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Influence of Black Cumin Fixed and Essential Oil Supplementation on Markers of Myocardial Necrosis in Normal and Diabetic Rats

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Abstract: The cardiovascular disorders are major cause of mortality in diabetic patients and myocardial necrosis, atherosclerosis and high blood pressure are major ailments in this regard. The core objective of the present research study was to explore the role of Black Cumin Seed Fixed Oil (BCFO) and Black Cumin Essential Oil (BCEO) on the markers of myocardial necrosis i.e. cardiac enzymes in normal and diabetic Sprague dawley rats. Diabetes mellitus was induced using streptozotocin @ 60 mg/Kg body weight. The three diets were prepared i.e. D_1 (control), D_2 (BCFO @ 4.0%) and D_3 (BCEO @ 0.30%) and added separately in the diets and fed to the respective groups for a period of 56 days. The results indicated that diets were insignificant and level of cardiac enzymes remained in the safe ranges in normal rats. However, diabetes affected deleteriously and markers of myocardial necrosis increased in control group. In comparison, BCFO and BCEO normalized the levels of markers of myocardial necrosis. However, BCEO was more effective in reduced the elevated levels of these enzymes. Moreover, increased levels of transferases in diabetic rats were also reduced as a function of BCFO and BCEO treatment. In the nutshell, it can be assumed that black cumin essential oil is more effective in reducing the elevated levels of enzymes thus reducing the onset of cardiovascular disorders in diabetes mellitus.

Key words: Black cumin, essential oil, phytochemicals, diabetes mellitus, myocardial necrosis

INTRODUCTION

Cardiovascular Disorders (CVD) are major cause of mortality and morbidity over the globe. The reasons behind are lack of physical activity, inadequate diets and hypertension (Butt *et al*., 2009) Sometime, diseases like diabetes mellitus are also accompanied with CVD incidence and according to estimates 50% of diabetic patients are died due to ill heart health. Diabetes mellitus induced metabolic changes leading to myocardial necrosis, cardiac hypertrophy owing to accumulation of cholesterol, triglycerides, phospholipids and glycated protein in the myocardium (Christopher *et al*., 2003).

Induction of diabetes mellitus increases levels of cholesterol, low density lipoprotein and total triglycerides while reducing high density lipoprotein contents (Kanter *et al*., 2003; Meral *et al*., 2001; Altan *et al*., 2007; El-Nekeetya *et al*., 2007). It is being hypothesized that the cholesterol deposition due to increased activities of cholesterol synthesizing enzyme and reduced activities of antioxidants leads to Low Density Lipoproteins (LDL) oxidation (Singh *et al*., 2007). Moreover, the depletion of enzymes due to increased free radical concentration could be considered one of the important complications of diabetes mellitus (Clarkson, 1995). Numerous studies have reported that the antioxidant supplementation could avert the chances of such malfunctioning of key human's systems. Tocopherol serve as an effective agent for reducing the extent of damage caused by free radicals along with its cholesterol lowering potential (Eder *et al*., 2002). Moreover, Van Acker *et al*. (2000) highlighted the importance of antioxidant and enumerated that they are equally effective as tocopherols in lowering the diabetes complications (Sesso *et al*., 2003).

Plants are naturally bestowed with variety of components commonly known as phytochemicals that holds potential to cure various maladies (Butt *et al*., 2008). Black cumin is one such example with ample of phytochemicals mainly concentrated in its fixed and essential oils (Sultan *et al*., 2009). The tocopherols and sterols in fixed oils and antioxidant and alkaloids in essential oils can be effective utilized in lowering the elevated levels of markers of myocardial necrosis. The improvement in serum lipid profile can also be beneficial in reducing diabetes complication like cardiovascular disorders resulting due to increased susceptibility towards atherosclerosis and atherogenesis.

MATERIALS AND METHODS

Black cumin seeds of indigenous variety were obtained from Barani Agricultural Research Institute (BARI), Chakwal. Raw materials were procured from local market while reagents (analytical and HPLC grade) and standards were purchased from Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan) and Merck (Merck KGaA, Darmstadt, Germany). Sprague dawley rats were procured from National Institute of Health (NIH), Islamabad, Pakistan.

Extraction of fixed and essential oil: The oil from the black cumin seed was extracted through solvent extraction technique as described in AOCS (1998); hexane used as solvent and after extraction was recovered by Rotary Evaporator (Eyela, Japan). Essential oil was extracted following the method of Kanter *et al.* (2003) using locally fabricated hydro-distillation apparatus. The extracted black cumin fixed oil was administered @ 4.0% and black cumin essential oil @ 0.30% to check their potential to reduce myocardial necrosis. The doses were selected on the basis of preliminary trials. The both oils were added in the diets and further fed to their respective groups for a period of 56 days.

Test animals and their housing: Sprague dawley rats were used during the present investigation as test animals. A total of 60 rats 6-7 weeks old, were selected after physical and behavioural examination. The animals were acclimatized by feeding basal diet for a period of one week. They were divided into 2 groups of 30 further divide into three of 10 rats each. The diets prepared from the selected treatments were fed for a period of 8 weeks. The experimental diets contained fixed amounts of oil (10%), protein (20%), corn starch (55%) and cellulose (10%). The mineral and vitamin mixtures (AIN-76) were added @ 3% and 1%, respectively.

Diabetic mellitus was induced in rats (Weight 150-200 grams) by injecting Streptozotocin (STZ) @ 60 mg/Kg body weight, dissolved in 0.01 M citrate buffer (pH 4.5), intravenously. The blood glucose of each rat was monitored after injecting STZ to check the glucose response.

The animals were maintained according to standard guidelines of Animal Institute of Nutrition (AIN), USA. Feed and water intakes were monitored on daily basis, while body weights were recorded weekly throughout the experimental period. Half of the overnight fasted rats were sacrificed after four weeks of feeding trail and the rest at the end of study. Blood samples were collected through cardiac puncture and serum was collection following the method of Uchida *et al.* (2001).

Biomarkers of myocardial necrosis: The activity of LDH was determined using a commercially available kit from Biocon® (Germany). Likewise, Creatine Phosphokinase (CPK) were estimated using commercial kits from Cayman Chemicals (Kairisto *et al.*, 1993) and serum creatine phosphokinase-MB (CK-MB) by using commercial kit from Stanbio Laboratory (Boerne, TX).

Biomarkers of multiple organ toxicity: Activities of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) were determined by the enzymatic methods using commercial kits and used as biochemical markers for the early acute damage (ChronoLab, Switzerland). Total bilirubin contents were also assayed using commercial kits.

Statistical analysis: Data obtained was analyzed statistically using statistical package i.e. Cohort V-6.1 (Co-Stat Statistical Software, 2003). Sample for each analysis was run quadruplet and values expressed are means ± standard deviation. The distribution of the data was checked and analysis of variance was applied afterwards. The significant differences were further compared through Duncan's Multiple Range test (DMRt).

RESULTS

Statistical analysis demonstrated that cardiac enzymes Lactate Dehydrogenase (LDH), creatine kinase and its isoenzyme MB (CPK and CK-MB) were affected non-significantly in relation to diets, study intervals and their interactions (Table 1). The means for LDH indicated that values were in the range of 340.89±17.15 to 382.38±15.15mg/dL. There was less activity of this enzyme in the group of rats fed on black cumin fixed oil as compared to control and black cumin essential oil groups. The similar patterns of results were recorded for the creatine kinase and its isoenzyme MB (CPK and CK-MB). Both these enzymes varied from 161.44±8.50 to 191.44±12.29 mg/dL and 20.53±1.08 to 29.41±1.32 mg/dL, respectively. Overall, the values for cardiac enzymes remained in the normal ranges as described in the literature.

Table 1: Effect of diets and study intervals on cardiac enzymes in normal rats

SOV	df	LDH	CPK	CK-MB
Diets (A)	2	2111.705^{NS}	366.074^{NS}	90.523^{NS}
Intervals (B)	2	653.801^{NS}	604.616^{NS}	19.000^{NS}
A x B	4	1298.673^{NS}	671.303^{NS}	22.203^{NS}
Error	36	2034.499	486.048	90.167
Total	44			

NS = Non-significant, LDH = Lactate dehydrogenase
CPK = Creatine phosphokinase CK-MB = Creatine kinase-MB

It is observed from Table 2 that diets, study intervals and their interactions exhibited non-significant differences in liver enzymes (alanine aminotransferase, ALT; aspartate aminotransferase, AST; alkaline phosphatase, ALP) and bilirubin. Activities of liver enzymes like AST, ALT and ALP ranged from 116.00±2.62 to 120.67±4.03, 43.06±0.76 to 45.88±1.28 and 169.65±4.54 to 186.84±6.30 IU/L, respectively in different diets groups. Moreover, bilirubin proteins varied non-significantly from 0.64±0.03 to 0.66±0.05 mg/dL.

Induction of diabetes by means of Streptozotocin injection resulted in marked increase in the activities of the biomarkers of myocardial necrosis. The diabetes mellitus is well characterized with the high levels of glucose and low level of insulin. Moreover, such abnormalities are more prominent when we look into

Table 2: Effect of diets and study intervals on liver and kidney function tests in normal rats

SOV	df	AST	ALT	ALP	Bilirubin
Diets (A)	2	90.695^{NS}	62.411^{NS}	1108.560^{NS}	0.002^{NS}
Intervals (B)	2	187.984^{NS}	61.294^{NS}	1536.640^{NS}	0.036^{NS}
A x B	4	90.091^{NS}	104.898^{NS}	298.381^{NS}	0.012^{NS}
Error	36	216.280	59.014	803.057	0.017
Total	44				

NS = Non-significant AST = Aspartate aminotransferase ALT = Alanine aminotransferase ALP = Alkaline phosphatase

Table 3: Effect of diets and study intervals on cardiac enzymes in diabetic rats

SOV	df	LDH	CPK	CK-MB
Diets (A)	2	67779.825**	24555.768**	110.303**
Intervals (B)	2	8202.317**	6124.330**	1.165^{NS}
A x B	4	23070.759**	8232.495**	31.747**
Error	36	2279.276	270.782	9.979
Total	44			

NS = Non-significant

cardiovascular health. The death count in diabetic patients is often due to CVD. In the present investigation, data pertaining to cardiac enzymes when subjected to statistical analysis (Analysis of variance) revealed that cardiac enzymes were significantly affected only as a function of study duration while diets and interaction did not imparted significant impact on same enzymes (Table 3). Cardiac enzymes LDH, CPK and CK-MB remained in the ranges of 365.36±16.22 to 610.35±30.94 mg/dL, 189.67±8.42 to 331.21±9.63 mg/dL and 28.17±1.25 to 36.53±1.85 mg/dL, respectively (Table 4). Cardiac enzymes concentrations increased with the passage of time and all enzymes gave maximum concentration at the end of study (56 days) while minimum values were recorded at the start of the study (Table 4) with the exception of CK-MB. It was further observed that groups of rats fed on control diets were shown increasing tendency for levels of cardiac enzymes, while black cumin fixed and essential oils attenuated the adverse situation and elevated levels of these enzymes due to induction of diabetes mellitus were normalized. These results indicated the possibility of utilization of these phytochemicals rich fraction in reducing the extent of myocardial necrosis.

It was deduced from mean squares (Table 5) that diets, study duration and their interaction affected liver enzymes significantly. Means (Table 6) indicated that control groups showed maximum values i.e. 131.35±2.685, 65.70±2.529, 326.83±62.192 IU/L and 1.09±0.089 mg/dL for AST, ALT, ALP and total bilirubin contents, respectively. Diets containing black cumin fixed and essential oils mitigated the adverse consequences of diabetes mellitus significantly; AST reduced from 126.05±5.273 to 102.88±4.531 and 128.03±7.388 to 97.66±4.951 IU/L whereas ALT from 65.87±2.756 to 54.56±2.403 and 70.98±4.096 to 46.81±2.373 IU/L, respectively. Moreover, ALP activity i.e. 251.36±22.654 and 238.46±21.717 IU/L was recorded for black cumin fixed and essential oils group, respectively. Whereas total bilirubin contents in these groups were found to be 0.86±0.041 and 0.82±0.026 mg/dL, correspondingly.

DISCUSSION

Nutrients present in the diets e.g. macro and micronutrients plays important role in normal functionality of the organisms (Mahmood *et al.*, 2008). The plants are important sources of several key nutrients. In the recent era consensus has been sought on the development of functional and nutraceuticals foods from plants origin for the treatments of various disorders. Prevention of diabetes mellitus and its complications is important target of such modern innovations. In this regard, animal modeling for safety evaluation, toxicity limits and healthiness of functional and nutraceutical foods are imperative before dissemination of knowledge to end users. Malley *et al.* (2007) suggested clinical pathological evaluation by means of blood biochemical screening as one of the safety assessment tools when some novel food sources are exploited for their appraisal as safe human food ingredient (Singh *et al.*, 2002). Sprague dawley rats are special strains of rats often used for safety assessment of food and rat modeling to study the effects of diets, special foods or efficacious study of bioactive compounds. In the present research investigation, same strain of rats was used in rat modeling studies to check the influence of black cumin seed fixed and essential oils in ameliorating the levels of cardiac and liver enzymes.

In the present investigation, diets containing black cumin fixed and essential oil did not change cardiac enzymes in normal rats and all values remained in the normal ranges. Morita *et al.* (2008) reported the normal values for the Sprague dawley rats and findings of present investigation were remained in the safe limits (Chengelis *et al.*, 2008).

Levels of cardiac enzymes and liver enzymes are important biomarkers of myocardial necrosis and their abnormal values indicate possible toxicity symptoms (Farag *et al.*, 2006; Patel *et al.*, 2008). It is concluded from the findings of present research that all these parameters remained in the normal ranges as described in safety studies conducted by Chengelis *et al.* (2008) and Morita *et al.* (2008). They reported that values of ALP, ALT and AST usually lie in the range of 139-260, 33-81 and 88-162 U/L respectively (Petterino and Argentino-Storino, 2006).

Streptozotocin induced diabetes mellitus results in abnormal values for kidney and livers enzymes. This phenomenon is attributed to free radical production that

Table 4: Levels of cardiac enzymes in diabetic rats

		Study intervals (Days)			
Parameters	Diets	0	28	56	Means
LDH (mg/dL)	D_1	501.03±28.91^{c}	543.29±17.60^{b}	610.35±30.94^{a}	551.56±31.83^{a}
	D_2	490.51±20.52^{c}	429.00±14.42^{d}	384.00±16.91^{e}	434.50±30.87^{b}
	D_3	506.37±19.28^{c}	435.55±21.20^{d}	365.36±16.22^{e}	435.76±40.71^{b}
	Means	499.30±4.66^{a}	469.28±37.05^{b}	453.24±78.74^{c}	
CPK (mg/dL)	D_1	280.44±9.00^{bc}	304.96±9.88^{b}	331.21±9.63^{a}	305.54±14.66^{a}
	D_2	272.89±6.96^{d}	216.49±3.93^{f}	205.42±4.31^{f}	231.60±20.89^{b}
	D_3	290.94±3.56^{cd}	239.64±7.07^{e}	189.67±8.42^{g}	240.08±29.23^{b}
	Means	281.42±5.23^{a}	253.70±26.49^{b}	242.10±44.78^{c}	
CK-MB (mg/dL)	D_1	32.35±1.87^{b}	36.30±1.18^{a}	36.53±1.85^{a}	35.06±1.36^{a}
	D_2	30.56±1.28^{c}	30.85±1.04^{c}	30.14±1.33^{cd}	30.52±0.21^{b}
	D_3	33.28±1.27^{bc}	29.22±1.42^{de}	28.17±1.25^{e}	30.22±1.56^{b}
	Means	32.06±0.80	32.12±2.14	31.61±2.52	

D_1 = Control diet; D_2 = Black cumin seed fixed oil; D_3 = Black cumin seed essential oil

Table 5: Effect of diets and study intervals on liver function tests in diabetic rats

SOV	df	AST	ALT	ALP	Bilirubin total
Diets (A)	2	1545.346**	237.572**	34182.243**	0.310**
Intervals (B)	2	1608.306**	555.336**	55537.275**	0.100*
A x B	4	165.737^{NS}	221.097**	8626.795**	0.027^{NS}
Error	36	141.681	37.663	765.621	0.026
Total	44				

NS = Non-significant; * = Significant; ** = Highly significant

AST = Aspartate aminotransferase ALT = Alanine aminotransferase ALP = Alkaline phosphatase

Table 6: Liver and kidney functions tests in diabetic rats

		Study intervals (Days)			
Parameters	Diets	0	28	56	Means
AST (IU/L)	D_1	136.68±5.205	129.25±6.292	128.12±5.687	131.35±2.685^{a}
	D_2	126.05±5.273	116.54±3.917	102.88±4.531	115.16±6.725^{b}
	D_3	128.03±7.388	112.26±3.638	97.66±4.951	112.65±8.769^{b}
	Means	130.25±3.26^{a}	119.35±5.10^{b}	109.55±9.40^{c}	
ALT (IU/L)	D_1	67.63±2.575^{ab}	60.69±2.955^{c}	68.79±3.053^{a}	65.70±2.529^{a}
	D_2	65.87±2.756^{b}	59.47±1.999^{c}	54.56±2.403^{d}	59.97±3.274^{b}
	D_3	70.98±4.096^{a}	56.38±1.827^{d}	46.81±2.373e	58.06±7.027^{b}
	Means	68.16±1.50^{a}	58.85±1.28^{b}	56.72±6.44^{b}	
ALP (IU/L)	D_1	205.83±11.878^{e}	362.39±11.743^{b}	412.28±20.901^{a}	326.83±62.192^{a}
	D_2	213.01±8.911^{e}	249.63±8.391^{d}	291.43±12.836^{c}	251.36±22.654^{b}
	D_3	195.45±7.443^{e}	254.72±12.400^{d}	265.21±11.771^{d}	238.46±21.717^{b}
	Means	204.76±5.10^{c}	288.91±36.77^{b}	322.97±45.29^{a}	
Bilirubin total (mg/dL)	D_1	0.92±0.077^{c}	1.13±0.062^{b}	1.22±0.103^{a}	1.09±0.089^{a}
	D_2	0.79±0.047^{d}	0.87±0.062^{c}	0.93±0.069^{c}	0.86±0.041^{b}
	D_3	0.82±0.063^{cd}	0.78±0.060^{d}	0.87±0.089^{c}	0.82±0.026^{b}
	Means	0.84±0.04^{c}	0.93±0.10^{b}	1.01±0.11^{a}	

D_1 = Control diet; D_2 = Black cumin seed fixed oil; D_3 = Black cumin seed essential oil.

Means sharing same letters in a column/row do not differ significantly at $p<0.05$

causes membrane damage especially in the liver and kidney tissues (Daba and Abdel-Rahman, 1998). In the present investigation, values for AST, ALT, ALP and total bilirubin were observed higher in control group as compared to lower in black cumin fixed and essential oils groups. The reduction in oxidative damage and improvement in antioxidant status of the body are possible due to influence of the experimental diets. Improvement in antioxidant status of the body certainly assures the normal functioning of the human body (Kökdil *et al*., 2006). Black cumin fixed oil is rich in tocopherols whereas thymoquinone, carvacrol, thymol, cymene, t-anethole and 4-terpineol are the major antioxidants of black cumin essential oil (Wajs *et al*., 2008). Antioxidant potential of experimental diets is certainly due to these functional ingredients. These findings are in agreement with Kanter *et al*. (2003), Zaoui *et al*. (2002) and Meral *et al*. (2001). Similarly, Shinde and Goyal (2003) reported significant decrease in creatinine, urea, AST and ALT levels in diabetic rats treated with antidiabetic agents (Sahin *et al*., 2007).

Conclusion: Myocardial necrosis is one of key cardiovascular disorder and its prevalence in diabetes mellitus is often attributed to LDL oxidation, free radical production and oxidative stress that cause myocardial membrane damage. Conclusive approach drawn from this part of efficacy study is black cumin fixed and essential oils hold potential to attenuate the elevated levels of biomarkers of myocardial necrosis. It was further observed that diets improved the health of normal rats while brought balance in the level of cardiac and liver enzymes. The findings should be tested in Cohort studies in human diabetic subjects for meticulousness.

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