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EFFICACY OF *GRIFFITHSIA PACIFICA KYLIN* ON *IN VIVO* ANTI DIABETIC AND ALTERATIONS IN ENZYMATIC ACTIVITIES OF STZ INDUCED RATS

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Keywords:

Griffithsia Pacifica Kylin, Red algae, Glycosylated hemoglobin, Streptozotocin (STZ), Hyperglycemia, Gluconeogenic Enzymes

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ABSTRACT: In recent years there is more research work taken in Marine sources for its pharmaceutically valuable compounds and active medicines for anticancer, anti diabetic, anti oxidant, antimicrobial, and anti-inflammatory. The present study investigated for the medicinal value of red algae *Griffithsia pacifica Kylin* (GPK) and its alterations in enzymatic activity of STZ induced diabetic rats. The results gave significant increase in hemoglobin and glycosylated hemoglobin along with the reduction in hyperglycemia. Thus the GPK might serve as an effective natural medicine for anti-hyperglycemic effects of diabetic rats and may be promising for development of phyto medicines for diabetes.

INTRODUCTION: In ancient periods Chinese and Japanese have used algae as food, because rich nutrient content in algae especially red algae. Nori known as nutritive food made with red algae for providing complete nutrient supplement by Japanese. The dairy industries like chocolate, milk, yoghurt are filled with high minerals, vitamins and nutrients, these nutrients are obtained from red algae [1]. Red algae can be used as body and blood purifier [2]. Red alga is the most valuable sea weed with many useful properties that has to be explored. Phytochemicals like Phenols, terpenes, polysaccharides and steroids are mostly found in the red algae [3].

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The live saving drugs were mainly found in marine algae and marine sources. Bioactive compounds of marine organisms have enourmous therapeutic potential. Marine products represent a vast untapped resource of bioactive molecules, especially antioxidant, antidiabetic, anticancer, antimicrobial [4].

Silver nanoparticles that synthesized using red marine alga which used as a capping agent and reducing agent [5, 6, 7] It has shown the good α-glucosidase inhibitor and α–amylase inhibitor [8]. Thus red algae have well anti diabetic activity.

Carbohydrate metabolic enzymes play a significant increase of glucose in diabetic patients. Glucose-6-Phosphatase is the most important key enzyme of glucose homeostasis, and catalysis the terminal step in gluconeogenesis and glycogenolysis [9, 10] the enzyme is mainly found in liver and kidney gluconeogenic tissues, there it plays an important role in glucose production [11].

Fuctose-1, 6-di Phosphatase is another key enzyme which catalysis the irreversible reaction in gluconeogenesis pathway and it regulates the pathway. The Glucokinase (Hexokinase 4) is a protein produced in hepatocytes [12, 13], it phospharylates glucose, preparing it for incorporation into glycogen, and is the key enzyme of glucose metabolism[14].

In general, the main activities of phenolic compounds are antioxidant and antidiabetic activity [15] Other attractive lipid bioactive compounds from red algae and other marine sources include the group known as sterols and omega fatty acids. The composition of sterols isolated from macro and microalgae [16] and other marine invertebrates have been extensively studied. Sterols and some of their derivatives were found previously to play an important role in lowering LDL cholesterol levels *in vivo* anti-inflammatory, antidiabetic and antiaterogenic activity [17]. Red Algae are orange to pink, 3-5cm tall, and thallus monosiphonus tuffed – filamentous, clearly visible to naked eye [18]. This species was first described by Harald Kylin in 1925.

MATERIALS AND METHODS:

Red Algae Collection: Algal materials were collected from the Rameswaram, Tamil Nadu, India and obtained fisher by catching method. The collected red algae were washed with tap water to remove salts and other adhering particles. The whole red algae was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol in sox let's apparatus to 60 °C. The solvent was completely removed by rotary vacuum evaporator. The extract was freeze dried and stored in vacuum desiccators.

Experimental Induction of Diabetes: Ethical Clearance No: VIT/IAEC/10th / March/14th /No.31. Dated: March31:2015. Diabetes was induced in the animals fasted overnight by a single intra peritoneal (ip) injection of freshly prepared solution of STZ (Sigma, USA) 35 mgkg^{-1} body weight in 0.1M cold citrate buffer pH 4.5 [19, 20, 21]. The animals were allowed to drink 5% glucose solution to overcome the drug-induced hyperglycemia [22]. Control rats were injected with citrate buffer alone as a placebo. Animals were considered diabetic if the blood glucose values were 4250 mg dL^{-1} on the third day after STZ injection. After a forth night, rats with moderate diabetes having glycosuria (indicated by Benedict's test for urine) and hyperglycemia with blood glucose range of 200– 300 mg dL^{-1} were used for the experiment. Blood was collected from the eyes (venous pool) by sino-ocular puncture.

Drugs and chemicals: Streptozotocin, nicotinamide, saline, acetonitrile (HPLC grade), potassium dihydrogen orthophosphate, and all substrates, buffers were purchased from Micro labs, Tamil Nadu, India. And the rest of chemicals like standard gallic acid (GA), ellagic acid, catechin, and epicatechin were received from Ranbaxy Research Laboratory, Hyderabad, India.

Acute Toxicity Test: The albino wistar rats were divided into six groups of six animals each. A group received saline (10 ml/kg) by gavage and kept as normal control. A single dose of GPK algae extract was administered orally to group 2, 3 and 4 at doses of 50, 500, and 5000 mg/kg b.wt. respectively. The extract did not produce any toxic symptoms of mortality up to the dose level of 5000 mg/kg body weight in the treated animals, and hence it was considered safe for further pharmacological screening. The mortality, measured body weight and behavioural screening were recorded daily during 14 days after the extract administration.

Experimental Design: Studies were carried out using Wistar albino male rats (150–200g), obtained from Indian Veterinary Preventive Medicine (IVPM) (Ranipet, Tamil Nadu, India). The animals were grouped and housed in polyacrylic cages (38 × 23 × 10 cm) and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by the Poultry Research Station (Nandhanam, India), and freshwater *ad libitum*. All the animals were acclimatized to laboratory conditions for 1 week before commencement of the experiment. Rats were fasted for 24 h prior to the experiment in mesh-bottomed cages to reduce coprophagia but allowed free access to water except for the last hour before the experiment. The animals were divided into six groups as follows after the induction of

STZ-induced diabetes. Group I animals were considered as control rats. Group II animals were treated as diabetic STZ-induced rats. Group III diabetic-induced animals were fed with 200 mg kg^{-1} of ethanol extract of GPK for six weeks. Group IV diabetic-induced animals were fed with 400 mg kg^{-1} of ethanol extract of GPK for six weeks. Group V diabetic rats were given glibenclamide orally (0.6 mg kg^{-1}) in distilled water daily for six weeks, Group VI GPK 400 mg kg^{-1} only GPK extract for six weeks.

Determination of Blood Glucose, Glycosylated hemoglobin (HBA1C) and hemoglobin levels: All the groups of rats were sacrificed by cervical dislocation after six weeks of treatment, blood was collected and processed for estimation of blood glucose (glycosylated hemoglobin (HbA1C) [23] and hemoglobin [24] levels.

Determination of Lipid Profile: Plasma samples were used to measure triglycerides (TG) and cholesterol levels. The biochemical estimation such as serum TC [25], TG, LDL, HDL [26] was carried out by the standard methods.

Determination of enzymatic activities: The activity of Glu-6-phosphatase, Fru-1, 6-Di phosphatase, Glucokinase was determined by liver and kidney supernatant fractions of 14 days experimental rats by the method of Baginsky *et al.*, Tashima and Yoshimura respectively [27]. About 1-2 mg of protein was used for both the assays. One enzyme unit is defined as the amount of inorganic phosphate, Pi [28] liberated per gram fresh weight per minute at 37°C for both the phosphatases. Phosphatases estimation was by the method of Fiske and Subbarow.

RESULTS:

Statistical Analysis: Results were analyzed for statistical significance using one way ANOVA followed by Dunnett's test using the graph pad statistical software for comparison with control group and STZ-treated group. A p50.05 was considered as significant. The results of the changes in general parameters including changes in body weight, tissue weight, are shown in **Fig. 1(a)-3(j)**.

Effect of GPK extract on Blood Glucose and Total Cholesterol: The blood glucose levels were significantly increased in STZ diabetic rats as compared to control. Administration of GPK and glibenclamide tended to lower the values close to those of control rats. The effect of GPK on blood glucose levels in diabetic rats was more evident than glibenclamide **Fig. 1 (a)**. The Total cholesterol levels were significantly increased in STZ diabetic rats compared to control. Administration of GPK and glibenclamide turns to lower the values as normal to those of control (**Fig. 1(a)**.

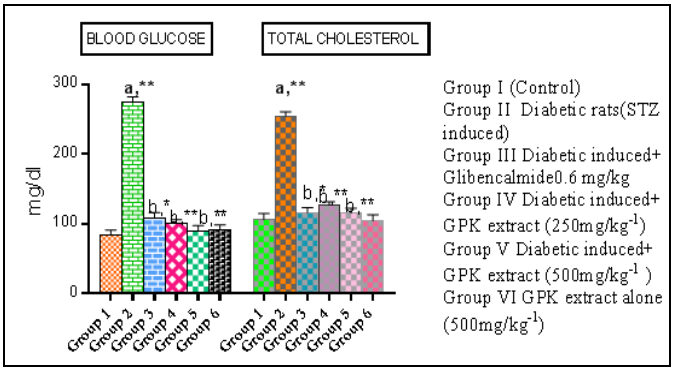

FIG. 1(a): EFFECT OF GPK EXTRACT ON BLOOD GLUCOSE AND TOTAL CHOLESTEROL OF STZ INDUCED DIABETIC RATS

Effect of GPK extract on hemoglobin (HB), and glycosylated hemoglobin (HBA1C): Alterations in the HB and HBA1C following treatment of diabetic rats with GPK extract and glibenclamide are given in (**Fig. 1 (b)**. There was no significant alteration in total hemoglobin levels, while the glycosylated hemoglobin (HBA1C) was significantly higher in diabetic rats compared to control (Group II **Fig. 1(b**). On treatment with GPK extract or glibenclamide, HBA1C level was lowered significantly, (Group IV or Group V) compared to untreated diabetic rats (Group II). The effect of GPK was more distinct than glibenclamide in lowering the HBA1C levels.

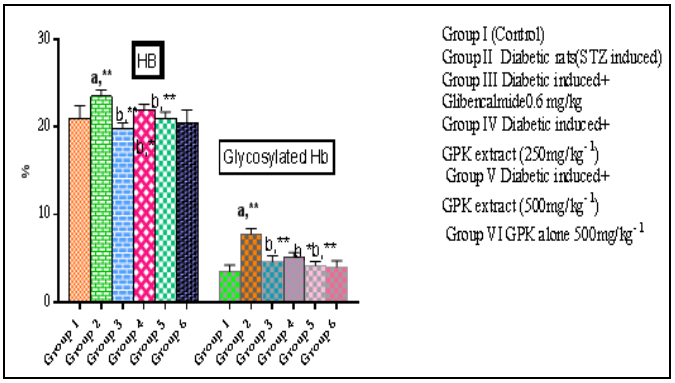

FIG. 1(B): EFFECT OF GPK EXTRACT ON HB AND HBA1C OF DIABETIC INDUCED RATS

Effects of GPK extract on blood lipid profile (TG, HDL, and LDL): The lipid profile such as TG, LDL (**Fig. 1(c)** levels were significantly increased in diabetic animals (DC), whereas HDL levels were decreased **(Fig. 1(c)**.

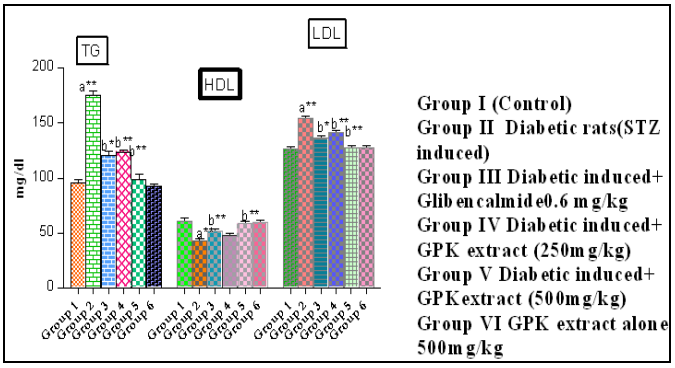

FIG. 1(c): EFFECT OF GPK EXTRACT ON TG, HDL, AND LDL ON STZ INDUCED DIABETIC RATS

Diabetic animals at given GPK 250mg kg^{-1} showed alterations in the TG, HDL and LDL levels compared to STZ. On the other hand, the dosage was increased from 250 to 500 mg kg^{-1} body wt., a significant fall in the TG and LDL levels was found compared to diabetic animals. When compared with standard drug (Group V) the fall in TG, LDL was dose dependent and highest reduction in cholesterol noted in 500 mg kg^{-1} group compared to STZ animals. The lower HDL in diabetic rats, increased significantly after administration of the ethanol extract of GPK. The increment was highest at 500 mg kg^{-1} GPK (Group VI) (**Fig. 1(c)**.

Effect of GPK extract on body weight of STZ induced diabetic rats: The body weight of STZ induced diabetic rats were significantly decreased than normal control rats. Administration of ethanol extract of GPK gave the alterations in the body weight when compared to diabetic induced rats.

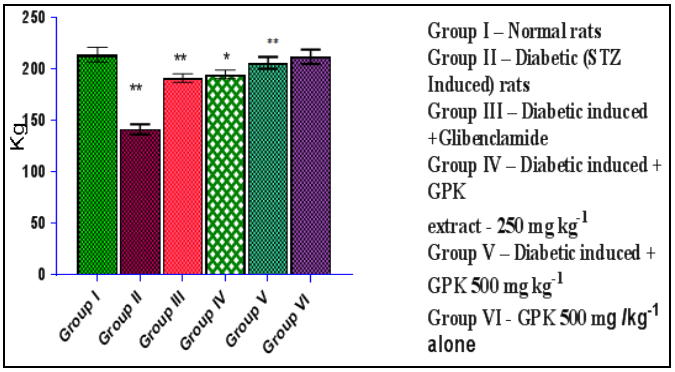

FIG. 2: EFFECT OF GPK EXTRACT ON BODY WEIGHT OF STZ INDUCED DIABETIC RATS

When compared to standard drug GPK increased the body weight at dose dependent level, the highest concentration of GPK 500mg kg^{-1} increased the body weight of STZ induced rats as control rats (**Fig. 2**).

Effects of GPK extract on enzymes: The activity of Glucose-6-Phosphatase was almost same in kidney and liver of control. The increased activity occurs during diabetes was very high in liver and kidney. The activity of fru-6-tase in control rats was same in liver and kidney. The diabetic liver and kidney exhibited 3-4 fold increased activity. The activity of these two enzymes was markedly decreased by administration of ethanol extract of GPK.

The remaining enzymes Hexokinase, Glucokinase, LDH activities were decreased in STZ induced diabetic rats, this was comes to normal after the administration of GPK extract in diabetic induced rats **Fig. 3(a)-(h).**

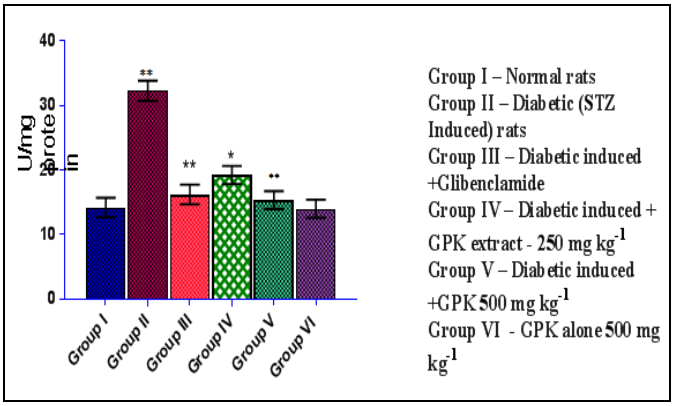

FIG. 3(a): EFFECT OF GPK EXTRACT ON GLUCOSE - 6 PHOSPHATASE IN LIVER OF STZ INDUCED DIABETIC RATS

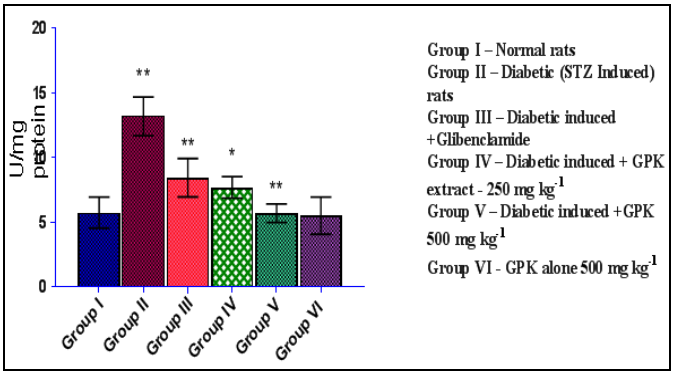

FIG. 3(b): EFFECT OF GPK EXTRACT ON THE ACTIVITY OF FRUCTOSE-1, 6-DIPHOSPHATASE IN LIVER OF STZ INDUCED DIABETIC RATS

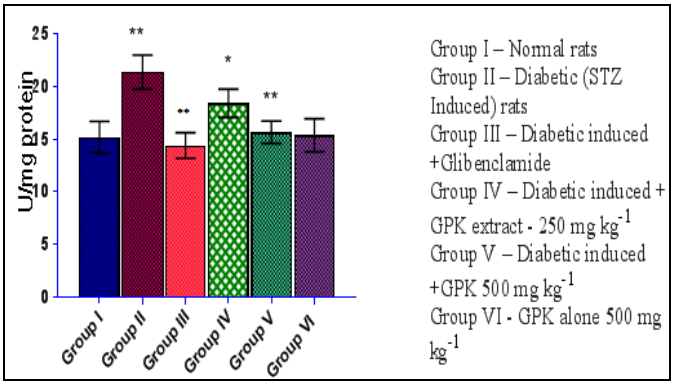

FIG. 3(c): EFFECT OF GPK EXTRACT ON ACTIVITY OF GLUCOSE-6-PHOSPHATASE IN KIDNEY OF STZ INDUCED DIABETIC RATS

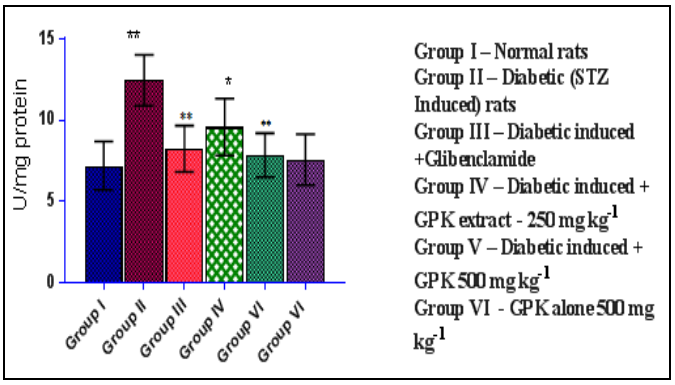

FIG. 3(d): EFFECT OF GPK EXTRACT ON THE ACTIVITY OF FRUCTOSE-1, 6-DIPHOSPHATASE IN KIDNEY

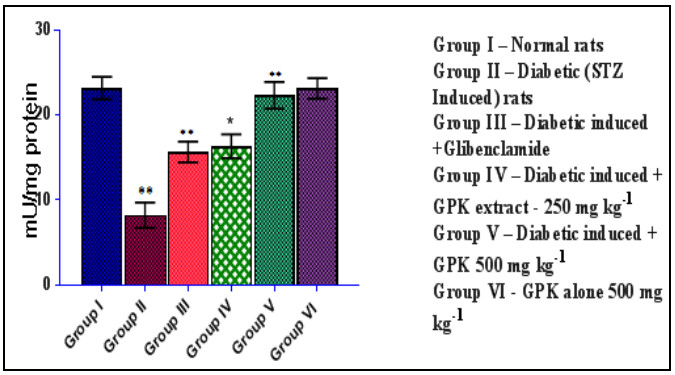

FIG. 3(e): EFFECT OF GPK EXTRACT ON THE ACTIVITY OF GLUCOKINASE IN LIVER

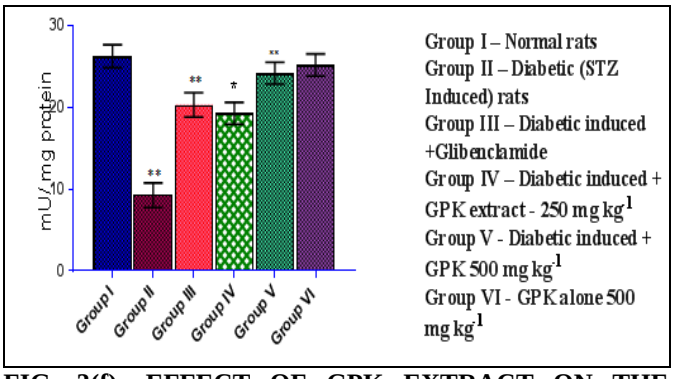

FIG. 3(f): EFFECT OF GPK EXTRACT ON THE ACTIVITY OF GLUCOKINASE IN KIDNEY

DISCUSSION: In diabetes the increased blood sugar levels might be due to either insulin resistance of the body cells or decreased secretion of insulin from β-cells manifested in the decreased serum insulin levels [29]. The reduction in the serum insulin levels in the STZ-treated rats might be attributed to the reduced secretion of the hormone which might be due to the damage of the β-cells of endocrine pancreas. The STZ selectively destroy the pancreatic cells and induce hyperglycemia. The blood glucose level of *GPK* extract fed animals was significantly reduced. The highest decrease was recorded in the 400 mg kg^{-1}GPK and STZ group.

In addition, during diabetes the excess glucose present in circulation reacts with hemoglobin to form glycosylated hemoglobin. These findings are in agreement with our studies, in which there was no significant changes observed in total hemoglobin but the glycosylated hemoglobin was significantly higher in diabetic rats. Administrations of GPK extract restored to normal the total hemoglobin and HBA1C in diabetic rats by reducing the glucose levels.

The lipoprotein levels in the STZ-induced diabetic rats in this study revealed significant alterations in lipoprotein metabolism. The serum TC content increased significantly in diabetic animals. Since insulin exerts a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency was associated with excess lipolysis and increased influx of free fatty acids to the liver [30, 31] the animal's revealed better proliferation from the STZ-induced damage when compared to control as well as 50mg kg^{-1} treated animal.

The findings of the present work indicate the usefulness of the extract of GPK; plasma glucose concentrations were lower than in the control group at 60 min ($p < 0.05$) following a single subcutaneous administration of insulin [32].

This study suggests that the ethanol extracts of GPK exerted anti hyperglycemic effect as evidenced by decreased glucose levels and decreased serum lipid levels. Therefore one can attribute therapeutic value of this ethanol extracts of GPK to combat the diabetic condition in rats.

CONCLUSION: We conclude from the above results and discussion on the ethanol extract of

Griffithsia pacifica Kylin has significant hypoglycemic, hypolipidemic potential. In the present investigation we demonstrated that GPK have beneficial effects on the altered serum glucose.

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