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ANTI-DIABETIC ACTIVITY OF EDIBLE MACROFUNGUS DACRYOPINAX SPATHULARIA IN STREPTOZOTOCIN-INDUCED HYPERGLYCEMIA IN ALBINO WISTAR RATS

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ABSTRACT

The present study was performed to evaluate the anti-diabetic efficacy of the edible macrofungus Dacryopinax spathularia against Streptozotocin (STZ)-induced diabetes in Wistar albino rats. STZ has been reported to cause hyperglycemic conditions by inducing various adverse impacts on Beta cells of Pancreatic islets, including enhanced oxidative stress and alterations in vital molecules like NAD (Nicotinamide Adenyl Dinucleotide) and DNA (deoxyribonucleic acid). The results of the present work revealed that the present macrofungus i.e. D. spathularia contains various biochemical constituent compounds having strong antioxidant properties. The results also revealed that the STZ-induced diabetic rats showed a significant depletion in blood glucose levels back towards the normal blood glucose levels after administration of aqueous extract of D. spathularia for 21 days of experimental time period. The present work reveals that the D. spathularia is an excellent nutraceutical source, and can be used as a natural hypoglycemic agent. The present work may further be extended to discover the precise biochemical constituent compound present in the macrofungal extract and the precise molecular and cellular mechanism underlying its strong anti-diabetic impact to develop new drugs or medicines for treatment of one of the most common disease across the globe i.e. Diabetes mellitus.

KEYWORDS: Macrofungus, Diabetes Mellitus, Anti-Diabetic, Streptozotocin, Hyperglycemia Hepatoprotective, Antioxidant, Silver Nanoparticles

INTRODUCTION

The macrofungi or Mushrooms are valued for more than just their exquisite flavor; they are also a rich source of nutrients. Mushrooms include various vital mineral nutrients that are thought to be crucial for the body's regular functioning in addition to proteins, carbohydrates, glycogen, fat, vitamins, amino acids, and crude fibre (Gbolagade *et al*., 2006; Kalac, 2009). Mushrooms can be used as a regular dietary component or their isolated bioactive mycochemical constituent chemicals can be consumed for health benefits, which is why they are frequently regarded as functional foods or nutraceutical goods (Lakhanpal and Rana, 2005; Preeti *et al*., 2012). When we consider the nutritional potentialities of edible mushrooms, several studies have reported that the edible mushrooms have significant vitamin content which includes riboflavin, folic acid, biotin, ascorbic acid, thiamine, pantothenic acid and niacin among others (Hossain *et al*., 2007). Several studies have also reported that mushrooms are a rich source of minerals like manganese, iron, magnesium, selenium, zinc, calcium etc. (Alam *et al*., 2007). Therefore, the mushrooms are considered as a food resource with higher nutritional qualities and a low-calorie food. The mushrooms have also been reported to contain several medicinally important biochemical compounds, and to possess marked biomedicinal properties (Cheung, 2010). Several studies have

reported that the mushrooms contain potent bioactive chemical compounds like alkaloids, flavonoids, phenolics, saponins, tannins, terpenoids, and fatty acids, among others, which have marked pharmacological properties like antioxidant, antibacterial, immuno-modulatory, and anti-viral among many others (Kalac, 2013).

One of the most common pathophysiological conditions that involve elevated blood glucose levels is diabetes mellitus, which is intricately related with aberrant liver physiological or metabolic mechanisms (Stephens, 2003). Diabetes mellitus is a chronic metabolic disease that is widely prevalent worldwide and closely linked to postprandial hyperglycemia. An abnormal high blood glucose level one to two hours after meal is known as postprandial hyperglycemia, which may be a contributing factor to the development of type 2 diabetes mellitus (Bang-sil et al., 2005). Postprandial glucose levels have been found to rise in tandem with the activity of the calcium metalloenzyme pancreatic α-amylase in the human gut. Therefore, regulating α-amylase activity could be a key component of diabetes treatment for hyperglycemic situations (Hwang et al., 2005). A pharmaceutical substance called streptozotocin (STZ) is commonly used to induce experimental diabetes in rats in order to create a model for the disease's investigation. STZ is found to be selectively targeting and destroying the beta cells of pancreatic islets, consequently leading to deficiency of insulin and hyperglycemia (Yang et al., 2008). The probable mechanism of induction of diabetes by STZ includes the depletion of cellular NAD+ levels, DNA alkylation and thereby energy deprivation and increasing oxidative stress, leading to pancreatic beta cell death (Akbarzadeh et al., 2007).

Dacryopinax spathularia is a wild edible macrofungus that belongs to the group Basidiomycota, and is being used as a traditional medicinal source or folk medicine for the treatment of many diseases including antibacterial, anti-diabetic, anti-inflammatory, and antiviral, among others (Mitko *et al*., 2008). Therefore, the present work had been undertaken to scientifically validate and to determine the anti-diabetic efficacy of the aqueous extract of fruiting bodies of the edible macrofungus i.e. *Dacryopinax spathularia.*

MATERIALS AND METHODS

Collection of Sample and Preparation of Extract

Fresh fruiting bodies of the macrofungus *Dacryopinax spathularia* were collected, washed with deionized water, disinfected with 0.1% $HgCl_2$ solution and again washed with deionized water repeatedly to remove the traces of $HgCl_2$. The fruiting bodies are then shed dried for 7-8 days and then grinded into powdered form with the help of an electric grinder. 25 gm of the powdered sample was then subjected for solvent extraction using distilled water (500 ml) as solvent a using the Soxlet apparatus.

Qualitative Analysis of the Extract

The preliminary qualitative analysis for the presence of different secondary metabolites was done according to previously established standard tests (Bhaskar and Kumar, 2012).

Animals and Acute Toxicity Studies

Adult albino wistar rats (*Rattus norvegicus*) weighing 175-225 gm were used for the study. The animals were maintained under standard laboratory conditions, under relative humidity of 50±15%, temperature 25±5ºC and a dark-light cycle of 12 hrs. A commercial pellet diet and unlimited water were given to the animals (Choudhury *et al*., 2013). The experiment was carried out with prior permission from Ranchi University's Ethics Committee (Proceeding No. 46, Page

no. 137). Tests for Acute Toxicity The stair case approach was used to conduct the acute toxicity studies (Mallory and Evelyn, 1956).

The LD50 of extracts was determined using 50 albino rats of either sex. The rats were divided into five groups of ten rats each, and acute toxicity tests were carried out using staircase method following OECD guidelines (2004). Each group was fed with the increasing concentration of the extract, and no mortality was observed upto a dose of 2000 mg/Kg Body Weight of the rats.

Experimental Design

The rats were divided into three groups with ten rats in each group as follows:

Group B (Control): received 1 ml of normal saline orally

Group B (STZ-treated): received 0.1 ml/kg/day i.p. of Streptozotocin (Streptozotocin solution was prepared in 0.1M sodium citrate buffer with pH of 4.5. STZ was administered at a dose of 5-60 mg/ kg body weight through intra-peritoneal route (Ghosh, 2008).

Group C (STZ + Extract treated): received 500 mg/kg body weight of the extract orally

Rats were given an intraperitoneal (i.p.) injection of STZ diluted in 0.1 M sodium citrate buffer (pH 4.5) at a dose of 50–60 mg/kg body weight to induce diabetes after 18 hours of fasting. For the first 24 hours after receiving a STZ injection, the animals were monitored for signs of convulsions, behavioural abnormalities, and allergic reactions. A 5% glucose solution was given to the animals in order to treat any adverse reaction brought on by STC. No animals displayed any reactions.

Following the 72-hour STZ injection, blood glucose levels were recorded. Only animals with blood glucose levels between 200 and 300 mg/dl with glycosurea were selected for the investigation, and they were split up into the three groups. There was no STZ administered to the control group. Group A (Control) received 1 ml of normal saline orally on daily basis for 21 days. Before the normal saline was administered on day 0 at 10 am, blood glucose levels were measured. They were then taken at 10 am on days 3, 7, 14, and 21 days. Group B (STZ treated) was given 0.5 ml of regular saline orally every day. The animals were watched for signs of seizures, hyperglycemia, and behavioral abnormalities. This group's blood glucose levels were measured at 9 a.m. on day 0 prior to the administration of regular saline. For 21 days, Group C was given a daily oral dose of the macrofungal extract at a concentration of 500 mg/kg. At 10 am in the morning on the day before the medication was administered, the blood glucose level was recorded. The blood glucose level was then measured at 10 a.m. on the third, seventh, fourteenth, and twenty-first days following drug delivery. The animals were monitored for signs of convulsions and hypoglycemia.

RESULTS

Qualitative Analysis of the Extract

Table 1: Showing Qualitative Analysis of Biochemical Constituent Compounds in the Extract

Biochemical Compound/Secondary Metabolite	Presence/Absence
Alakaloids	+
Flavonoids	+
Phenolics	+
Fatty acids	+
Terpenoids	+
Saponins	+
Tannins	+
Quinons	-

Anti-Diabetic Activity

Table 2: Showing Blood Glucose Levels In Different Groups of Experimental Rats (n=10, Mean±SE)

Animal groups	Blood Glucose level (mg/dl) on days			
	3rd Day	7th Day	14th Day	21st Day
Group A (Control)	72.68±8.39	78.26±12.14	76.82±11.49	74.92±9.87
Group B (STZ)	284.67±32.89	268±26.38	259±27.63	234±29.12
Group C (STZ+Extract 500mg/Kg Body Weight/Day)	274±28.56	216±21.93	169±17.33	114±11.39

DISCUSSION

Several studies have reported that the edible mushrooms are rich in their secondary metabolite content, which have potent hypoglycemic effects (Hwang *et al*., 2005). The hypoglycemic or anti-diabetic effect of various mushrooms have been studied, like *Agaricus compestris* (Gray and Flatt, 1998), *Cordiceps militaris* (Kwon *et al*., 2001), *Ganoderma lucidum* (Yang *et al*., 2000), *Lentinus edodes* (Yang *et al*., 2002), *Pleurotus eryngii* (Kang *et al*., 2001), *Inonotus obliquus* (Bang-sil *et al*., 2005), among many others. Previous studies have reported that Diabetes is associated with an abnormal imbalance between the free radicals and the antioxidant system of the cells, which leads to the oxidative stress (Sancho and Pastore, 2012). Lack of insulin in the diabetes condition disturbs or hinders the glucose utilization and raises the levels of oxygen-derived free radicals (Zhang and tan, 2000). Therefore, as an additional treatment, the reduction or inhibition of oxidative process can stop or postpone the onset of diabetes-related problems.

The present work revealed that *Dacryopinax spathularia* contains the biochemical constituent compounds, which have strong antioxidant properties. Antioxidants can help prevent problems from diabetes and can be obtained by dietary supplements or natural antioxidant consumption (Bajaj and Khan, 2012). Table 2 clearly shows that the *Dacryopinax spathularia* has strong hypoglycemic impact on STZ-induced diabetes in mammalian animal model, which can be attributed to its biochemical constituent compounds with strong antioxidant activity. The present work may pave the way for further research in future to discover the exact biochemical compound and its underlying molecular and cellular mechanism responsible for its marked anti-diabetic effect.

CONCLUSION

Diabetes mellitus is a chronic metabolic disorder, which mainly involves hyperglycemic conditions due to destruction of beta cells of pancreatic islets and thereby insulin deficiency. The extract of *D. spathularia* showed marked

efficacy in improvement of abnormal hyperglycemic condition, which may be attributed to its bioactive secondary metabolite content which are reported to have marked antioxidant properties. The present work may be further extended to identify and isolate specific biochemical compound from the extract and to explore the exact molecular and cellular mechanisms underlying the hypoglycemic efficacy of the extract. The present work may pave the way for the discovery or development of new drugs or medicinal agents with least side effects for the treatment of one of the most prevalent disease across the globe, i.e., Diabetes mellitus.

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