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RESEARCH ARTICLE

ANTIDIABETIC EVALUATION OF BILE CONTENT OF *CHANNA PUNCTATUS* AGAINST ALLOXAN MONOHYDRATE INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus is a metabolic disorder which results due to chronic hyperglycemia associated with the imbalance in carbohydrate, fat and protein metabolism. Presently available several drugs reduce the hyperglycemia in diabetes mellitus; unfortunately these drugs have side effects. While, herbal drugs are mostly out of toxic or side effect than the chemical drug. Hence, the aim of the present study was designed to compare the possible therapeutic effects of *Channa punctatus* bile content against Alloxan induced diabetic rats. The Bile content of *Channa punctatus* was administered orally in an aqueous solution at a dose of 5ml/kg body wt. to diabetic rats. Applied doses did not cause any acute toxicity or behavioural changes. The blood glucose levels was significantly ($P<0.05$) reduced when compared to the alloxan induced diabetic rats. In conclusion, *Channa punctatus* bile content produced a significant hypoglycemic effect and also hypolipidemic activity at dose level of 5ml/kg.

INTRODUCTION

Diabetes mellitus is an endocrine metabolic disorder characterized by hyperglycemia, altered lipids, carbohydrates, proteins metabolism and it increases risk of cardiovascular diseases complications (Davis, 2006). The two forms of diabetes, type 1 and 2, differ in their basic mechanisms of development and in physiologic characteristics such as associations with obesity, age, and insulin. But, both types of the diabetes share the common characteristics of hyperglycemia, microvascular and macrovascular complications. Moreover, the alterations of lipoproteins metabolism are involved to the pathogenesis of the cardiovascular disease in both forms of diabetes in a similar way (Howard, 1987).

Diabetes has a considerable impact on the health, life style, life expectancy of patients and its related complications are major healthcare problems. *Channa punctatus* is very rich in fish albumin, a type of protein that speeds healing after surgery and childbirth. It also helps the child's growth and weight gain people. There was also show fish extract of snakehead fish for 10-14 days can increase blood albumin levels of 0.6 to 0.8 g/dl. The PLWHA (people living with HIV/AIDS) who were given fish extract the cork on a regular basis, can increase levels of albumin in the blood, so the weight will rise slowly.

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In addition to helping the formation of new tissue, which is albumin in the blood also serves to regulate water balance in cells, providing nutrients in the cells and helps remove waste products. Albumin also serves to maintain of fluid in the body. In India, traditionally numbers of plants are used to manage the diabetic conditions and their active principles were isolated but few plants have been scientifically studied. Therefore, the present study was carried out to evaluate the antidiabetic activity of *Channa punctatus* bile content in alloxan induced diabetes and to probe into the mechanism of its antidiabetic property.

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain approximately weighing 160-200g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2ºC and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water. They were acclimatized to the environment for one week prior to experimental use.

Chemicals

Alloxan monohydrate, Ethylene Diamine Tetra Acetic Acid (EDTA)), Chloroform were purchased for Sigma chemical company, Mumbai All other chemicals and reagents used in this study was of analytical grade with high purity and were obtained from Glaxo laboratories and Sisco Research laboratories, Mumbai, India.

Induction of Insulin-Dependent Diabetes Mellitus (IDDM)

IDDM was induced in overnight fasted adults male Wistar albino rats weighing 150-200g by a intraperitoneal injection of 120 mg/kg alloxan monohydrate (Loba chemie) (Mukhtar *et al.,* 2004). This model has been used in earlier studies to induce type I diabetes in rats (Neeli *et al.,* 2007). Glibenclamide (2.5mg/kg) was used as the standard drug. After 72 hrs of alloxan injection, stable hyperglycemia was confirmed by glucose in urine of rats by Benedict's qualitative test (Nagarajan *et al.,* 2005). The 5ml/kg of bile samples (Biplab De *et al.,* 2012) at 100% concentration was administered orally given once a day for 15 days after hyperglycemia was confirmed by the elevated glucose levels in urine determined at 72 hrs.

Experimental Design

The animals were divided into six groups of six animals each as follows. Each animal was marked for identification and regularly monitoring.

Group I: Served as normal control and received distilled water with Tween 80
Group II: Alloxan treated control (120mg/kg b.w)
Group III: Alloxan (120mg/kg b.w) + Bile content (5g/kg p.o)
Group IV: Alloxan (120mg/kg i.p) + std. drug Gilibenclamide (2.5mg/kg p.o)

Collection of blood and preparation of serum sample

At the end of the experimental period, the animals were anaesthetized using chloroform vapour prior to dissection. Blood was collected by cardiac puncture into serum separator tubes. The blood was allowed to clot by standing at room temperature for 30 minutes and then refrigerated for another 30 minutes. The resultant clear part was centrifuged at 3000 rpm for 10 minutes, and then the serum (supernatant) was isolated and stored at refrigerated until required for analysis.

Estimation of Glucose

Glucose was estimated by GOD/POD method (Trinder, 1969). 0.1 ml of serum was mixed with 1.0 ml of buffered enzyme reagent and incubated at 370C for 25 minutes. Absorbance of working standard and test were measured at 505 nm against blank in a spectrophotometer. Values were expressed as mg/dl serum.

Statistical Analysis

Values were expressed as mean ± standard deviation for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for post-hoc multiple comparison tests. Statistical Package for Social Studies (SPSS) 9.0 version was used and $p<0.05$ was considered to be significant.

RESULTS

Antidiabetic activity of alloxan induced albino rats

The remarkable antidiabetic activity was observed in alloxan induced hyperglycemic rat after oral administration of bile sample. The results were given in Table – 1 oral administration of bile content of Channa punctatus in 100% concentration showed average of bile content in 100% concentration after 24 hrs of administration showed maximum 45.83% diabetes reduction respectively, when compared with standard drug. The blood glucose levels was significantly ($P<0.05$) reduced when compared to the specific diabetic control animals.

Table 1. Effect of bile content glucose (mg/dl) level in alloxan induced diabetic rats

Group	Glucose (mg/dl)		% of antidiabetic activity	
	12 hrs	24 hrs	12 hrs	24 hrs
Group I	87.4 ± 2.14	87.4 ± 2.14	---	---
Group II	151.8 ± 18.67#	144 ± 16.77#	---	---
Group III	105.8 ± 12.83*	78.2 ± 12.51*	30.46	45.83
Group IV	100.6 ± 8.046*	89.2 ± 7.053*	33.77	38.19

Values were expressed as mean ± SD
#Significantly different from group I.
*Significantly different from group II ($p<0.05$)

DISCUSSION

Alloxan monohydrate is commonly used for experimental induction of type-I diabetes mellitus, which causes selective pancreatic islet β-cell cytotoxicity mediated through the release of nitric oxide (NO). This results in rapid reduction in pancreatic islet pyridine nucleotide concentration and subsequent β -cell necrosis. The action of Alloxan on mitochondria generates SOD anions, which leads to diabetic complications (Papaccio *et al.,* 2000).

Based on the above perspectives, in the present study, the antidiabetic activity has been assessed in rats made diabetic by alloxan. Sulfonyl ureas such as glibenclamide are often used as a standard antidiabetic drug in alloxan-induced diabetes to compare the efficacy of variety of antihyperglycemic compounds (Anderson *et al.,* 1974).

Previously reported that the bile content of fish contain cholesterol, bile salt of deoxycholic acid and cholic acid, bile pigment, fatty acid (Biplab De *et al.,* 2012). Bile acids such as cholic acid (CA) and chenodeoxycholic acid (CDCA) are natural ligands for the farnesoid X receptor (FXR) (Lefebvre *et al.,* 2009), and activation of FXR in liver may increase the production of small heterodimer partner (SHP) (Brendel *et al.,* 2002), a protein that plays a central role in lipid and glucose metabolism via regulation of various downstream molecules (Ma *et al.,* 2006).

Diabetes affects both glucose and lipid metabolism (Sperling *et al*., 2000). The insulin deficiency depletes the activity level of lipoprotein lipase, thus leading to deranged lipoprotein metabolism during diabetes (Ranganathan *et al.*, 2000). Alloxan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycaemia (Chattopadhyay *et al.*, 1997). During antidiabetic study it was found that in maximum cases individual animal was showing droeshiness before death, through no abnormality was found. Among freshwater fishes spotted snake head, *Channa punctatus* distributed throughout the south east – asian countries, and are most widely cultivated in Tamil Nadu, India. Stability of bile contents was not appreciable. It can also be concluded that fresh bile content of *Channa punctatus* showed remarkable antidiabetic activity. However more investigation is necessary to confirm the antidiabetic activity of bile content of *Channa punctatus* and to purify and characterize the activity components.

The present study suggests that the *Channa punctatus* bile content had synergetic hypoglycemic effect revealed by decreased blood glucose levels restored heamoglobin and therefore attribute to therapeutic value of the bile content of *Channa punctatus* to combat the diabetic condition in rats.

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