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ANTI-DIABETOGENIC IMPACT OF *Allium sativum* (GARLIC) AND *Nigella sativa* (BLACK CUMIN) ON DIABETIC RAT MODEL

M. M. Hossain[1], M. S. Islam[2], A. N. M. I. Rahman[3], M. E. Kabir[4] and M. R. Islam[5]

ABSTRACT

A study was conducted to determine the single and combined effects of black cumin and garlic on streptozotocin (STZ) induced diabetic rat model. Sixty (60) albino rats at 3-4 weeks of age were obtained from local market and divided into five groups. The groups were normal control (A), diabetic control (B), diabetic treated with garlic (C), diabetic treated with black cumin (D) and diabetic treated with black cumin and garlic (E) extract at a dose rate of 250, 500 and 750 mg/kg body weight respectively for 3 weeks. On day 0, 7, 14 and 21 blood samples were collected and blood glucose level was determined using diabetic kit. The blood glucose level was reduced from 177.5 ± 10.6 to 96.1 ± 2.4, 183.5 ± 9.4 to 101.3 ± 2.5 and 178.6 ± 7.7 to 91.1 ± 1.4 and the average body weight were increased from 257.6 ± 6.3 to 288.1 ± 2.5, 254.3 ± 10.7 to 282.5 ± 8.8 and 257.5 ± 9.0 to 286.8 ± 7.3 in group C, D, E, respectively after 3 weeks of treatment. Moreover, blood cholesterol level was reduced from 177.5 ± 10.6 to 96.1 ± 2.4, 183.5 ± 9.4 to 101.3 ± 2.5 and 178.6 ± 7.7 to 91.1 ± 1.4 in group C, D, E, respectively after 3 weeks of treatment. Our findings concluded that the combination of garlic and black cumin could be used as anti-diabetogenic agent in diet.

Keywords: diabetes, herbs, rat, streptozotocin

INTRODUCTION

Recently herbs have been used as a medicine in the field of herbal medicine. Many of the oral anti-diabetic agents have several serious adverse effects thus, managing of diabetes without any side effects is still a challenge. Therefore, the search for many effective and safer hypoglycemic agents has continued to be an important area of investigation. Besides drug classically used for the treatment of diabetes (insulin, sulphonylureas, biguanides and thazolidinediones), several species of plants have been described in the scientific and popular literatures having a hypoglycemic activity.

Diabetes mellitus (DM) is a chronic progressive metabolic disorder characterized by hyperglycemia mainly due to absolute (Type 1 DM) or relative (Type 2 DM) deficiency of insulin hormone (WHO, 1999). DM virtually affects every system of the body mainly due to metabolic disturbances caused by hyperglycemia, especially if diabetes control over a period of time proves to be suboptimal (WHO, 1999). Until recently it was believed to be a disease occurring mainly in developed countries, but recent findings reveal a rise in number of new cases of type 2 DM with an earlier onset and associated complications in developing countries (Kinra *et al.,* 2010; Chuang *et al.,* 1998; Narayanapa *et al.,* 2011). Diabetes is associated with complications such as cardiovascular diseases, nephropathy, retinopathy and neuropathy, which can lead to chronic morbidities and mortality (American Diabetes Association, 2004). The World Health Organization estimates that 346 million people suffer from diabetes worldwide (WHO, 1999). Without urgent action, this number is likely to double by 2030 (Shrivastava *et al.,* 2013). Moreover, DM is a predisposing factor for renal disorder progression and is referred to as diabetic kidney disease (Junod *et al*., 1996; Hiramatsu *et al*., 2021). Generally, diabetes is classified into two main types: type-1 diabetes, a state of insulin deficiency because of defect in islet β-cell function and type-2 diabetes which mainly characterized by resistance to the actions of insulin. The overall prevalence of DM in the global population is approximately 6%, of which 90% is type 2 diabetes (Zheng *et al*., 2018). Pharmacological agents, including sulfonylureas, biguanides, alpha-glucosidase inhibitors, thiazolidinediones, and meglitinide, are also used; however, long-term

[1]Associate Professor, [2] Professor, Dept. of Anatomy, Histology and Physiology, [3]Associate Professor, Dept. of Animal Nutrition, Genetics and Breeding [4]Assistant Professor, Dept. of Animal Production and Management, [5] Associate Professor, Dept. of Surgery and Theriogenology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

complications of type 2 diabetes mellitus are unaltered with these agents (De Fronzo, 1999). Metformin is currently being used in type 2 diabetes as the first-choice oral agent, along with appropriate diet control and lifestyle advice. Metformin acts primarily by reducing the hepatic glucose output and improving insulin sensitivity in the liver and muscle. Metformin has pleiotropic vascular effects that act on endothelial imbalance, probably increasing nitric oxide bioavailability, decreasing atheroma plaque growth, improving the atherogenic lipid profile, and inhibiting lipid incorporation into vessel walls, thereby inhibiting vascular smooth muscle cell proliferation (Lima *et al.*, 2009). The American Diabetic Association has recommended metformin as a first-line agent for the treatment of type 2 diabetes, as metformin helps in weight loss and lowers fasting plasma insulin concentrations, total and low-density lipoprotein cholesterol, and free fatty acids (American Diabetes Association, 2011). However, long-term complications are not altered with metformin therapy. Moreover, the hypoglycemic drugs lead to some unpleasant side effects such as lactic acidosis, peripheral edema, severe hypoglycemia, and abdominal discomfort (Lorenzati *et al.*, 2010).

Therefore, the search for new anti-diabetic agent's preferably herbal medicinal product is to be the main challenge in the modern world to protect this silent killer type of metabolic disease without creating health hazard. On the other hand, WHO Expert Committee on diabetes has recommended that traditional medicinal herbs can be further investigated for the treatment of diabetes. On this regard, choice to solve the problem is the main challenge for the betterment of the mankind. Considering the above facts, the present study was designed to compare the single and combined effect of garlic and black cumin on blood glucose level in streptozotocin (STZ) induced diabetic rats.

MATERIALS AND METHODS

This research work was conducted at the Department of Anatomy, Histology and Physiology, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka for a period of 3 weeks to evaluate the efficacy of garlic and black cumin on STZ induced diabetic rats.

Collection and acclimatization of rats

Total 60 mixed albino rats (aged 3- 4 months) having weighing (200 to 300 g) were collected from local market. All the rats were divided into 5 groups having 12 rats in each group. Each group of rats was housed at serene, bottomed wire cages arranged in rows and kept in the animal house of this department. The animals were fed pellet at a recommended dose of 100 g/kg body weight. Drinking water was supplied *ad libitum*. The rats were reared in this condition for a period of two weeks to acclimatize them prior to experimental uses.

Induction of diabetes

DM was induced, STZ injection was given through intra-peritoneal route which increased the blood glucose level and decreased body weight. Single dose of STZ administered intra-peritoneally @ 55 mg/kg body weight (Anderson *et al.,* 1974). In this experiment, polyuria, polydipsia and polyphagia after 24 hours of STZ injection were observed.

Experimental design

In this study, a total of 60 rats (12 normal rats and 48 STZ induced diabetic rats) were used for trial. The rats were divided into 5 groups each containing 12 individuals as follows:

- Group A: Normal control group
- Group B: Diabetic control group
- Group C: Diabetic with garlic treated group
- Group D: Diabetic with black cumin treated group
- Group E: Diabetic with both garlic and black cumin treated group

After 18 hours of starvation, body weights and blood glucose level were measured after acclimatization of rats. Then STZ was injected at a dose rate of 55 mg/kg body weight in intra-peritoneal route to each rat to induce diabetes in groups B, C, D and E. All the group of rats was reared under normal diet and water ad libitum from day 0-15, on 15^{th} day blood glucose level and the body weights were measured for the first time to ensure diabetic induction. Then all the rats of this group were kept for more 21 days for the treatment of diabetic condition. Aqueous extract of garlic, black cumin, and combination of garlic and black cumin were fed at a dose of 250 mg/kg, 500 mg/kg and 750 mg/kg body weight daily for 21 days in groups C, D and E respectively. During that period on day 0, 7, 14 and 21 blood glucose level and body weight were measured.

Statistical analysis

The data obtained from this study were expressed as mean ± standard deviation using Microsoft Excel 2016. The data were tabulated, analyzed and compared by pared t -test using the statistical software MINITAB.

RESULTS AND DISCUSSION

The differences in the levels of blood glucose and body weight in garlic, black cumin and garlic and black cumin combined treatment for 21 days in group C, D and E as compared to control group of animals shown in Table 1. Briefly, the blood glucose level was reduced from 177.5 ± 10.6 to 96.1 ± 2.4, 183.5 ± 9.4 to 101.3 ± 2.5 and 178.6 ± 7.7 to 91.1 ± 1.4 and the average body weight were increased from 257.6 ± 6.3 to 288.1 ± 2.5, 254.3 ± 10.7 to 282.5 ± 8.8 and 257.5 ± 9.0 to 286.8 ± 7.3 in group C, D, E, respectively, after 3 weeks of treatment compared with control groups. On the other hand, the differences in the levels of blood cholesterol and body weight in garlic, black cumin and garlic and black cumin combined treatment for 21 days in group C, D and E as compared to control group of animals shown in Table 2. Briefly, blood cholesterol level was reduced from 177.5 ± 10.6 to 96.1 ± 2.4, 183.5 ± 9.4 to 101.3 ± 2.5 and 178.6 ± 7.7 to 91.1 ± 1.4 and the average body weight were increased from 81.6 ± 5.1 to 126.6 ± 6.5, 82.5 ± 6.2 to 128.7 ± 6.9 and 81.2 ± 5.6 to 113.8 ± 6.1 in group C, D, E, respectively, after 3 weeks of treatment compared with control. These results agree with previous study as the effects of wild cherry & cumin on erythromycin-induced hepatic inflammation in diabetic rats (Al-Shaikh *et al.*, 2011; Amanullah, 2007; Ismail *et al.*, 2010). The combined treatment with garlic (C) and black cumin (D) were more effective than individual treatment of garlic and black cumin (E) treated group.

Table 1. Descriptive statistics of mean values of body weight (B. wt.) (g) and blood glucose level (BGL) (mg/dl) with standard deviation in different rat groups

Group	Day 0		Day 7		Day 14		Day 21	
	B. Wt (g)	BGL (mg/ dl)	B. Wt (g)	BGL (mg/ dl)	B. Wt (g)	BGL (mg/ dl)	B. Wt (g)	BGL (mg/ dl)
A	303.6 ± 9.6	94.5 ± 3.3	306.5 ± 9.4	96.0 ± 2.2	307.0 ± 8.2	96.3 ± 1.8	308.6 ± 7.0	96.1 ± 2.2
B	260.8 ± 8.1	177.0 ± 10.5	237.5 ± 8.2	210.6 ± 9.4	220.5 ± 6.7	234.5 ± 6.5	210.0 ± 6.4	247.0 ± 6.1
C	257.6 ± 6.3	177.5 ± 10.6	267.3 ± 5.5	160.5 ± 5.0	277.6 ± 4.6	130.3 ± 6.3	288.1 ± 2.5	96.1 ± 2.4
D	254.3 ± 10.7	183.5 ± 9.4	263.0 ± 8.9	165.0 ± 5.7	272.8 ± 9.6	135.1 ± 6.6	282.5 ± 8.8	101.3 ± 2.5
E	257.5 ± 9.0	178.6 ± 7.7	267.1 ± 8.6	156.8 ± 2.4	278.3 ± 8.2	123.1 ± 2.1	286.8 ± 7.3	91.1 ± 1.4

Our results supported some other authors finding such as post-treatment and pre-treatment of STZ-induced diabetic rats with ginger extract significantly decreased the blood glucose level and increased the insulin level (Akhani *et al.*, 2004). Garlic extracts reduced blood glucose levels in a dose dependent manner producing its best effects at 300 mg/kg body weight (Ozougwu and Eyo, 2010). Researchers also showed that studies with raw garlic and onion have shown that they significantly reduce the total serum cholesterol (Ugwu and Omale, 2011; Effendy *et al.,* 1997). Also, some earlier reports supported our study (Banerjee and Maulik, 2002; Augusti and Sheela, 1992). In conclusion, the present study reinforces the findings of previous studies that the combined effect of garlic and black cumin reducing the blood glucose level and could be used as an anti-diabetigenic agent in the diet.

Table 2. Descriptive statistics of mean values of body weight (B. wt.) (g) and blood cholesterol level (BCL) (mg/dl) with standard deviation in different rat groups

Group	Day 0		Day 7		Day 14		Day 21	
	B. Wt (g)	BCL (mg/ dl)	B. Wt (g)	BCL (mg/ dl)	B. Wt (g)	BCL (mg/ dl)	B. Wt (g)	BCL (mg/ dl)
A	94.3 ± 5.3	120.3 ± 5.6	94.1 ± 2.4	118.3 ± 6.4	94.3 ± 3.5	109.3 ± 6.7	94.6 ± 2.8	116.1 ± 6.5
B	83.2 ± 4.2	205.1 ± 5.3	79.5 ± 1.6	224.3 ± 6.3	74.1 ± 4.2	232.1 ± 6.5	68.2 ± 3.2	240.7 ± 5.7
C	81.6 ± 5.1	209.1 ± 5.2	84.4 ± 2.3	180.5 ± 5.7	89.1 ± 2.7	154.4 ± 6.3	92.1 ± 3.5	126.6 ± 6.5
D	82.5 ± 6.2	213.4 ± 6.2	84.6 ± 3.2	183.1 ± 5.8	88.2 ± 3.3	158.3 ± 5.9	92.5 ± 4.1	128.7 ± 6.9
E	81.2 ± 5.6	218.1 ± 6.3	86.2 ± 1.8	176.2 ± 6.2	92.2 ± 3.5	147.3 ± 6.5	96.4 ± 4.5	113.8 ± 6.1

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