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RESEARCH ARTICLE

Synergetic Potency of *Butea monosperma*'s Leaf Extract and Metformin on Tissue Protein and Hepatic Glycogen of Alloxan Induced Diabetic Albino Rat

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Abstract

The antidiabetic activity of several combinations of aqueous extract of plant Butea monospermea and a potent antidiabetic agent Metformin was studied. Tissue protein (mg/ml tissue homogenate) is an easily altered biochemical system and was measured at regular intervals during the experiment period. According to the findings, all of the treatments investigated had an optimistic consequence on tissue total protein concentration in Diabetes Mellites. Glycogen content, which is a recovered form of carbohydrate (glucose), is a direct indicator of insulin action in the body. Treatment with Butea monosperma aqueous extract at various dosages aimed at 15 days exhibited a substantial surge in the liver carbohydrate (glycogen) amount as compared to normal control. When the hepatic glycogen concentration of all the extracts was analysed independently, it was found that the extract at a dose concentration of 600 mg kg-1 body weight was most efficient in recovering the hepatic glycogen content.

Introduction:

Diabetes is a major threat to community well-being and the socio-economic status of most parts of the world. This is because of the increasing death rate and illness results which are related to these diseases. Recent data have shown that about 425 million people worldwide (62 million in India) are living with diabetes, which may increase by 48% in 2045. (International Diabetes Federation, 2017). According to the World Health Organization (WHO), DM affects at least 171 million people globally or 2.8% of the population. Its frequency is rapidly increasing, with projections that it will more than double by 2030 (Roglic *et al*., 2004). In 2013, the International Diabetes Federation (IDF) reported that nearly 382 million people, or 8.3% of the global population, had diabetes, and that number is expected to rise to 592 million by 2035. Insulin Dependent Diabetes Mellitus (IDDM) is caused by cellular autoimmune destruction of the -cell, which results in absolute insulin insufficiency in about 5% to 10% of the population. Polyuria, polydipsia, and fast weight loss are common acute symptoms; diabetics have insulinopenia (an insulin shortage) due to the failure of pancreatic islet beta cells and must rely on insulin to stay alive and avoid ketosis. (Mooradian 2009; Patel & Sharma, 2014). Glycosuria, polyurea, polyphagia, and polydipsia are the four fundamental signs of DM. Epidemiologic data have proven that diabetes mellitus is an independent cardiovascular disease risk factor, bolstering the impact of other known risk factors like smoking, hypercholesterolemia, and hypertension (Stamler *et al*., 1993; Almdal *et al*., 2004).

Non-Insulin Dependent Diabetes Mellitus (NIDDM) refers to those who have insulin resistance as well as insulin insufficiency. In the beginning, and typically throughout their lives, patients with NIDDM do not require insulin treatment to survive. Approximately 90% of all people with diabetes have this kind of diabetes. Minimal symptom individuals are not prone to ketosis and do not require insulin to avoid ketonuria. The most common sign of this form of diabetes is obesity (Ceriello *et al*., 2001). Obesity, on the other hand, is a source of insulin resistance. Insulin concentrations in patients with type 2 diabetes might be normal, low, or high. The majority of persons with this kind of diabetes, however, have impaired insulin action. Both type 1 and type 2 diabetes are polygenic illnesses, meaning they are caused by a combination of genes and environmental factors. There are a few types of diabetes (for example, young-onset diabetes and neonatal diabetes) that

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are single-gene diseases affecting the pancreatic beta cells, accounting for just 1 to 2% of all cases. Individuals with these genetic disparities have a 10 to 15% higher risk of diabetes than those who do not have a discrepancy. (Aliciguzel *et al*., 2003). Numerous medications, such as sulfonylureas, biguanides, thiazolidinediones, alpha-glucosidase, and meglitinides, are commercially available for the treatment of diabetes and its complications. Herbal remedy also called phytomedicine denotes using various products of plants like seeds, berries, roots, leaves, bark, or flowers for therapeutic purposes. Therapeutic herbs have a significant function in the human and animal health systems around the world, both in the diseased state and in maintaining proper health. India has long been known as a rich source of medicinal flora (Joy *et al*., 1998). Furthermore, ethano-botanical research of herbal formulations used for diabetes mellitus has identified approximately 1200 species of plants having hypoglycemic properties (Singh, 2001).

Mathodology

The fresh leaves of *Butea monosperma* were collected from the natural habitat in Nanded Maharashtra India during the month of July 2021. After collection, those were washed with distilled water to remove the dust and debris. After wash leaves were shade dried for a week and dried leaves were fine powdered with a manual blender. A portion of powder (300 grams) was soaked with distilled water for 24 h at room temperature. The mixture was filtered by using a Whatman filter paper. The collected filtrate then was air dried and kept at a low temperature for further use as BMAE (Buteamonosperma aqueous extract). During the test, the crude extract was made diluted with distilled water just prior to being given to animals (Shirzad *et al*., 2011).

Alloxan monohydrate was brought from SRL chemicals for induction of diabetes in the albino rats. Another chemical Metformin (Merk) was purchased from the market. The tablets were crushed into a fine powder and an appropriate concentration of it was dissolved in distilled water prior to administration to experimental animals and orally administered at a dose of 100 mg kg-1 day-1 (Morales *et al.*, 2010).

Albino rats of both sexes, 3 months old, weighing approx. 150-250 g were purchased from the animal house of Pharmacy college and Research Centre, Nanded Maharashtra. Rats were accommodated in iron cages in standard conditions (temperature 25±2EC and 12:12 light: dark cycle). All groups of rats were acclimatized for two weeks before starting the experiment. They were fed every day with a standard diet and were given free access to distilled water. Animals described as fasting were deprived of food for at least 18 h but were allowed free access to drinking water (Zhao *et al*., 2016).

After overnight fasting (deprived of food for 16 hours had been allowed free access to water), hyperglycaemia was induced by intraperitoneal injection of 60 mg kg-1 of freshly prepared solution of Alloxan (0.1 g dissolved in 5 mL of freshly prepared sodium citrate buffer 0.1 M, pH 4.5) (Eidi, *et al*., 2006). The Alloxan-injected animals were given 20% of glucose solution for 24 h to prevent mortality which occurs as a result of hypoglycaemic effects of drugs. The diabetic status of animals was confirmed by monitoring Fasting Blood Glucose (FBG) using a glucometer (Accu check, Roche, USA). Rats with FBG levels of more than 200 mg dL-1 were considered as diabetic and selected for further study.

For both the Normal non-diabetic (normoglycemic) and Diabetic (hyperglycaemic) categories, the rats were divided into the following groups each group consisting of six rats.

Group A: Distilled water (Normal control)
Group B: Alloxan treated (Diabetic Control)
Group C: Metformin 60 mg/kg (Standard Diabetic)
Group D: Metformin + BMAE 200 mg/kg
Group E: Metformin + BMAE 400 mg/kg
Group F: Metformin + BMAE 600 mg/kg

The administration of both drug and plant extract was done by oral route.

Results are expressed as Mean ± Standard Deviation (SD). Data were analysed according to the procedures described by Gomez & Gomez (1984) by SPSS Version 18.0 (SPSS Inc., Chicago, IL, USA). Probability $p<0.05$ indicated significance.

Results and Discussion:

Effect on Tissue total protein: Table 1 reveal the result of alloxan and successive metformin therapy on tissue total protein, as well as other crude extracts. It is obvious from the statistics that the administration of alloxan to the Diabetic Control group lead to a substantial decrease in activity 64.44%, 56.48%, 51.25% in the liver, kidney and pancreas separately. Furthermore, the Standard Diabetic group raised its action by 14.7%, 26.87%, and 10.95% in each of the three tissues. Co-administration of 60 mg Butea monosperma aqueous extract(BMAE) to alloxan-induced diabetic mice increased tissue protein content in the liver, kidney, and pancreas by 60.6%, 42.4%, and 47.5%, respectively. However, 15 days of therapy with 200 mg BMAE extract significantly increased the decreased content of tissue protein in the liver, kidney, and pancreas, respectively, by 58.08%, 0.6%, and 42.03%. In the three tissues of the 400 mg and 600 mg BMAE extracts treated groups, the same trend of substantial amelioration of tissue total protein content was found. Hepatic tissue protein content increased by 62.8% and 69.2% in these two groups, renal tissue protein content increased by 48.7% and 57.5%, and pancreatic tissue protein content increased by 52.4% and 58.2%. According to the findings, 600 mg BMAE was shown to be the most effective in overall relief of tissue total protein content drop, followed by 400 mg, 200 mg, 100 mg,

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60 mg, and SD treatments, and metformin was found to be less effective than the plant extracts.

Result on hepatic glycogen content: According to the statistics given in Table-2, after the development of diabetes, the concentration of hepatic glycogen was considerably reduced in the DC group to 20.25 +7.50 mg/g tissues, compared to 49.8 +10.3 mg/g tissues in the NC group. Treatment with metformin and BM extract, on the other hand, results in a significant increase in hepatic glycogen content, with concentrations of 95.64 +15.5 mg/g tissue in the SD group and 72.8 +6.8, 90.6 +7.2, and 97.25 +8.5 mg/g tissue in the 200 mg, 400 mg, and 600 mg treated groups, respectively.

Table 1: Effect of crude extract of *Butea monosperma* and Metformin on Total tissue Protein (mg/ml Tissue Homogenate) on Alloxan induced diabetic albino rat.

Groups	Tissue Total Protein (mg/ml)		
	Liver	Kidney	Pancreas
Control			
Normal Control (NC)	14.77 ±2.4	5.75 ±2.5	3.50±0.75
Diabetic Control (DC)	5.03±1.34	2.08±1.70	1.60±0.45
Treated			
60 mg (SD)	6.75±0.9	2.74±1.50	2.05±0.5
200 mg	12.4±2.5	2.9±2.7	2.6±1.9
400 mg	13.2±2.0	4.2±1.5	3.7±0.75
600 mg	15.5±4.1	5.8±1.8	4.5±2.1

Table 2: Effect of crude extract of *Butea monosperma* and Metformin on Hepatic glycogen content (mg/g tissue) on Alloxan induced diabetic albino rat. Data represent mean ± SD of 6 observations. Values are significantly at P<0.05.

GROUPS	Hepatic Glycogen Content (mg/g tissue)	
Control	Normal Control (NC)	49.8 +10.3
	Diabetic Control (DC)	20.25 +7.50
Treated	60 mg (SD)	95.64 +15.5
	200 mg	72.8 +6.8
	400 mg	90.6 +7.2
	600 mg	97.25 +8.5

The effect of the BMAE on rat tissue total protein and the Hepatic glycogen content has implications for its use for nutritional and therapeutic purposes.

Diabetic circumstance ends in hypo proteinemia that is, the lower in blood serum general proteins. Moreover, the consumption of amino acids or protein synthesis within the hepatocytes has been stated to be blocked because of the abnormal function of the liver (El-Shenawy & Nabi, 2006). According to the findings, all of the therapies investigated had a favourable effect on the concentration of protein level of the particular tissue in Diabetes Mellites.

Chitra *et al.* (2010) also discussed the anti-diabetic and free radical scavenging properties of Strychnosnuxvomica seed extract. In alloxan-induced diabetic rats, they found a significant rise in SOD, CAT, and total protein levels and a reduction in LPO, TC, blood serum creatinine, and blood urea nitrogen levels, demonstrating the extract's antioxidant properties.

In alloxan-induced diabetic rats, Rajaram (2013) examined the antioxidant and antidiabetic properties of the methanolic bark extract of Tectona grandis Linn and found that protein formation is reduced in major organs due to complete or virtual insulin hormone insufficiency. In albino rats made diabetic by alloxan induction, the methanolic bark extract of the plant Tectona grandis Linn. demonstrated substantial antioxidant and antidiabetic activity, and it could be employed in drug development. According to the findings, all of the therapies investigated had a favourable effect on tissue total protein concentration in Diabetes Mellites. Ethanolic extract of plant Butea monosperma(BMEE), at a dose of 600 mg, was found to be the most effective in restoring protein content in the liver, pancreas, and kidney. At the same time, metformin was also found to be the most effective in all three tissues.

The direct measurement of insulin action in the body is glycogen, a reconstituted form of glucose. Insulin induces the storage of carbohydrates in the form of glycogen in the hepatocytes and body muscles through the coordinated activity of two enzymes, glycogen phosphorylase and glycogen synthase (Taylor, 1996; 1993). The lack of glycogen synthetase activating systems (Annamal & Augusti, 1980) and improved glycogen phosphorylase activity (Abdel-moneim, 1997) reveal a malfunction in the insulin process. It resulted in a significant drop in insulin levels, which could be a primary cause of postprandial hyperglycaemia. The decrease in hepatic glycogen level after alloxan administration could be related to the alloxan-induced death of Langerhans beta cells (Wilson & Leiter, 1990). Sharma & Garg (2012) studied the liver glycogen content in Butea monosperma hydroethanolic bark extract. The level of liver carbohydrate(glycogen) was dramatically lowered after the administration of the diabetic drug alloxan at a dose of 150 mg kg-1 body weight. The liver carbohydrate amount increased significantly after 15 days of treatment with BMEE at doses of 200mg, 400mg, and 600mg kg-1 body weight. When the hepatic glycogen content of all the extracts was analysed independently, it was determined that the extract with a dosage of 600 mg kg-1 body weight was the most effective in improving the amount of liver carbohydrate. When all of the extract-treated groups were compared to the metformin-treated Standard Diabetic group, and hence was discovered that metformin proves the maximum effectiveness.

Conclusively, the use of a combination of the extract from the leaves of *Butea monosperma* and metformin with a great part of the extract and a reduced dose of metformin is quite additive and equally enriching the declined level of total tissue protein and hepatic glycogen content, for managing of diabetes mellites. So, there is a need for collaboration between the orthodox and herbal medical

practitioners to promote and increase the intake of leaves of such plants which are having a wide medicinal role in controlling diabetes mellites.

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