

Original Research Article**Effect of dietary supplementation of Turmeric (*Curcuma Longa*) and/or Black
cumin seed (*Nigella Sativa*) in streptozotocin induced diabetic rats****Abstract**

Aim: The objective of the present study was to investigate the effect of Turmeric (*Curcuma Longa*) and Black cumin seed (*Nigella Sativa*) either alone or in combination in streptozotocin (STZ) induced diabetic rats.

Study design: Thirty five healthy adult albino rats kept on ordinary ration, received water *ad libitum* and divided into five groups (7 rats in each). Rats of first and second groups were non diabetic and diabetic respectively. Rats of 3-5 groups were diabetic and received Turmeric (1 g/kg b.wt.), Black cumin seed (2 g/kg b.wt.), half dose of black cumin seed and Turmeric as a mixture, respectively. All treatments were orally administered by intragastric intubations once a day for six weeks.

Methodology: Changes in blood glucose concentration, body weight and water and food intake were measured. In addition, selected biochemical parameters were also determined.

Results: The present study revealed that, dietary supplementation of either Turmeric or Black cumin seed to diabetic rats were significantly ($P<0.05$) reduced the blood glucose level as well as water and food intake accompanied by an increase in body weight gain when compared with untreated diabetic rats. Proteins were not affected whereas liver and kidney functions were disrupted in STZ-diabetic rats and restored whenever treated by medicinal plants.

Conclusion: Hypercholesterolemia and hyperlipidemia observed in STZ-diabetic rats were attenuated by administration of medicinal plants. These results also appeared to reveal that, Turmeric was the most effective drug over Black cumin seed and the mixture of both plants.

Keywords: Diabetes mellitus, blood glucose, body weight, water intake, food intake.

1. INTRODUCTION

Diabetes is one of the most common chronic diseases affecting more than 100 million people worldwide. The two major types of diabetes mellitus are characterized by hyperglycemia, abnormal lipid and protein metabolism along with specific long term complications affecting the retina, kidney and nervous system [1]. Hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus [2]. There are many anti-diabetic plants, which might provide useful sources for the development of drugs, in the treatment of diabetes mellitus. The literature on medicinal plants with hypoglycemic activity is vast. As many of these plants were used for many centuries and sometimes as regular constituents of the diet, it is assumed that they do not have many side effects. However chronic consumption of large amounts of traditional remedies must always be taken with caution as toxicity studies have not been conducted for most of these plants [3].

Turmeric (*Curcuma longa*) is a perennial herb that grows to a height of three to five feet and is cultivated extensively in Asia (India and China) and other countries with a

tropical climate. *Curcumin*, the active ingredient from the spice turmeric is a potent antioxidant and anti-inflammatory agent with hepatoprotective, anticarcinogenic and antimicrobial properties [4,5]. *Curcumin* also has a beneficial effect on blood glucose in diabetics and increases gastric mucosal secretion in rabbits [6]. The Turmeric rhizomes have been reported to possess antidiabetic properties as its alcohol extract possesses active constituents showing blood glucose lowering activity in alloxan induced diabetic rats [3]. Black cumin seed (*Nigella sativa*) is herbaceous plant which is a member of the Ranunculacea family. Significant reduction in blood glucose and cholesterol levels in humans was also reported [7]. Black cumin seed has been found to rank high among the antidiabetic plants most recommended by traditional practitioners [8]. Many studies have also examined the antidiabetic effect of Black cumin seed (*Nigella sativa*) in normal and in diabetic animal models. Aside from the effect of its crude aqueous extract to restore glucose homeostasis [9] *Nigella sativa* petroleum ether extract significantly lowered fasting plasma levels of insulin and triglycerides and normalized HDL-cholesterol [10].

The effect of Black cumin seed and Turmeric mixture, hereafter, abbreviated as BTM on the blood glucose level are not available and to the authors knowledge this is the first report in rats. Therefore, the present research work was conducted to study the comparative efficacy of both plants alone or as a mixture (BTM) on blood glucose level; water and food intake, body weight and some biochemical parameters in STZ induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Plant materials

Turmeric (*Curcuma longa*) and Black cumin seed (*Nigella sativa*) were purchased from a local market in Al-Ahsa, Saudi Arabia and identified by botanists in the faculty of Agriculture, King Faisal University, Saudi Arabia. Both plants were analyzed and their ingredients are presented in Table 1.

2.2 Preparation of plant suspension

The whole Black cumin seeds were crushed in a blender and 12.5 g of the seeds was added to 100 ml distilled water at room temperature to prepare a crude suspension a few minutes before experiment [11]. The Black cumin seed was used in a dose of 2 g/kg body weight daily [9,10,12]. Turmeric powder was mixed with distilled water [13] to obtain a dose of 1 g/kg body weight [6].

2.3 Experimental animals

A total of thirty five adult albino rats weighing between 185–200 g were obtained from the laboratory animal house of the College of Veterinary Medicine, King Faisal University, Saudi Arabia. They were maintained as performed by national guidelines and protocols, approved by the University Scientific Research Ethics Committee. They were housed in clean and disinfected cages. Commercial basal diet and water were provided *ad libitum*. Rats were subjected to natural photoperiod of 12hr light:dark cycle throughout the experimental period (5 weeks). All rats received basal diet for two weeks before the start of the experiment for adaptation and to ensure normal growth and behavior. They were maintained in their respective groups for 42 days, monitored closely every day and

weighed every week. The rat food was weighed every day before and after feeding the animals to determine the daily food intake.

2.4 Induction of experimental diabetes

Diabetes was induced by administering intraperitoneal injection of a freshly prepared solution of STZ (60 mg/kg b. w.) in 0.1 M cold citrate buffer (pH 4.5) to the overnight fasted rats [14]. Because of the instability of STZ in aqueous media, the solution was made using cold citrate buffer (pH 4.5) immediately before administration. Control rats were injected with citrate buffer alone. The rats were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After 72 hours, fasting blood glucose levels were monitored. Animals having blood glucose levels 145 mg/dl were excluded from the experiment and animals having blood glucose values above 250mg/dl on the third day after STZ injection were considered as diabetic rats [15]. Then the treatment was started on the third day after STZ injection and it was considered as first day of treatment.

2.5 Experimental design and sampling analysis

The rats were divided into five groups (7 rats each).

Group 1: non diabetic control rats (positive control).

Group 2: Diabetic control rats (negative control).

Group 3: Diabetic rats treated with turmeric (1 g/kg b.wt. per day) in aqueous solution orally for six weeks [6].

Group 4: Diabetic rats treated with black cumin seed (2 g/kg b.wt. per day) in aqueous solution orally for six weeks [12].

Group 5: Diabetic rats treated with black cumin seed (1 g/kg b.wt. per day) mixed with turmeric (0.5 g/kg b.wt. per day) in aqueous solution orally for six weeks.

Daily measurements of body weight, and water and food intake were recorded. For blood glucose level estimation, blood samples were collected from the fasted rats of five groups prior to the treatment with plants and three times per weeks after oral administration of the treatments up to 6 weeks. Blood samples were collected by snipping tail with sharp razor and blood glucose level was then measured immediately by glucose strips (haemo-glucotest). In addition, blood samples were collected without anticoagulant for serum separation. Serum was separated by centrifugation for 10 min at 1200 g and was immediately frozen at -20°C until the time of analysis. The sera were used for spectrophotometric determination of the activities of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) [16]. In addition, total protein [17], albumin [18], globulin, uric acid [19], creatinine [20], triacylglycerol (TAG) [21] and total cholesterol [22] were also determined. Very low density lipoprotein cholesterol (VLDL-c) was calculated by division of TAG by 5 [23]. Calcium, phosphorus and magnesium were determined by using commercial kits on chemistry analyzer according to the manufacturer instructions.

2.6 Statistical analysis

All the grouped data were statistically evaluated and the significance of changes caused by various treatments was determined using Students t-test [24]. The results have been expressed as means $\pm$ SD from seven rats in each group. A one way ANOVA was also done wherever appropriate. The level of statistical significance was set at $P < 0.05$.

3. RESULTS

The present findings (Table 2) showed that the control rats (group I) gained weight over the six weeks of the experimental period, with the mean body weight increasing by 60 grams. Moreover, the untreated diabetic rats (group II) lost an average of 30 grams of their weight after six weeks ($P<0.05$). When these diabetic rats treated with Turmeric alone, they did not lost their weight but gained 25 grams of body weight which represented 41.7% of weight gained in normal non diabetic control rats. Also, diabetic rats treated with BTM did not lost their weight but gained 20 grams of body weight which represented 33.3% of weight gained in normal non diabetic control rats. Loss of body weight observed in non-treated diabetic rats was improved by 50% when they treated with Black cumin seed only.

Data summarized in Table 3 revealed that, water intake in untreated diabetic groups (Group II) were significantly ($P<0.05$) increased by 100% than non-diabetic normal rats (group I). When these diabetic rats treated with Turmeric alone, this increase in water intake was decreased significantly ($P<0.05$) till reached the control value. However, 30% of the increase in water intake in diabetic rats was decreased ($P<0.05$) whenever treated with Black cumin seed only or BTM throughout the entire intervals of the experiment.

Data summarized in Table 4 revealed that feed intake in untreated diabetic groups (Group II) were significantly ($P<0.05$) increased by 153% than non-diabetic normal rats (group I). When these diabetic rats treated with Turmeric alone this increase in feed intake was decreased significantly ($P<0.05$) till reached the control value. However, 39.5% of the increase in feed intake in diabetic rats was reduced ($P<0.05$) whenever

163 treated with Black cumin seed only or BTM throughout the entire intervals of the
164 experiment.

165 The results for the effects of Turmeric and/or Black cumin seed on blood glucose
166 concentration of diabetic rats are shown in Table 5. At the start of the experiment or at
167 zero time, there were no statistically significant ($P>0.05$) differences in the mean values
168 of blood glucose level between all experimental groups. After injection of STZ, the mean
169 values of blood glucose levels in untreated diabetic rats (group II) were remained above
170 350 mg/dl during the entire period of the study, which were significantly ($P<0.05$) higher
171 than those of the non-diabetic normal control rats by 253.3%. The percentage of increase
172 in glucose level of diabetic rats (253.3%) was reduced by 45.6% (201 mg/dl), 25.7% (260
173 mg/dl) and 28.6% (250 mg/dl) when they treated with Turmeric, Black cumin seed and
174 BTM respectively.

175 The results presented the effect of Turmeric and/or Black cumin seed on protein
176 patterns, lipid profile, liver and kidney functions and electrolytes were presented in Table
177 6. These findings indicated that, STZ induced significant ($P<0.05$) increase in total
178 cholesterol values by 31.3% compare with normal non diabetic rats. Turmeric and Black
179 cumin seed administration reduced this increase till reached lower than the normal
180 control level. In addition, BTM also restored the cholesterol to normal level. The present
181 findings indicated also that, STZ induced significant ($P<0.05$) increase in TAG values by
182 9% (142 ± 3.4 mg/dl) compare with normal non diabetic rats (130 ± 4.1 mg/dl). Turmeric,
183 Black cumin seed and BTM administration reduced this increase till reached 50.8% ($64 \pm$
184 4.1 mg/dl), 30.8% (90 ± 4.1 mg/dl) and 41.5% (76 ± 4.1 mg/dl) lower than the normal
185 control level (130 ± 4.1 mg/dl). The other examined biochemical values remained

186 unchanged throughout the experimental period.

187

188 4. DISCUSSION

189 Growth promoters are commonly added to the animals feed for growth
 190 enhancement and efficient feed utilization. They are chemical products, antibiotics,
 191 enzymes and/or natural extractives. Since the use of chemical products antibiotics might
 192 have some unfavorable side effects, therefore researchers tended to use natural additives
 193 which meet the requirements of good growth promoting agents [25]. Medicinal plants
 194 have received increasing attention as spices for human and additive in diets for animals.
 195 In the present study, we tried to examine the antidiabetic effect of Black cumin seed and
 196 Turmeric alone or as a mixture in experimentally induced diabetic rats, since the
 197 publication concerning this issue are limited. Injection of STZ in rats caused significant
 198 increase in blood glucose level. Using 60 mg/kg STZ dose induced an autoimmune
 199 process that resulted in the destruction of the β -cells of the islets of Langerhans and 60
 200 mg/kg STZ dose resulted in the toxicity of β -cells with emergence of clinical diabetes
 201 within 2-4 days [26]. Previous articles [27,28] indicated that, STZ diabetic rats are
 202 hyperglycemic and have increased oxidative stress. This is also in consistent with the fact
 203 that blood glucose level might have increased due to gluconeogenesis in the absence of
 204 insulin [29]. In other hand, the present study revealed that Turmeric and/or Black cumin
 205 seed were effective in reducing blood glucose concentration. The hypoglycemic effect of
 206 Turmeric observed in the current study comes in accordance with those obtained in rats
 207 [6,30]. Authors reported that *curcumin*, the active principles of Turmeric had a beneficial
 208 effect on blood glucose in diabetics and increases gastric mucosal secretion in rabbits.

The inclusion of *curcumin* at 0.6-0.9 g/kg diet improved the adverse effect of aflatoxins in blood glucose values of broiler chicken [31]. The present results concerned the hypoglycemic effect of Turmeric agrees with that reported the antidiabetic effect of Turmeric rhizomes in alloxan induced diabetic rats [3]. The antidiabetic effect of Turmeric perhaps attributed to the antioxidant activity of *curcumin* against oxidative tissue damage [32]. In consistence with the previous studies [8,9,33], the present study confirmed that, Black cumin seed had hypoglycemic effect. The antidiabetic effect of Black cumin seed perhaps explained by an insulin-like stimulation of glucose uptake by muscle and adipose tissue [34] or inhibition of intestinal glucose absorption [12]. Interestingly, the present study revealed for the first time that Turmeric was effective in lowering blood glucose concentration than Black cumin seed and the mixture of both plants (BTM). The first possibility to interpret such effect is that the active ingredients in Turmeric that affecting blood glucose level might be antagonized with Black cumin seed active ingredients in the mixture. The second possibility is that the efficiency of Turmeric active ingredients might be lower beyond 1 g/kg body weight/day. The third possibility is that the antioxidant activity of Turmeric might be is more potential than those of Black cumin seed to reverse the toxic action of STZ which necessitate molecular study of antioxidants.

The present study also showed that, injection of rats with STZ lead to reduction of mean value of body weight accompanied by an increase in the mean values of food and water intake. These results further support the fact that the most classic symptoms for diabetes mellitus are Polyuria, Polydipsia and Polyphagia. Poor or defect of glucose utilization can also lead to weight loss and sense of fatigue despite of normal or even

increased of food intake [35]. In addition, body weight loss and fatigue can also result due to loss of fluid. To compensate for loss of body weight and fluid, the animals should increase food and water intake as a consequence to such changes [35]. On other hand, the present data also revealed that treatment of diabetic rats with Turmeric and/or Black cumin seed appears to have positive effects on body weight gain as well as in reduction of both food and water intake. These improvements might be explained by the partial reduction in blood glucose levels in STZ induced diabetic rats. As Turmeric was the best in lowering blood glucose concentration over Black cumin seed and their mixture (Table 5), this was reflected in body weight and water and food intake. However, another study [31] reported that, oral administration of Turmeric (0.3 or 0.6 g/kg diet) had no effect on growth performance of broiler chicken. An increase in body weight gain in rats fed different concentration of Black cumin seed was reported [33] whereas in the contrary, oral administration of methanol extracts of Black cumin seed lowered body weight in mice [36]. Interestingly, the effect of combined mixture of Black cumin seed and Turmeric was investigated only in fish [37]. The authors concluded that dietary supplementation of Black cumin seed and Turmeric (5g/kg diet) improved performance and biochemistry of *Mugil Cephalus* [37]. The synergistic effect of BTM reported above was not correct in the present study as Turmeric was superior over BTM regarding performance and biochemical parameters in rats.

The unchanged serum total proteins, albumin and globulin values in STZ diabetic rats treated with BTM disagree with the findings observed in *Mugil cephalus* fish [37] when the same dose of BTM was used. The present results concerning biochemistry disagree with previous researches demonstrating the positive effect of Black cumin seed

in biochemical parameters of Nile tilapia [27], Catfish [38] and Pekin ducklings [39] and *Mugil Cephalus* fish [37] and disagree also with the results concerning the positive effect of Turmeric in biochemical values of mice [40], rats [41] and *Mugil Cephalus* fish [37]. However, this difference perhaps attributed to different species, dose and experimental period. The increased activity of AST in STZ induced diabetic rats indicated liver dysfunction as reported before in rats [42]. Liver damage was observed after oral administration of aqueous extract of *Nigella sativa* (10 ml/kg of body weight for 14 consecutive days) in rats [43] or in mice after oral administration of an aqueous extract of the Black cumin seeds in 4 different doses, 6, 9, 14 and 21 g/kg [36]. In addition the same liver damage was observed in ducklings kept on powdered Black cumin seed (20 gm/kg diet) [37]. The liver damage observed in the previous studies [36,39,43] disappeared in the present study and that might be attributed to difference in plant source, dosage, route of administration, species, age and administered ingredients. The present study reported non-significant changes in liver and kidney function in all groups. These results come in accordance with the previous findings [44] demonstrated that diabetes induction caused insignificant changes in serum levels of ALT, AST, urea and creatinine. In the contrary, the present findings also disagree with others [42] demonstrated an increase in blood urea nitrogen and uric acid in STZ induced diabetic rats indicated kidney dysfunction.

Diabetes induced many changes in lipid profile as hyperlipidemia and hypercholesterolemia observed in STZ diabetic rats come in accordance with the previous studies in rats [27,45]. The antihyperlipidemic and antihypercholesterolemic effect of Turmeric was reported before in rats [27,46]. Administration of 500 mg Curcumin to human volunteers for one week did not produce significant change in serum cholesterol

level, with Curcumin tending to lower serum cholesterol and TAG levels [47]. Contrary to these results, consumption of Curcumin over 1 month or 6 months was reported not to affect blood concentrations of TG, or TC, LDL, and HDL-c [48]. The hypocholesterolemic effect of Turmeric reported to be due to up-regulation of cholesterol 7 α -hydroxylase in rats [46]. Hypolipidemic and hypocholesterolemic effect of Black cumin seed observed in the present study come in accordance with previous research in rats [33,49,50]. In the contrary, demonstrated hypercholesterolemic and hyperlipidemic effect followed oral administration of Black cumin seed (2%) in Pekin ducklings was recorded [37]. The hypolipidemic effect of Black cumin seed may be attributed to the synergistic effect of its different constituents, soluble fiber, sterols, flavenoids and high content of polyunsaturated fatty acids. Hypolipidemic effect of soluble fibers contents of Black cumin seed might be related to decreased cholesterol absorption and increased bile acid synthesis and degradation [51]. The hypocholesterolemic effect of black cumin seed may relate to up-regulation of low density lipoprotein receptors and inhibition of 3-hydroxy 3-methylglutaryl coenzyme A reductase [52].

5. CONCLUSION

The present study can concluded that, dietary supplementation of either Turmeric or Black seed to diabetic rats were effective in reducing blood glucose, water and food intake, TAG and Cholesterol accompanied by an increase in body weight gain in STZ diabetic rats. Turmeric was the most effective medicinal plant used over black cumin seed and plants mixture.

301

302 **COMPETING INTERESTS**

303 The authors declare that they have no conflict of interest

304

305 **REFERENCES**

306

307 1. Mathur ML, Gaur J, Sharma R, Haldiya KR.. Antidiabetic Properties of a spice
308 Plant *Nigella Sativa*. The Journal of Endocrinological Metabolism. 2011;1:1-8.

309 2. Luzi L. Pancreas transplantation and diabetic complications. The new England
310 Journal of Medicine. 1998;339:115-117.

311 3. Shnkar TNB, Shanta NV, Ramesh HP., Murthy IAS, Murthy VS. Toxicity Studies
312 on Turmeric (*Curcuma longa*): Acute Toxicity studies in rats, Guineapigs and
313 Monkeys. Indian Journal of Experimental Biology. 1980;18:73-75.

314 4. Duvoix A, Blasius R, Delhalle S, Schnekenburger M, Morceau E, Henry E,
315 Dicato M, Diederich M. Chemopreventive and therapeutic effects of curcumin.
316 Cancer Letters. 2005;223:181-190.

317 5. Aggarwal BB, Kumar A, Aggarwal MS, Shishodia S. Curcumin derived from
318 turmeric (*Curcuma longa*): a spice for all seasons. In: Preuss, H. G. editor,
319 Phytopharmaceuticals in Cancer Chemoprevention. CRC, USA. 2005;349-387.

320 6. Arun N, Nalini N. Efficacy of turmeric on blood glucose and polyol pathway in
321 diabetic albino rats. Plant Foods for Human Nutrition. 2002;57:41-52.

322 7. Bamosa AO, Ali BA, Sowayan SA. Effect of oral ingestion of *Nigella sativa*
323 seeds on some blood parameters. Saudi Pharmatheutical Journal. 1997;5:126-129.

- 324 8. Haddad PS, Depot M, Settaf A, Chabli A, Cherrah Y. Comparative survey on the
325 medicinal plants most recommended by traditional practitioners in Morocco and
326 Canada. *Journal of Herbs Spices and Medicinal Plants*. 2003;10:25-45.
- 327 9. Labhal A, Settaf A, Bennani N, Cherrah Y, Slaoui A, Hassar M. Action anti-
328 obésité et hypocholestérolémiant de *Nigella sativa* chez le rat des sables.
329 *Caducée*. 1997;27:26–28.
- 330 10. Le PM, Benhaddou-Andaloussi A, Elimadi A, Settaf A, Cherrah Y, Haddad P.
331 The petroleum ether extract of *Nigella sativa* exerts lipid-lowering and insulin-
332 sensitizing actions in the rat. *Journal of Ethnopharmacology*. 2004;94:251–259.
- 333 11. Al-Ghamdi MS. The anti-inflammatory, analgesic and antipyretic activity of
334 *Nigella sativa*. *Journal of Ethnopharmacology*. 2001;76:45–48.
- 335 12. Meddah B, Ducroc R, Faouzi M, Eto B, Mahraoui L, Benhaddou-Andaloussi A,
336 Martineau LC, Cherrah Y, Haddad PS. *Nigella sativa* inhibits intestinal glucose
337 absorption and improves glucose tolerance in rats. *Journal of Ethnopharmacology*.
338 2009;121:419–424.
- 339 13. Mills S, Bone K. *Principles and Practice of Phytotherapy*. Toronto (ON):
340 Churchill Livingstone. 2000.
- 341 14. Sekar N, Kanthasamy S, William S, Subramanian S, Govindasamy S. Insulinic
342 actions of vanadate in diabetic rats. *Pharmacological Research* 1990;22:207-217.
- 343 15. Tank R, Sharma N, Sharma I, Dixit VP. Anti-diabetic activity of *Curcuma longa*
344 in alloxan Induced diabetic rats. *Indian drugs*. 1989;27:587-589.

- 345 16. Reitman S, Frankel SA. Colorimetric method for the determination of serum
346 glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of
347 Clinical Pathology. 1957;28:56-63.
- 348 17. Doumas BT, Bayson DD, Carter RJ, Peters T, Schaffer R.. Estimation of total
349 serum protein. Clinical Chemistry. 1981;27:1642–1643.
- 350 18. Reinhold RP. Determination of serum albumin. Clinical Chemistry.
351 1953;21:1370–1372.
- 352 19. Tabacco A, Meiathini F, Moda E, Tarli P. Simplified enzymic/colorimetric serum
353 urea nitrogen determination. Clinical Chemistry. 1979;25:336-337.
- 354 20. Henry JB. Clinical Diagnosis and management, 17th edition, Saunder Publisher.
355 1984.
- 356 21. Sidney PG, Bernard R. Improved manual spectrophotometric procedure for
357 determination of serum triglycerides. Clinical Chemistry. 1973;19:1077–1078.
- 358 22. Zak B, Dickenman RC, White EG, Burnett H, Cherney PJ. Rapid estimation of
359 free and total cholesterol. American Journal of Clinical Pathology 1954;24:1307-
360 1315.
- 361 23. Bauer JD. Clinical laboratory methods 9th edition. The C.V. Company II 1830,
362 Westline industrial, Missouri. 1982.
- 363 24. Chou YH. Experimental design and the analysis of variance, Statistical Analysis-
364 I. New York, Holt, Reinhart and Winston Publication. 1975;340-352.
- 365 25. Fluck H, Schib RJ, Rowson JM. Medicinal Plants and Their Uses. W. Foulsham
366 and Co. Ltd, England. 1976;168.

- 367 26. Weiss RB. Streptozocin: A review of its pharmacology, efficacy and toxicity.
368 Cancer Treatment Report. 1982;66:427-438.
- 369 27. Hussain HEMA.. Hypoglycemic, hypolipidemic and antioxidant properties of
370 combination of curcumin from curcuma longa, linn. and partiallt purified product
371 from Abroma augusta, linn. in streptozotocin induced diabetes. Indian Journal of
372 Clinical Biochemistry. 2002;17:33-43.
- 373 28. Sellamuthu PS, Muniappan BP, Perumal SM, Kandasamy M. Antihyperglycemic
374 effect of mangiferin in streptozotocin induced diabetic rats. Journal of Health
375 Sciences. 2009;55:206-214.
- 376 29. Yao XH, Chen L, Nyomba BLG. Adult rats prenatally exposed to ethanol have
377 increased gluconeogenesis and impaired insulin response of hepatic
378 gluconeogenic genes. Journal of Applied Physiology. 2006;100: 642-648.
- 379 30. Sharma S, Kulkarni SK, Chopra K. Curcumin, the active principle of turmeric
380 (Curcuma longa), ameliorates diabetic nephropathy in rats. Clinical and
381 Experimental Pharmacology and Physiology 2006;33:940–945.
- 382 31. Ahmadi F. Effect of turmeric (*Curcumin Longa*) powder on performance,
383 oxidative stress state and some of blood parameters in broiler fed on diets
384 containing aflatoxin B1. Global Veterinaria. 2010;5:312-317.
- 385 32. Joe B, Lokesh BR. Role of capsaicin, curcumin and dietary n-3 fatty acids in
386 lowering the generation of reactive oxygen species in rat peritoneal macrophages.
387 Biochemica Biophysica Acta. 1994;1224:255-263.

- 388 33. El-Bagir NM, Farah ITO, Alhaidary A, Mohamed HE, Beynen AC.. Clinical
389 laboratory serum values in rabbits fed diets containing black cumin seed. Journal
390 of Animal and Veterinary Advances. 2010;19:2532-2536.
- 391 34. Benhaddou-Andaloussi A, Martineau LC, Vallerand D, Haddad Y, Afshar A,
392 Settaf A, Haddad PS. Multiple molecular targets underlie the antidiabetic effect of
393 *Nigella sativa* seed extract in skeletal muscle, adipocyte and liver cells. Diabetes,
394 Obesity and Metabolism. 2010;12:148-157.
- 395 35. Irshaid F, Mansi K, Aburjai T. Antidiabetic effect of essential oil from *Artemisia*
396 *sieberi* growing in Jordan in normal and alloxan induced diabetic rats. Pakistan
397 Journal of Biological Sciences. 2010;13:423-430.
- 398 36. Vahdati-Mashhadian N, Rakhshandeh H, Omid A. An investigation on LD50 and
399 subacute hepatic toxicity of *Nigella sativa* seed extracts in mice. Pharmazie.
400 2005;60:544-547.
- 401 37. El-Bahr SM, Saad TT. Effect of Black cumin seeds (*Nigella sativa*) and/or
402 Turmeric (*Curcumin*) on hematological, biochemical and immunological
403 parameters of *Mugil cephalus* fish vaccinated with *Aeromonas hydrophila*
404 bacterin. The Thirteen scientific congress, Faculty of Veterinary Medicine, Assuit
405 University, 25-28, November. 2008;365-388.
- 406 38. Ezat AM. Effect of black seed (*Nigella sativa*) on the immune status of catfish
407 (*Clarias gariepinus*). Ph.D. (Thesis). Faculty of Veterinary Medicine, Assuit
408 University, Egypt. 2005.

- 409 39. El-Bahr SM. Effect of black cumin seeds (*Nigella sativa*) on the profile of serum
410 lipids, lipoproteins and fatty acids in pekin ducklings. International Journal of
411 Applied Chemistry (IJAC), 2007;3:221–230.
- 412 40. Antony S, Kuttan R, Kuttan G. Immunomodulatory Activity of Curcumin.
413 Immunological Investigations. 1999;28:291-303.
- 414 41. Salama AF, El-Bahr SM. Effect of curcumin on cadmium-induced oxidative
415 testicular damage in rats. Journal of the Medical Research Institute (JMRI).
416 2007;28:130-136.
- 417 42. Saeed MK, Deng Y, Dai R. Attenuation of Biochemical Parameters in
418 Streptozotocin-induced Diabetic Rats by Oral Administration of Extracts and
419 Fractions of *Cephalotaxus sinensis*. Journal of Clinical Biochemistry and
420 Nutrition. 2008;42:21–28.
- 421 43. Tennekoon KH, Jeevathayaparan S, Kurukulasooriya AP, Karunanayake EH.
422 Possible hepatotoxicity of *Nigella sativa* seeds and *Dregea volubilis* leaves.
423 Journal of Ethnopharmacology. 1991;31: 283-289.
- 424 44. Abubakar G, Danladi AA, Sunday EA, Sani I. Antidiabetic Effect of *Nauclea*
425 *latifolia* Leaf Ethanolic Extract in Streptozotocin-induced Diabetic Rats.
426 Pharmacognoc Research. 2009;1:392-395.
- 427 45. El-Sayed EM, Abo-Salem OM, Aly HA, Mansour AM. Potential antidiabetic and
428 hypolipidemic effects of propolis extract in streptozotocin-induced diabetic rats.
429 Pakistan Journal of Pharmaceutical Sciences. 2009;22:168-174.

- 430 46. Kim M, Kim Y. Hypocholesterolemic effects of curcumin via up-regulation of
431 cholesterol 7 α -hydroxylase in rats fed a high fat diet. Nutrition Research and
432 Practice. 2010;4:191-195.
- 433 47. Soni KB, Kuttan R. Effect of oral curcumin administration on serum peroxides
434 and cholesterol levels in human volunteers. Indian Journal of Physiology and
435 Pharmacology. 1992;36:273-275.
- 436 48. Baum L, Cheung SK, Mok VC, Lam LC, Leung VP, Hui E, Ng CC, Chow M, Ho
437 PV, Lam S, Woo J, Chiu HF, Goggins W, Zee B, Wong A, Mok H, Cheng WK,
438 Fong C, Lee JS, Chan MH, Szeto SS, Lui VW, Tsoh J, Kwok TC, Chan IH, Lam
439 CW. Curcumin effects on blood lipid profile in a 6-month human study.
440 Pharmacological Research. 2007;56:509-514.
- 441 49. Kocyigit Y, Atamer Y, Uysal E. The effect of dietary supplementation of *Nigella*
442 *sativa* L. on serum lipid profile in rats. Saudi Medical Journal. 2009;30:893-896.
- 443 50. Nader MA, El-Agamy DS, Suddek GM. Protective effects of propolis and
444 thymoquinone on development of atherosclerosis in cholesterol-fed rabbits.
445 Archive of Pharmacal Research. 2010;33:637-643.
- 446 51. Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella*
447 *sativa*. Phytotherapy Research. 2003;17:299-305.
- 448 52. Al-Naqeep G, Ismail M, Allaudin Z. Regulation of low-density lipoprotein
449 receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase gene expression
450 by thymoquinone-rich fraction and thymoquinone in HepG2 cells. Journal of
451 Nutrigenetic and Nutrigenomics. 2009;2:163-172.

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Table 1. Proximate analysis of ingredients used in the diets of the experiment, dry matter basis

Ingredients	Moisture	CP	EE	CF	NFE*	Ash
	%	%	%	%	%	%
Black cumin seed	5.8	19.54	34.4	6.1	36.16	3.8
Turmeric	13.5	11.74	6.4	7.2	70.07	4.59

CP (crude protein), EE (ether extract), CF (crude fiber)

*NFE (Nitrogen free extract) is calculated by differences

Table 2. Effect of oral administration of Turmeric and/or Black cumin seed for six weeks on body weight gain (g) in streptozotocine diabetic rats

Groups	Body weight (g)		Gain in body weight (g)
	Initial	Sixth weeks	
I	195 ± 5.1	255 ± 6.2 ^a	60
II	194 ± 7.2	164 ± 7.1 ^c	-30
III	196 ± 4.2	221 ± 3.1 ^b	25
IV	190 ± 5.2	175 ± 8.1 ^c	-15
V	194 ± 6.1	214 ± 5.2 ^d	20

I (non diabetic), II (diabetic), III (diabetic treated with turmeric), IV (diabetic treated with Black cumin seed), V (diabetic treated with plant mixture; BTM)

Values are mean ± SD of 7 rats

Means within the same column with different letters are significantly differed ($P \leq 0.05$).

Note: a, is the highest value, decreased via b, c to the lowest one is d.

Table 3. Effect of oral administration of Turmeric and/or Black cumin seed for six weeks on water intake (ml/day) in streptozotocine diabetic rats

Groups	Water intake (ml/day)			
	1 st week	2 nd week	4 th week	6 th week
I	50 ± 3.3 ^d	51 ± 5.1 ^c	55 ± 4.1 ^c	60 ± 4.6 ^c
II	100 ± 5.2 ^a	95 ± 5.2 ^a	90 ± 5.1 ^a	87 ± 4.7 ^a
III	40 ± 4.1 ^c	45 ± 4.3 ^c	45 ± 5.3 ^c	50 ± 3.9 ^c
IV	70 ± 6.2 ^b	75 ± 3.2 ^b	75 ± 4.4 ^b	75 ± 3.4 ^b
V	70 ± 5.5 ^b	75 ± 2.1 ^b	75 ± 5.1 ^b	75 ± 2.9 ^b

I (non diabetic), II (diabetic), III (diabetic treated with turmeric), IV (diabetic treated with Black cumin seed), V (diabetic treated with plant mixture)

Values are mean ± SD of 7 rats

Means within the same column with different letters are significantly differed ($P \leq 0.05$).

Note: a, is the highest value, decreased via b, c to the lowest one is d.

Table 4. Effect of oral administration of Turmeric and/or Black cumin seed for six weeks on feed intake (g/day) in streptozotocine diabetic rats

Groups	Food intake (g/day)			
	1 st week	2 nd week	4 th week	6 th week
I	15 ± 2.1 ^c	17 ± 1.2 ^c	20 ± 1.1 ^c	22 ± 2.1 ^c
II	38 ± 1.9 ^a	40 ± 1.5 ^a	39 ± 1.4 ^a	41 ± 1.7 ^a
III	17 ± 1.1 ^c	19 ± 1.0 ^c	21 ± 1.3 ^c	25 ± 1.2 ^c
IV	23 ± 1.2 ^b	24 ± 2.1 ^b	28 ± 2.1 ^b	32 ± 1.1 ^b
V	22 ± 1.7 ^b	26 ± 1.1 ^b	25 ± 2.1 ^b	30 ± 1.4 ^b

I (non diabetic), II (diabetic), III (diabetic treated with turmeric), IV (diabetic treated with Black cumin seed), V (diabetic treated with plant mixture)

Values are mean ± SD of 7 rats

Means within the same column with different letters are significantly differed ($P \leq 0.05$).

Note: a, is the highest value, decreased via b, c to the lowest one is d.

Table 5. Effect of oral administration of Turmeric and/or Black cumin seed for six weeks on blood glucose concentration (mg/dl) in streptozotocine diabetic rats

Groups	Blood glucose concentration (mg/dl)			
	1 st week	2 nd week	4 th week	6 th week
I	99 ± 2.1 ^e	100 ± 1.2 ^e	98 ± 1.1 ^e	96 ± 2.1 ^e
II	350 ± 1.9 ^a	355 ± 1.5 ^a	360 ± 1.4 ^a	355 ± 1.7 ^a
III	201 ± 1.1 ^d	190 ± 1.0 ^d	188 ± 1.3 ^d	200 ± 1.2 ^d
IV	260 ± 1.2 ^b	255 ± 2.1 ^b	250 ± 2.1 ^b	244 ± 1.1 ^b
V	250 ± 1.7 ^c	240 ± 1.1 ^c	245 ± 2.1 ^c	240 ± 1.4 ^c

I (non diabetic), II (diabetic), III (diabetic treated with turmeric), IV (diabetic treated with Black cumin seed), V (diabetic treated with plant mixture)

Values are mean ± SD of 7 rats

Means within the same column with different letters are significantly differed ($P \leq 0.05$).

Note: a, is the highest value, decreased via b, c to the lowest one is d.

Table 6. Effect of oral administration of Turmeric and/or Black cumin seed for six weeks on blood biochemistry in streptozotocine diabetic rats

Parameters	Group I	Group II	Group III	Group IV	Group V
Total Protein (g/l)	5.5 ± 0.2	5.4 ± 0.1	5.4 ± 0.2	5.4 ± 0.2	5.6 ± 0.1
Albumin (g/l)	3.2 ± 0.2	3.3 ± 0.1	3.1 ± 0.1	3.2 ± 0.1	3.4 ± 0.2
Globulin (g/l)	2.3 ± 0.2	2.1 ± 0.1	2.3 ± 0.1	2.2 ± 0.2	2.2 ± 0.1
A/G ratio	1.4 ± 0.1	1.6 ± 0.1	1.3 ± 0.2	1.5 ± 0.1	1.5 ± 0.1
Total cholesterol (mg/dl)	48 ± 2.2 ^b	63 ± 3.5 ^a	39 ± 2.1 ^c	37 ± 1.2 ^c	49 ± 2.3 ^a
TAG (mg/dl)	130 ± 4.1 ^b	142 ± 3.4 ^a	64 ± 4.1 ^c	90 ± 4.4 ^c	76 ± 4.4 ^d
ALT (U/l)	19 ± 1.2	18 ± 1.4	19 ± 2.1	19 ± 2.1	20 ± 2.4
AST (U/l)	100 ± 4.5	102 ± 4.1	99 ± 5.2	105 ± 3.4	100 ± 4.4
BUN (mg/dl)	4.1 ± 1.6	3.8 ± 2.1	3.3 ± 2.2	4.2 ± 1.9	5.1 ± 2.0
Uric acid (mg/dl)	2.1 ± 0.3	2.3 ± 0.2	1.6 ± 0.4	1.6 ± 0.3	2.0 ± 0.1
Creatinine (mg/dl)	0.5 ± 0.1	0.6 ± 0.2	0.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.1
Calcium	10.6 ± 2	11.5 ± 0.8	10.7 ± 0.7	9.9 ± 1.4	10.5 ± 1
Phosphorus	2.1 ± 0.2	4.5 ± 1.9	2.0 ± 0.1	2.3 ± 0.6	2.6 ± 0.8
Magnesium	1.5 ± 0.9	0.8 ± 0.2	1.0 ± 0.1	0.8 ± 0.3	1.0 ± 0.3
Chloride	109.6 ± 16	104.6 ± 5.5	114 ± 7.7	107.7 ± 13	107.2 ± 4.5

I (non diabetic), II (diabetic), III (diabetic treated with turmeric), IV (diabetic treated with Black cumin seed), V (diabetic treated with plant mixture).

Values are mean ± SD of 7 rats

Means within the same row with different letters are significantly differed ($P \leq 0.05$).

Note: a, is the highest value, decreased via b, c to the lowest one is d.