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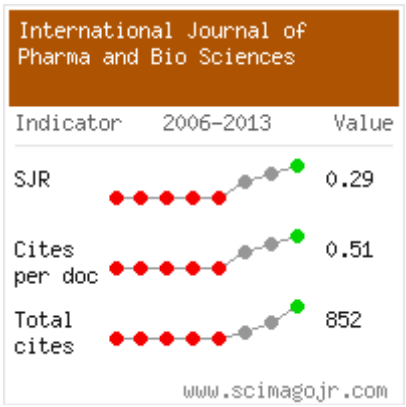

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EVALUATION OF BIOCHEMICAL PROFILE OF CHRYSIN IN STREPTOZOTOCIN –NICTONIMAIDE INDUCED DIABETIC RATS

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ABSTRACT

To evaluate the effect of Chrysin, a flavone, biologically active compound extracted from many plants, honey and propolis on Streptozotocin(STZ) – nicotinamide induced diabetic rat models. On administration of graded doses of Chrysin (25, 50 &100 mg/Kg body wt.) to normal and diabetic rats for 45 days, a significant ($p<0.05$) reduction in the levels of blood glucose, urea, uric acid and creatinine. The treatment with Chrysin significantly increased the level of total protein, albumin and bilirubin. Significant changes were also observed in plasma lipid profile in Chrysin treated diabetic control and normal animals. This present investigation suggests that for all the above biochemical studies performed, Chrysin treated rats showed more hypoglycemic activity.

KEYWORDS: **Chrysin, STZ, Biochemical, flavonoids**

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INTRODUCTION

Diabetes mellitus (DM) is an increasingly prevalent metabolic disorder in humans and it is characterized by disruption of normal carbohydrate, protein and fat metabolisms[1]. It is caused by a decrease in the insulin secretion by the pancreatic β cells and a decrease in the body's ability to respond against insulin. The resulting hyperglycemia may lead to metabolic complications, including ketoacidosis and the development over time of micro-vascular and macro-vascular complications and neuropathies[2]. The majority of diabetes (90%) is type 2 diabetes and it was also documented that the number of people diagnosed with this globally estimated to be at 2-3% of the world population and is rising at a rate of 4-5% per year [3,4]. In India, the statistical projection of diabetes will raise to 57 million in 2025, which was only 15 million in 1995[5]. In recent studies, STZ- nicotinamide is a method to induce diabetes in animals that resemble non obese type 2 diabetes mellitus in man[6]. The administration of STZ increases the production of free radicals that damage the pancreatic DNA and thus affect insulin production. This is because of depletion in the level of nicotinamide which is the substrate of poly ADP ribose synthetase, an enzyme involved in DNA repair mechanism[7]. The prior treatment with nicotinamide into experimental rats allows minor damage to pancreatic cells by inhibiting poly ADP ribose synthetase activity and prevents depletion of NAD[8]. Therefore, the administration of STZ- nicotinamide induces diabetes with moderate hyperglycemia associated with loss of early phase insulin secretion. This form of diabetes is very common in people over 40 years of age. The pathogenesis of DM is managed by insulin and oral administration of hypoglycemic drugs. Insulin therapy have several draw backs, which include insulin allergy, insulin antibodies, lipodystropy, autoimmunity and other delayed complications like morphological changes in kidney and severe complications. Other oral synthetic hypoglycemic drugs, apart from side effects, none of them have been successful in maintaining blood glucose and controlling long term microvascular and macrovascular complications[9,10]. Therefore, there is the need for new treatment to prevent or delay these complications. There is a growing interest in phytomedicine because of their effectiveness, fewer side effects and lower costs. Moreover, from the past few years, some of the new bioactive drugs isolated from hypoglycemic plants showed antidiabetic activity with more efficacy than oral hypoglycemic agents used in clinical therapy. Flavonoids are widely recognized for their ability to improve diabetic conditions by decreasing blood glucose levels[11]. They are natural polyphenolic phytochemicals present in the average human diet. Flavonoids are comprised of several classes, including flavonals, flavones, flavonols and flavans. Chrysin (5,7dihydroxy flavone) is a natural, biologically active compound derived from passiflora, pelargonium and pinacaeae. It is also naturally present in honey, some plant extracts, propolis and pine wood. Several recent studies have shown that chrysin has multiple biological activities, such as anti-inflammation[12], anti-cancer[13], antihypertension[14] and anti-oxidation effects[15]. Chrysin also has the potential for clinical and therapeutic applications against the physiological and biochemical effects of aging[16]. Also, studies on hepatotoxicity demonstrate that promising hepatoprotective action of Chrysin[17]. The present study is to investigate the Evaluation of Biochemical profile of Chrysin in STZ – nicotinamide induced diabetic rat models.

MATERIALS AND METHODS

Chemicals

Streptozotocin(STZ) and Chrysin were purchased from Sigma Aldrich chemicals Pvt. Ltd., USA. All other chemicals and reagents used were of analytical grade.

Animals

Albino Wistar rats (170-200g) were purchased from Central animal house, Annamalai University, Chidambaram, Tamil Nadu. The

animals were acclimatized to the conditions or about 7 days prior to dosing. The animals were fed under standard diet and water ad libitum maintained under standard laboratory conditions. The temperature 20^0-25^0C was maintained for 12 hrs each at the dark and light cycle was maintained. All the protocols were performed in accordance with the Institutional Animal Ethical Committee (Regd.No:MCAS/03/2012-2013) as per the directions of the CPCSEA.

Induction of diabetes mellitus

Streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5) and nicotinamide in normal physiological saline solution (0.9% NaCl solution). Diabetes was induced in overnight fasted rats by a single intraperitonial (i.p) injection of 65mg/kg of STZ, 15 min after the i.p. administration of 110mg/kg of nicotinamide. Hyperglycemia was confirmed by the elevated levels of plasma glucose, determined 72 hrs after diabetes induction. The animals with blood glucose concentration more than 230 mg/dl was used for the study[18].

Experimental Design

The rats were divided into 6 groups with six rats in each group. Diabetes was induced in four groups of rats. The experimental periods were 45 days. Group I : Normal Control, Group II : Normal rats + Chrysin(100 mg/kg), Group III : Diabetic control, Group IV : Diabetic rats + Chrysin (25mg/Kg), Group V : Diabetic rats + Chrysin (50 mg/Kg) and Group VI : Diabetic rats + Chrysin (100 mg /Kg). Chrysin was suspended in 0.5% dimethylsulfoxide (DMSO). After 45 days of treatment, the overnight fasted rats were sacrificed by cervical decapitation and the blood was collected for carrying out various biochemical parameters. Body weights of all the rats were taken before and after the treatment by electronic balance. At the end of the experiment, the rats were sacrificed under sodium pentobarbitone anaesthesia[19]. Whole blood was collected via cardiac puncture using sterile syringes and needles and emptied into plain tubes, allowed to clot for about two hours. The clotted blood was thereafter centrifuged at 3, 500 rpm for 30 minutes to recover serum from clotted blood. Serum was separated with sterile syringes and needles and stored frozen until used for biochemical analysis.

Biochemical Investigations

The level of glucose in serum was determined as described by Trinder (1969)[20]. Total bilirubin concentrations were determined by the method Pearlman and Lee (1974)[21]. Protein in the serum was determined after trichloro acetic acid precipitation by the method of Lowry *et al*, 1951[22]. Serum urea, uric acid and creatinine levels were determined by Barker, 1944; Fossatai *et al.,* 1980; Bonsness and Taussky, 1945 respectively [23, 24, 25].

Measurement of plasma lipid profile

Total cholesterol (TC) in plasma was measured by the enzymatic procedure described by Allain *et al*.,1974[26]. Triglycerides (TG) and Phospholipids (PL) were estimated by Mcgowan *et al*.,(1983), and Zilversmit and Davis.,1950 respectively[27,28].Plasma HDL-cholesterol (HDL-C) and LDL-cholesterol (LDL-C) were determined using Diagnostic Kit (Qualigens Diagnostics, Mumbai, India). LDL-C, VLDL-C in the plasma was calculated by Friedwald's ,1972) formula[29]. VLDL-C and LDL-C fractions were calculated as VLDL-C = TG/5 and LDL-C = TC − (HDL-C + VLDL-C), respectively.

Statistical analysis

The data are expressed as mean ± SD. Readings within a group were compared using the one- way ANOVA analysis and readings between groups were compared using the Independent sample t- test. Statistical analysis was performed using SPSS. A level of $p < 0.05$ was considered to be significant results.

RESULTS

In the present study, the STZ- nicotinamide induced diabetic rats significantly elevates the level of blood glucose. Table 1 shows that elevated glucose level was controlled by Chrysin (100mg/kg b.w.) significantly ($p<0.05$) and also the treatment of STZ-nicotinamide induced diabetic rats with the Chrysin significantly ($p<0.05$) increased the levels of

total protein and albumin in diabetic group. The level of bilirubin in diabetic rats significantly increased ($p<0.05$) when compared to normal control rats. Treatment with Chrysin (100mg/kg b.w.) significantly ($p<0.05$) decreased the bilirubin level to near normal. The concentration of serum creatinine, urea and uric acid were found to be significantly increased in diabetic control rats when compared to normal control rats. Table 2 summarizes that the significant ($p<0.05$) increase in the levels of serum urea, uric acid and creatinine in the diabetic groups and were significantly ($p<0.05$) decreased by Chrysin administration. Table 3 shows that there was a significant increase in the level of cholesterol, triglycerides, phospholipids, LDL, and decrease in the level of HDL Cholesterol in diabetic control rats when compared to normal control rats. The above parameters were significantly ($p<0.05$) reversed on diabetic rats treated with Chrysin.

TABLE 1

Effect of Chrysin on the levels of glucose, Total protein, Albumin and Bilirubin in serum of control and experimental animals

	Glucose (mg/dl)	Total protein (g/L)	Albumin(mg/dl)	Bilirubin (mg/dl)
Group I	92.0433±1.00719 ^{b}	7.3450±0.78816 ^{b,c}	4.7250±0.81652^{b,c}	0.8350±0.12012 ^{c}
Group II	90.3200±1.03863 ^{a}	7.8083±0.70596 ^{c}	5.6367±0.90853 ^{c}	0.8017±0.09745^{b,c}
Group III	190.57±0.96872 ^{f}	4.7850±0.89234 ^{a}	2.8517±0.69286 ^{a}	0.5050±0.09670 ^{a}
Group IV	163.63±0.91595 ^{e}	6.5017±0.99654 ^{b}	3.3850±0.85795 ^{a}	0.7417±0.08976^{b,c}
Group V	143.02±0.75192 ^{d}	6.6217±0.75505 ^{b}	3.4917±.81693 ^{a}	0.7033±0.06121 ^{b}
Group VI	102.50±0.84916 ^{c}	6.7300±0.84716 ^{b}	4.4917±0.75271 ^{b}	0.8233±0.06439 ^{c}

Each value is expressed as Means±SD for six rats in each groups. Value that have different superscript letter(a,b,c,d,e,f)

TABLE 2

Effect of Chrysin on the levels of Creatinine, Urea and Uric acid in serum of control and experimental animals

	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Group I	0.5417±0.08134 ^{a}	25.2950±0.66 ^{b}	3.1383±0.65064 ^{a}
Group II	0.5350±0.10035 ^{a}	23.0667±0.5±0.56 ^{a}	3.1417±0.84447 ^{a}
Group III	1.4750±0.65884 ^{c}	42.9200±1.246 ^{f}	6.9983±0.97493 ^{c}
Group IV	1.0300±0.32193 ^{b}	39.1083±1.26 ^{e}	4.5033±0.58126 ^{b}
Group V	0.9017±0.43278^{a,b}	30.9733±1.05 ^{d}	3.2033±0.53339 ^{a}
Group VI	0.5200±0.12712 ^{a}	29.6517±1.21 ^{c}	2.7750±1.09099 ^{a}

Each value is expressed as Means±SD for six rats in each groups. Value that have different superscript letter (a,b,c,d,e,f)

TABLE III

Effect of Chrysin on plasma lipid profile of control and experiment rats.

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
TG (mg/dl)	62.66±4.96^{a}	60.73±4.38^{a}	153.16±4.97^{e}	126.25±4.20^{d}	91.60±2.77^{c}	74.95±4.27^{b}
PL (mg/dl)	100.80±3.80^{b}	93.84±3.60^{a}	182.84±5.88	165.52±4.50^{e}	131.86±4.79^{d}	125.52±3.53^{c}
TC (mg/dl)	72.39±4.97 ^{a}	73.36±3.70 ^{a}	183.60±4.16 ^{e}	110.46±3.51 ^{d}	92.18±2.99 ^{c}	82.74±3.96^{b}
HDL (mg/dl)	45.85±3.33 ^{d}	49.46±3.40 ^{e}	24.32±2.93 ^{a}	27.29±2.75 ^{b}	37.92±2.34 ^{c}	43.83±3.63 ^{d}
LDL (mg/dl)	14.01±3.45 ^{a}	11.76±5.44 ^{a}	128.65±4.1 ^{e}	56.61±3.45 ^{d}	35.94±3.51 ^{c}	23.92±5.11 ^{b}
VLDL(mg/dl)	12.53±0.91 ^{a}	12.15±0.88 ^{a}	30.63±0.99 ^{e}	25.55±1.12 ^{d}	18.32±0.55 ^{c}	14.98±0.85 ^{b}

Each value is expressed as Means±SD for six rats in each groups. Value that have different superscript letter (a,b,c,d,e,f)

DISCUSSION

Type II diabetes is characterized by a progressive loss of β-cell function that results in a hyperglycemia and its related complications. Despite the presence of known antidiabetic prescription medicines, evaluation of plant products in the treatment of DM is profitable because of the presence of several bioactive compounds with less toxicity and free from adverse effects. Administration of STZ alone in experimental rats causes diabetes by the rapid depletion of β-cells of pancreas. However, it is attenuated by pretreatment of animals with nicotinamide prior to STZ administration[8]. The advantage of NA on blood glucose is due to the protection of β-cells against STZ-induced injury and is accompanied by increased blood insulin [30, 31]. This study manifested a significant decrease in serum total proteins levels in the diabetic rats. The decrease in serum protein in diabetic animals indicates proximal tubular dysfunction[32]. Nevertheless the total protein levels significantly increased after the administration of a flavone Chrysin. Hyperbilirubinemia may resulted from the decrease in liver uptake, conjugation or increase bilirubin production from hemolysis and the elevation indicates liver damage[33]. However, the decrease in bilirubin levels in Chrysin treated rats is indicative of a reversal of liver damage. Urea is the main end product of protein catabolism in the body, which was found to increase in diabetic rats. This accumulation in diabetic rats may due to the enhanced break down of both liver and plasma proteins. Alteration in nitrogen homeostasis may lead to increased hepatic elimination of urea nitrogen and increased peripheral release of nitrogenous substances [34]. Thus, the observed negative nitrogen balance may partly because of changes occurring within the hepatocytes. Uric acid, one of the major endogenous water soluble antioxidant in the body has been thought to be a metabolically inert end product of purine metabolism. The results of the present study show that significant increase in the level of uric acid in diabetic rats. This elevation may be due to either an increase in uric acid production or a decrease in its excretion[35]. In the current investigation, the increased level of uric acid observed in STZ-NA induced diabetic rats were maintained to normal levels by the administration of a flavone, Chrysin Chronic hyperglycemia induces advancement of serum creatinine leading to renal dysfunction. Creatinine is a byproduct of the breakdown of creatine and phosphocreatine, which are considered as an energy storage compound in muscle. Creatinuria occurs in any condition associated with extensive muscle breakdown as in starvation and poorly controlled diabetes mellitus[36]. The result from the present study show that Chrysin can also reduce the level of serum creatinine in STZ-NA induced diabetic rats that further helps in treating and preventing

the renal system from damage. Diabetes induced dyslipidaemia have been observed in diabetic experimental models due to increase in the mobilization of free fatty acids from peripheral fat depots and due to the inability to utilize glucose properly as well as increased cholesterol biosynthesis, which in turn leads to accumulation of lipids such as TC, TG and phospholipids in diabetic condition[37]. Acute insulin deficiency or the insulin resistance may be responsible for hyperlipidaemia owing to the insulin inhibiting action on 3-hydroxy-3-methylglutaryl coenzyme-A- a key enzyme in the cholesterol biosynthesis[38]. Also an increase in plasma LDL-C and VLDL-C fractions along with a decrease in HDL-C were observed in diabetic rats[39]. These abnormalities are the major risk factors for the development of cardiovascular diseases in DM[40]. In this study, administration of Chrysin reduced the elevated levels of plasma total cholesterol and triglycerides. It is possible to suggest that the mechanisms of antihyperlipidemic effect of the Chrysin might be similar to some of those suggested for antidiabetic plants exhibiting antihyperlipidemic activity, such as activation of lipoprotein lipase, insulin-mediated lipolytic activity or inhibition of lipogenic enzyme or hormone-sensitive lipase.

CONCLUSION

The present study endorsed the traditional rights of use of Chrysin in the treatment of diabetes. Chrysin significantly reversed STZ-nicotinamide induced raise in glucose levels, indicating Type-II antidiabetic activity. The antidiabetic activity may be due to improvement in glucose tolerance, refurbishment of liver functions, thus, decreasing the risk of secondary complications associated with diabetes. However, further studies are warranted for elucidation of their molecular mechanisms.

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