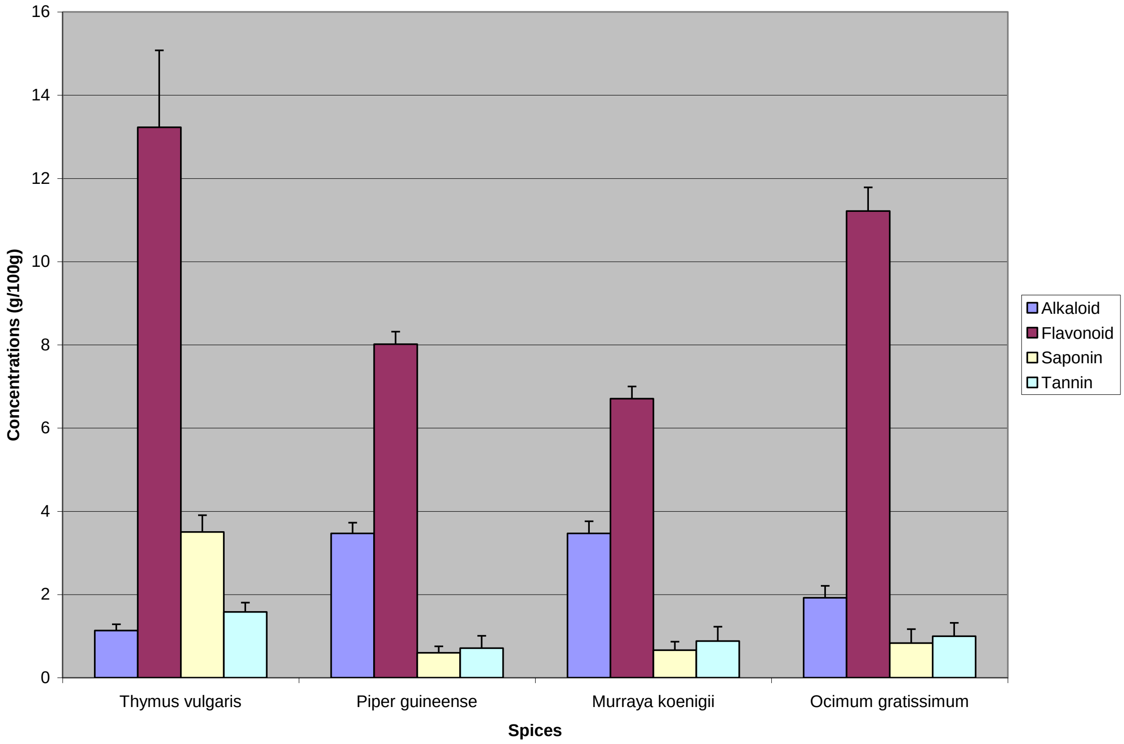
Fig. 1 showed the Phytochemical Composition (flavonoid, alkaloid, saponin and tannin) of the Spices. Bars with variable letters a-j showed significant difference ($p<0.05$) while those with common letters showed no significant difference ($p<0.05$).

Fig. 2 showed proximate Composition (moisture, ash, protein and sugar) of the Spices. Bars with variable letters a-l showed significant difference ($p<0.05$) in composition while those with common letters showed no significant difference ($p<0.05$).

Table 1. Effect of aqueous extract of spices on lipid profile (mmol/l).

Parameters	Group A	Group B	Group C	Group D	Group E	Group F
Total cholesterol	2.48 ± 0.47^b	3.65 ± 0.37^c	2.62 ± 0.08^b	2.57 ± 0.37^b	2.67 ± 0.34^b	2.12 ± 0.53^a
Triglyceride	0.75 ± 0.18^a	1.22 ± 0.13^b	0.75 ± 0.14^a	0.97 ± 0.20^a	0.86 ± 0.33^a	0.76 ± 0.23^a
HDL	1.14 ± 0.26^b	0.63 ± 0.36^a	1.12 ± 0.08^b	1.24 ± 0.55^b	1.29 ± 0.14^b	1.03 ± 0.11^b
LDL	0.97 ± 0.16^a	2.24 ± 0.32^c	1.20 ± 0.21^b	0.87 ± 0.27^a	0.86 ± 0.36^a	0.87 ± 0.23^a
VLDL	0.38 ± 0.09^a	0.61 ± 0.06^b	0.38 ± 0.07^a	0.53 ± 0.10^b	0.47 ± 0.06^a	0.39 ± 0.13^a

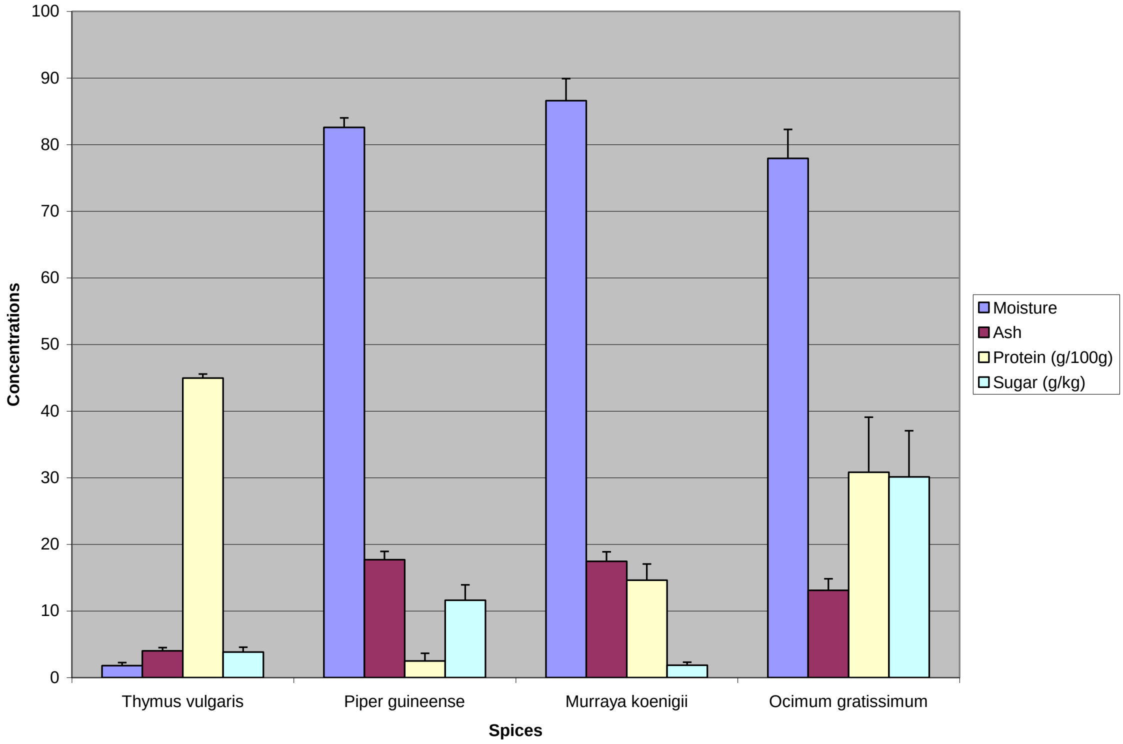
Values are mean ± standard deviation of five determinations. Values with different superscript alphabet are significantly different at $P<0.05$.



Data shown are means ± SD (n=3).

Fig. 1. Phytochemical Composition of the Spices.

Fig. 3 showed the blood glucose of rats before and after feeding with spices extracts. Before feeding with the spices extracts, the sugar level of the experimental groups of rats showed no significant difference ($P<0.05$) when compared with diabetic rats. After treating the rats with spices extracts, the sugar level showed significant decrease ($P<0.05$) compared to diabetic control rats.



Data shown are means ± SD (n=3).

Fig. 2. Proximate Composition of the Spices.

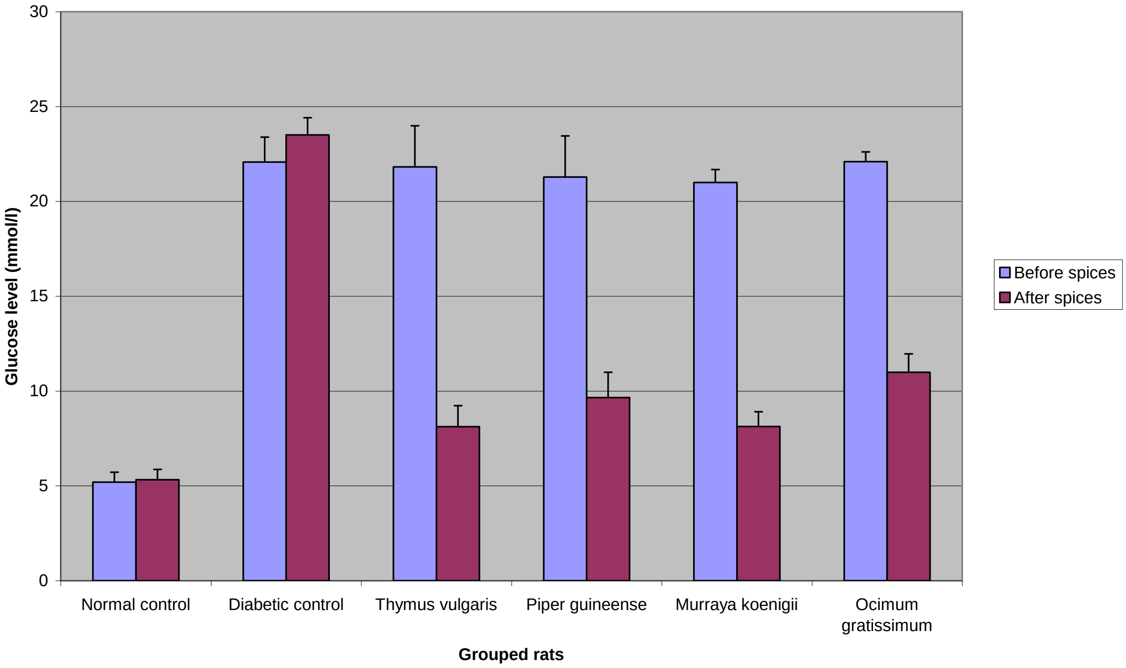
The effects of aqueous extract of the spices on lipid profile of alloxan-induced diabetic rats are as shown in table 1. The result showed that total cholesterol, triglyceride, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) significantly ($P<0.05$) decreased compared to diabetic control while high density lipoprotein (HDL) significantly ($P<0.05$) increased compared to diabetic control.

Discussion

The result in fig. 1 showed that flavonoids were well distributed in all the spices with *T. vulgaris and O. gratissimum* having 13.23g/100g dry material and 11.21g/100g dry material respectively. Flavonoids are known for its antioxidant properties. Nijveldt *et al.* (2001) reported that flavonoid is a direct scavenger of free radicals; it stabilizes the reactive oxygen species by reacting with the reactive compounds of the radical. *P. guineense* and *M. koenigii* contain largest quantity of alkaloid with about three times the alkaloid content of *T. vulgaris*. Saponin and tannin were also present at various concentrations in the spices. It has been reported that alkaloids extracted from the leaves of *Aegle marmelose* progressively reduced blood glucose from 158mg/dl to 90mg/dl in 30days (Ponnachan *et al.*, 1993) and, saponin and tannin extracted from *Hunteria umbelleta* also showed anti-diabetic effect (Adeneye and Adeyemi, 2009).

All the spices except *T. vulgaris* (which was purchased as a dry product) showed high moisture content. High moisture content is an indication of reduced shelve life (Otunola *et al.*, 2010).There were no significant difference ($P<0.05$) in the ash content of *P. guineense*, *M. Koenigii* and *O. gratissimum* however, they differed significantly ($P<0.05$) with *T. vulgaris*. Ash content is an indication of mineral content of the spices. Researchers have reported that certain inorganic mineral elements play important role in the maintenance of the normal glucose tolerance and in the release of insulin from β-cells of islets of Langerhans (Otunola *et al.*, 2010, Abolaji *et al.*, 2007).

T. vulgaris have high protein content, 45g/100g dry material (DM) followed by *O. gratisssimum*, 30.8g/100g DM, *M. Keonigii*, 14.8/100g DM and the least, *P. guineense*, 2.5g/ DM. The normal daily protein requirements for a normal adult is 45–50g, therefore, *T. vulgaris* and *O. gratissimum* can serve as a protein supplement since they are used as flavors in our foods (Dashak *et al.*, 2001). The carbohydrate contents are low. Only *O. gratissimum* have appreciable carbohydrate content as shown in fig. 2. Low carbohydrate content of the spices is advantageous to the diabetic patient because the consumption of the spices will contribute insignificant value of sugar to the patient's total glucose value.



Data shown are means ± SD (n=5).

Fig. 3. Effect of the aqueous extracts of these Spices on Blood Glucose (FBS).

Fig. 3 showed that before the treatment with spices extracts, the induced rats showed glucose level on the range of 15.0mmol/L to 30mmol/L. After treating with spices extract, the glucose level reduced from 4mmol/L to 14mmol/l. Average percentage reduction by the spices was 60%. This showed that the spices have potent anti-diabetic effect. However, the degree of hypoglycemic effect varies from spices to spices. *M. koenigii* had the highest hypoglycemic effect with about 63.72% reduction while *O. gratissimum* showed least effect with about 50.23% reduction as shown in fig.3.

The variation in the degree of anti-hyperglycemic effect might be as a result of variation in the phytochemical and nutritional properties of the spices (Mensah *et al.*, 2008). Our work is in agreement with the work of Dearlove *et al.* (2008), Zeggwagh *et al.* (2007) and Karim *et al.* (2011), who suggested that the bioactive constituent of herbs and spices certainly makes foods more medicinal and anti-inflammatory. The protective effect of *M. koenigii* on pancreatic β-cells and their antioxidant activities are major factors that contribute towards its hypoglycemic activity (Arulselvan and Subramanian, 2007). The reduced hypoglycemic effect of *O. gratissimum* may be due to its relative high glucose content as shown in fig. 2. However, hypoglycemic effect of the extracts largely depends on its antioxidant properties (Mensah *et al.*, 2008; Zeggwah *et al.*, 2007; Okwu and Josiah, 2006; Meliani *et al.*, 2011; Adeneye and Adeyemi, 2009). The extract might have exerted its anti-diabetic effect by inhibiting glycogenolysis and gluconeogenesis, thereby preventing production of glucose from glycogen or other sources. The observed increase in the activity of insulin when diabetic rats were treated with *M. koenigii* were suggested to be due to the effect of the extract probably exerted on the rat by stimulating insulin secretion from the remnant β-cells or from the regenerated β-cells (Arulselvan and Subramanian, 2007) .

The observed high level of total cholesterol, VLDL, LDL and triglycerides before administration of aqueous extract of the spices may be as a result of over production of endogenous substrate and/or defect in the clearance of the products (VLDL and triglycerides). During hyperglycemia, there are increases in the production of free fatty acid (FFA). The excess FFA condenses with glycerol to form VLDL, thereby increasing the plasma VLDL and triglyceride. The increase in triglyceride of diabetic animals may be as a result of insulin deficiency which results to hyperglycemia in which fatty acids from adipose tissues are mobilized for energy purpose followed by accumulation of the excess fatty acids in the liver which are converted to triglyceride (Shih *et al.*, 1997).

Increase in low density lipoprotein (LDL) could be as a result of decrease in its clearance. The lysine residue of apolipoprotein B tends to be glycated proportionately with increase in blood glucose. Glycation changes the structural configuration of LDL leading to defect in clearance of LDL. It was showed that approximately 40% glycation of apolipoprotein B completely blocks receptor mediated LDL catabolism in vivo (Kesaniemi *et al.*, 1983). The finding of reduced high density lipoprotein turnover is in keeping with reduced VLDL clearance and lipoprotein lipase activity. There are increases in the levels of HDL during lipolytic processes. An elevated hepatic lipase may also contribute to decreased HDL concentration, since it also plays key role in metabolism of HDL (Howard, 1987, Eisenberg, 1984). Lipoprotein metabolism normalizes when the rats were treated with the extracts and this could be attributed to the antioxidant properties of flavonoids which were widely distributed in the aqueous extract of all the spices. The metabolism of lipoproteins correlates well with glycemic control of the spices. The extract promotes insulin activity and/or production and might also interfere with the hepatic insulin resistance. Boden *et al.*, (2002), reported that free fatty acid cause hepatic insulin resistance by inhibiting insulin suppression of glycogenolysis and gluconeogensis.

Conclusion

It was observed that the spices were enriched with chemical and nutritional properties, exerted hypoglycemic effect on diabetic rats, and normalized the high lipid profile of diabetic rats. This study showed that these spices do not just impact flavor to our foods, but may be useful in reducing the risk of cardiovascular complication arising from diabetes and other metabolic diseases.

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