ORIGINAL ARTICLE

ANTIDIABETIC EVALUATION OF BILE CONTENT OF CHANNA PUNCTATUS

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Abstract
Fish is one of the popular health foods worldwide and has huge medicinal importance. Several traditional medical practitioners claimed that bile content of fresh water fish may useful in diabetes. Therefore the present work was undertaken to evaluate antidiabetic activity the bile content of fresh water carps, *Channa punctatus*. The animals were divided into four groups of four animals each as follows. Each animal was marked for identification and regularly monitoring. Group I Served as normal Control and received distilled water. Group II Alloxan treated control (120mg/kg b.w). Group III Alloxan (120mg/kg b.w) and bile content (5mg/kg p.o). Group IV Alloxan (120mg/kg i.p) and standard drug Glibenclamide (2.5mg/kg p.o). Blood Glucose and qualitative determination of Urine sugar were analysed. Oral administration of fresh bile content of *Channa punctatus* possesses remarkable antidiabetic activity.

Key words: Bile, Diabetic, *Channa punctatus*, Fish.

1. INTRODUCTION
Diabetes is a metabolic disorder of carbohydrate, fat and protein, affecting a large number of populations in the world (Pareek et al., 2009). Diabetes mellitus is not a single disorder but it is a group of metabolic disorder characterised by chronic hyperglycemia, resulting from defects in insulin secretion, insulin action, or both. Increased thirst, increased urinary output, ketonemia and ketonuria are the common symptoms of diabetes mellitus, which occur due to the abnormalities in carbohydrate, fat, and protein metabolism. (Craig et al., 2009). Diabetes mellitus has caused significant morbidity and mortality due to microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications (Thevenod, 2008). Diabetes is mainly attributed to the rapid rise in unhealthy life style, urbanization and aging. The number of people suffering from the disease worldwide is increasing at an alarming rate with a projected 366 million peoples likely to be diabetic by the year 2030 as against 191 million estimated in 2000. In India the prevalence rate of diabetes estimated to be 1-5% complication are the major cause of morbidity and mortality in diabetic mellitus. There is an increasing demand by the use of animal products of natural products due to the side effects associated with used of insulin and oral hypoglycemic agents.

The treatment of diabetes mellitus is considered as the main global problem and successful treatment has yet to be discovered. Even though insulin therapy and oral hypoglycemic agents are the first line of treatment for the diabetes mellitus they have some side effects and fail to significantly alter the course of diabetic complications (Venkatesh et al., 2010). Currently available oral therapies for treatment of diabetes mellitus are sulfonylureas, biguanides, α-glucosidase inhibitors, and glinides, which can be used alone or combined with other drugs to achieve better effect. Many of these oral antidiabetic agents have a number of serious adverse effects, thus, the management of diabetes without any side effects is still a challenge (Pareek et al., 2009, Meenakshi et al., 2010).

Fish is one of the most popular food items of the non-vegetarians. It is unique animal meat that is rich in all essential amino acids and fatty acids and many other nutrients. Approximately 75% of world populations are dependant directly or indirectly depends on fishes for protein food (Salim, 2006). Fish contains protein (15-26%), fat (0.8-19.7%), minerals (0.9-2.2%), vitamin A, D, C and water (70-80%) (Chandy, 1994). Several researchers have proved the beneficial effect of fish in heart diseases.
inflammatory diseases, mineral deficiency etc. An antioxidant ω-3-fatty acid is abundant in fish, which is also essential for healthy development of the eyes and brain. Recent studies also reported its importance in treatment or management of diabetes, cardiovascular diseases. Several species of fresh water carps and snake headed fishes which are very copiously found in Tamil nadu includes Catla catla (Katla), Labeo rohita (Rohu), Labeo calbasu (Kalibous), Cirrhinus mrigala (Mrigal) Channa punctatus, Channa striatus etc. The importance of fish in diet lies in the chemical composition of the flesh, which is rich in proteins and minerals like calcium, phosphorous, iron etc. We also observed that few traditional medical practitioners of tribal communities (locally known as auchai or kabiraj) of Tamil Nadu claimed the therapeutic action of other parts excluding flesh of the fish, such as bile of the fresh water fish in diabetes. The bile known as Bakhwl in Tripuri community and them sometime takes this by frying with chilies and onion.

Keeping this in view, the work was undertaken to investigate the antidiabetic activity of bile content of Snake heated fish, Channa punctatus that are very abundantly found in the market of Tamil Nadu, India.

2. MATERIALS AND METHODS

2.1 Collection of samples

The sample fishes used in this work were collected from the local Water bodies of Nachiarkovil, Kumbakonam, Tamil Nadu. The carp, Snake head fish (Channa punctatus) 200-250g (Fig-1) were chosen as per availability in the market for the study. The bile was collected from intact gall bladder of fish. The collected bile was then preserved at 0°C for further study.

![Snake head fish and collection of bile](image)

Fig 1 Shows Snake head fish and collection of bile

2.3 Antidiabetic activity

2.3.1 Animals

Male albino rats of Wistar strain approximately weighing 180-200g 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 ± 2°C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water. They were adapted to the environment for one week prior to experimental use.

2.3.2 Induction of insulin-dependent diabetes mellitus (IDDM)

IDDM was induced in overnight fasted adult male Wistar albino rats weighing 150–200g by a single 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 (2.5 mg/kg) was used as the standard drug. After 72 h 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 (crude bile content) was administered orally given once a day for 15 days after hyperglycemia was confirmed by the elevated glucose levels in urine determined at 72 hours (Fig 2).

![Benedict test for confirmation of diabetics](image)

Fig 2 Shows Benedict test for confirmation of diabetics

2.4 Experimental Design

2.4.1 Alloxan induced Diabetic

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Served as normal Control and received distilled water.</td>
</tr>
<tr>
<td>II</td>
<td>Alloxan treated control (120mg/kg b.w)</td>
</tr>
<tr>
<td>III</td>
<td>Alloxan (120mg/kg b.w) + bile content (5mg/kg p.o)</td>
</tr>
<tr>
<td>IV</td>
<td>Alloxan (120mg/kg i.p) + standard drug Glibenclamide (2.5mg/kg p.o)</td>
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</tbody>
</table>

2.4.2 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 minute. The resultant clear part was centrifuged at 3000rpm for 10minutes, and then the serum (supernatant) was isolated and stored at refrigerated until required for analysis.

2.4.5 Biochemical estimation

2.4.5.1 Quantitative estimation of glucose: Glucose was estimated by GOD/POD method (Trinder, 1969).

2.4.5.2 Qualitative Determination of Urine Sugar: Glucose was detected in the urine by the method of Benedict (1908). Benedict’s reagent (5ml) was taken in a test tube and 8 drops of urine was added to it. Tubes were boiled for 1-2 min and then cooled slowly. The solutions...
turned into greenish/yellow/red or no precipitate depending upon the quantity of glucose present. Reddish precipitate would indicate high amount of glucose. The solution remained clear (blue colour) where no glucose was there.

3. RESULTS

3.1 Antidiabetics 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 reduction in diabetes by 30.46% after 12hrs respectively. The oral administration of bile content in 100% concentration after 24hrs of administration showed maximum 45.83% diabetes reduction respectively, when compared with standard drug.

4. DISCUSSION

The traditional people are depends on natural resources from time immemorial for their health care needs. This knowledge usually transmitted orally from generation to generation without any written document. Therefore research based on such information could lead the discovery of new treatment strategy or drug molecules. It was also well proved that bile acids in human confer gut mucosal protection against bacteria. (Hofmann and Eckmann., 2006) Several researches also proved that several fish part like epidermal mucus of cat fish, fish oil may exert antimicrobial activity (Rybin et al., 2004; Abid and Salim, 2004).

Previously reported that the bile content of fish contain cholesterol, bile salt of deoxycholic acid and cholic acid, bile pigment, fatty acid. Chloride, sodium, potassium, calcium, phosphate, nitrate, carbonate and bicarbonate are present in bile as inorganic constituents (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). The increase in SHP due to FXR activation increases glucose metabolism by inhibiting production of phosphoenol pyruvate carboxykinase (PEPCK), an enzyme associated with gluconeogenesis (although conversely FXR activation has been shown to increase PEPCK activity and glucose levels (Stayrook et al., 2005). FXR activation also represses glucose levels in a diabetic rat model (Zhang et al., 2006). Bile acids can also increase glucose metabolism by regulating energy homeostasis via activation of the G protein-coupled receptor 5 (TGR5)- cAMP-type 2 iodothyronine deiodinase (D2) pathway in brown adipose tissue or skeletal muscles independently of FXR (Watanabe et al., 2006). These observations suggest that BAS might worsen glycemic control because of potential deactivation of hepatic FXR through a decrease of bile acids in liver, in contrast to the established beneficial effects of BASs for glucose metabolism. However, there is a report showing that BAS treatment does not change the level of total bile acids in serum, but increases the absolute level of CA as well as the CA level relative to total bile acids (Kajiyama et al., 1996). The relative increase in circulating CA may itself influence glucose metabolism through a decrease of glucose levels via the TGR5-cAMP-D2 pathway. However, even if this mechanism occurs it is unlikely to improve overall glycemic control because the level of CDCA relative to total bile acids may be decreased by BAS treatment, and CDCA has similar effects on TGR5 to those of CA. The most plausible mechanism for the glucose-lowering effect of BASs may be associated with effects on the liver X receptor (LXR), as proposed by (Bays and Goldberg, 2007). LXR is a nuclear transcription factor that mainly regulates lipid metabolism, and its natural ligands are oxysterols such as 22(R)-hydroxycholesterol, 24(S)- hydroxycholesterol, and 27-hydroxycholesterol (Fu et al., 2001). Reduction of bile acid flux in the portal vein by BAS treatment decreases FXR activity in liver, and this decreased FXR activity may induce an increase of LXR activity due to decreased SHP production. LXR activation in liver results in improved glucose sensitivity by preventing gluconeogenesis based on inhibition of the activity of PEPCK and G6Pase (Cao et al., 2003). LXR activation may also improve glucose metabolism by promoting expression of glucokinase and glucose transporter 4 (GLUT4) in adipocytes (Laffitte et al., 2003) or by promoting insulin secretion in β cells in the pancreas (Efanov et al., 2004).

Alloxan, induces “chemical diabetes” in a wide variety of animal species by damaging the insulin secreting cells of the pancreas. This damages a large number of β cells, resulting in a decrease in endogenous insulin release, which paves the ways for the decreased use of glucose by the tissues. Alloxan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycaemia (Chattopadhyay et al., 1997). Sulfonylureas such as glibenclamide are often used as a standard antidiabetic drug in alloxan-induced diabetes to

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mg/dl)</th>
<th>% of Antidiabetic activity</th>
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<tr>
<td></td>
<td>12 hrs</td>
<td>24 hrs</td>
</tr>
<tr>
<td>Group I</td>
<td>87.4 ± 2.14</td>
<td>87.4 ± 2.14</td>
</tr>
<tr>
<td>Group II</td>
<td>151.8 ± 18.67*</td>
<td>144 ± 16.77*</td>
</tr>
<tr>
<td>Group III</td>
<td>105.8 ± 12.83*</td>
<td>78.2 ± 12.51*</td>
</tr>
<tr>
<td>Group IV</td>
<td>100.6 ± 8.046*</td>
<td>89.2 ± 7.053*</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD

* Significantly different from group I
*Significantly different from group II (p < 0.05)
compare the efficacy of variety of antihyperglycemic compounds. Glucose was measured as a metabolic control of insulin action. The impairment of glucose homeostasis and increase in glucose level are associated with diabetic (Nagarajan et al. 2005). In present study found that administration of bile to diabetic rats reversed their blood glucose.

Diabetes is associated with impaired peripheral glucose clearance and increased hepatic glucose production during fasting, which lead to postprandial and fasting hyperglycemia. Initial evidence that bile acids may regulate glucose metabolism came from studies showing that FXR agonist induced phosphoenolpyruvate Carboxykinase (PEPCK) mRNA expression and glucose output in human and rat hepatocytes (Stayrook et al., 2005). Treating mice with an FXR agonist also induced hepatic PEPCK mRNA expression in mice in vivo. A FXR binding site has been identified in the promoter of PEPCK gene. In contrast, later studies carried out in fxr knockout mice revealed that FXR-deficient mice had insulin resistance and hyperglycemia phenotypes. Administration of a FXR agonist GW4064 decreased serum glucose, increased liver glycogen, and improved insulin sensitivity in diabetic db/db mice (Zhang et al., 2006). A number of recent studies showed that bile acids and FXR repressed hepatocyte PEPCK and G6Pase gene expression and thus liver gluconeogenesis. In this case, it is shown that bile acids may induce the repressor SHP, which inhibits PEPCK via inhibiting C/EBP FoxO1 (Parks et al., 2007) and Glucocorticoid receptor (Yamagata et al., 2004). Although these liver effects of FXR activation may prevent fasting hyperglycemia, it does not sufficiently explain the increased insulin sensitivity and glucose disposal in FXR agonist-treated mice as determined by glucose and insulin tolerance tests.

The results of this study indicate that the bile of these fishes contains one are several components with antibacterial activity. During antidiabetic study it was found that in maximum cases individual animal was showing drowsiness before death, though no abnormality was found. Among freshwater fishes spotted snake head, Channa Punctatus (Bloch) 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 along with certain level of antibacterial activity. However more investigation is necessary to confirm the ant diabetic and antibacterial activity of bile content of Channa Punctatus and to purify and characterize the activite components.

In the present study to administration of bile to alloxan induced diabetic rats restored the glucose level. This study suggested that bile content of fish can be utilized with its medicinal importance particularly in diabetic condition. The antidiabetic activity of bile may be due to LXR activation improve glucose metabolism by promoting expression of glucokinase and glucose transporter 4 (GLUT4) in adipocytes or by promoting insulin secretion in β cells in the pancreas or insulin sensitivity to tissues.

5. Acknowledgement
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6. References


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