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Research

# Modulation of Carbohydrate Metabolic Enzymes by *Portulacaoleracea* Aqueous Extract in Alloxan Induced Diabetic Rats

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## ABSTRACT

Also known as "grape sugar"; Glucose (C6H12O6) is a simple sugar that forms a major constituent of carbohydrate and fatty foods. It is often given off as by-product of carbohydrate metabolism with defiant breakdown manifesting in clinical pathologies like diabetes mellitus (DM) and its related complications. Current study was designed to determine the effect of PotaricaOleracea extract on selected enzymes of carbohydrate metabolism in diabetic wistar rats. Twenty one (21) Wistar rats of between 150 – 200 g were assigned into three groups of seven (7) rats each. While group I received standard rat diets and water ad libitum (control), groups II and III were administered with 140 mg/kg body weight of Alloxan Monohydrate (DM induced) and Alloxan Monohydrate + P. oleracea aqueous extract (400 mg/kg body weight) respectively. After four (4) weeks of administration of test substances, rats were euthanized, and blood samples obtained (via cardiac puncture) for biochemical analysis. Liver was then harvested and homogenate was prepared for analysis of liver enzyme levels. After carefully subjecting obtained data to student t-test and one way analysis of variance (ANOVA); using the statistical package for social sciences (SPSS version 21). In the end, study observed a maintained, steady growth throughout a period of 14 days of experimentation, with a significant reduction in the growth of diabetic untreated rats.

Also, group II rats showed a statistically significant increase with maintained higher blood glucose level compared to control on the third, seventh and fourteenth days of experimentation, while group III had a had a significant decrease in blood glucose levels over the period of experiment, suggestive of a consistent destruction of the beta cells of the islet of langherans. Again, there was an insignificant change in Hexokinase and Fructose 1, 6, Bisphosphatase of group II, compared to group I rats, implicative that alloxan diabetes may increase the rate of glycolysis. Further studies aimed at corroborating efforts from this work is recommended.

**Keywords:**: Carbohydrates, Potaricaoleracea and Metabolic enzymes

## INTRODUCTION

Diabetes Mellitus (DM) is one of the world's dreadful, and top leading causes of death. It is a clinical manifestation of the body's defiant performance of carbohydrate metabolism, resulting in a rapid rise in plasma glucose levels (hyperglycemia) [1].

At least four polypeptides with regulatory activity are known to be secreted by the islets of Langerhans in the pancreas with respect to regulation of carbohydrate metabolism. Two of these are hormones insulin and glucagon, and reportedly have important functions in the regulation of the intermediary breakdown of carbohydrates, proteins, and fats [2]. The third polypeptide, somatostatin, plays a role in the regulation of islet cell secretions itself. A variety of other catalyst systems (enzymes) also have important roles to play in carbohydrate metabolism. For example, enzymes from the salivary glands attack carbohydrates (and fats in some species); enzymes from the

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stomach attack proteins and fats; and enzymes from the exocrine portion of the pancreas attack carbohydrates, proteins, lipids, DNA, and RNA. Other enzymes that complete the digestive process are found in the luminal membranes and the cytoplasm of the cells that line the small intestine. The action of the enzymes is aided by the hydrochloric acid secreted by the stomach and the bile secreted by the liver [3].

The principal product of carbohydrate digestion and the principal circulating sugar is glucose. The normal fasting level of plasma glucose in peripheral venous blood is 70 to 110 mg/dL (3.9–6.1 mmol/L). In arterial blood, the plasma glucose level is 15 to 30 mg/ dL higher than in venous blood. Once it enters the cells, glucose is normally phosphorylated to form glucose 6-phosphate at the very onset of glycolysis. The enzyme that catalyzes this reaction is hexokinase. In the liver, there is an additional enzyme called glucokinase, which has greater specificity for glucose and which, unlike hexokinase, is increased by insulin and decreased in starvation and diabetes [4].

Once complete, obtained pyruvate (bye product of glycolysis) is sent through the citric acid cycle (Krebs cycle, tricarboxylic acid cycle); which is a sequence of reactions in which acetyl-CoA is metabolized to CO2 and H atoms. Acetyl-CoA is first condensed with the anion of a four-carbon acid, oxaloacetate, to form citrate and HS-CoA. In a series of seven subsequent reactions, 2CO2 molecules are split off, regenerating oxaloacetate. Though efforts have been made to remedy the life threatening complications (in diabetics) that may emanate from any of the hormonal and/or enzyme systems that regulate carbohydrate metabolism [5].

Diabetes remains an incurable disorder of carbohydrate metabolism; with High cost and undesirable adverse effects associated with treatment drugs. This has supposedly promote the use of several herbs with minimal effect on hypoglycemic activities. In recent times, over 50% of such herbs now serve traditional medics in management and amelioration of numerous defiant carbohydrate metabolic ailments that may affect humans. One of such plants often alleged to be of great importance is Potaricaoleracea.

Portulacaoleracea (common purslane [1], also known as verdolaga, red root, or pursley) is an annual succulent plant of the Portulacaceae family. It may reach 40 cm (16 in) in height, with extensive distribution assumed to be mostly anthropogenic [3]. It extends from North Africa and Southern Europe through the Middle East and the Indian subcontinent to Malesia and Australasia. The species status in the Americas is uncertain, and is often considered an exotic weed with evidence of deposition in Crawford Lakes. Recently, Portulacaoleracea has been shown to be non-toxic to humans, with leaves containing such phytochemicals as; alkaloids, tannins, flavonoids, triperpenes, carotenoids, phenols, cellulose, sateroids, polyuronoids, b-galactoside and volatile oil. It is known to serve as anti-oxidant in some traditional foods due to its anti-oxidative properties and efficacious in the management of hyperglycaemia and several other related ailments of carbohydrate metabolism [6, 7]. Naiho (2012) in his study on the "effect of Portulacaoleracea on glucose tolerance" reported Portulacaoleracea to pose a hypoglycemic effect on diabetic wistar rats fed with its extract. Naiho further suggested that the extract possibly lowers blood sugar levels (hypoglycemic effect) by stimulating insulin secretion postprandial; making it a potential agent in the management of type II diabetes.

## Aim of Study

This study aimed at determining the effect of ofPortulacaoleracea aqueous extract on selected enzymes of carbohydrate metabolism, using Wistar rats as experimental model. Specifically, Study:

i. Accessed the effect(s) of Portulacaoleracea extract treatments on blood glucose levels.

ii. Examined the effect(s) of Portulacaoleracea extract treatments on body weights of alloxan induced diabetic wistar rats.

iii. Determined the effect(s) of Portulacaoleracea extract treatments on selected enzymes of glycolytic, citric acid and pentose phosphate pathways

iv. Determined the effect(s) of Portulacaoleracea extract on the liver's histo-architecture.

## Methodology

### Location of Study

The study was conducted in the Department of Human Physiology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State, Nigeria. Due to the sensitive and invasive nature of the study, Wistar rats were used as experimental model. The study investigated the possible effects and/or mechanism(s) of its action of Portulacaoleracea on selected enzymes of carbohydrate metabolism.

### Study Design

The research adopted an experimental type of research design

**Identification and Collection of Plants:** Portulacaoleracea plant was obtained from a wide growing habitat in Abraka, Delta State and authenticated by expert taxonomists from the Department of Animal and Environmental Biology, Delta State University, Abraka, Delta State.

**Preparation of Extract:** Extract was prepared using the method of Naiho A. O, (2012)

**Sample Collection:** After an overnight fast, rats were anaesthetized under chloroform vapour. Blood samples were obtained by cardiac puncture using 5ml syringe and 23G needle. Obtained blood samples were then centrifuged (at 3500rpm) for 10 minutes to obtain serum for biochemical analysis. Ten percent (10%) homogenates of the organs (Liver of each rat) was prepared by homogenizing appropriate weight of the organ in ice-cold phosphate buffer at pH 7.2. The homogenate was centrifuged at 4000g for 10 minutes and the supernatant obtained was used for biochemical analysis.

**Diabetes Induction:** Diabetes was induced by intraperitoneal injection of 140mg/kg body weight of alloxan monohydrate on rats after 24 hours fast. Diabetes was then confirmed after 72

hours of injection; and blood glucose≥ 150 mg/dL was accepted as diabetic.

**Experimental Grouping:** Twenty one (21) wistar rats of between 150 -200 g procured for the study. The animals were divided into three (3) groups of seven (7) rats each. Control group received standard rat diet and water ad libitum. Groups II and III received 60% fructose diet and water ad libitum. After 28 days, blood glucose levels were assayed to confirm hyperglyceamia. Group III received daily doses of aqueous extract of P. Oleracea of about 400mg/kg body weight using an endogastric tube for another two weeks.

**Laboratory Analysis:**Laboratory analysis was performed for selected enzymes of carbohydrate metabolism under stringent conditions and observation of laboratory rules. Total carbohydrate was estimated by the method of Carrol et al., (1956), serum lactate dehydrogenase activity was estimated by the method of Wroblewski et al., (1955). Pyruvate was estimated by the method of Friedemann and Haugen, (1943), while Phosphorylase activity was assayed by the method of Cori et al. (1955) in the direction of glycogen synthesis by estimating the inorganic phosphate formed from g|ucose-1- phosphate. Also, Glucose-6-phosphate dehydrogenase activity in tissue was estimated by the method of Kornberg and Horecker, 1955, Succinate dehydrogenase activity was estimated by the method of Nachlas et al. (1960), and Malate dehydrogenase activity was estimated by the method of Nachlas et al. (1960).

**Ethical Clearance:**Ethical clearance was obtained from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State. All animals were treated in line with guidelines, stipulated by the National Institute for Health Guide on the Care and Use of Laboratory Animals (1985).

**Statistical Analysis:** Statistical significance of treatment effect(s) was analyzed with the students' t-test, with values expressed as Mean ± SEM (Standard Error of Mean). All of these were automated and achieved with the Statistical Package For Social Sciences (SPSS) version 20. Differences between means were considered at $p < 0.05$.

## RESULTS

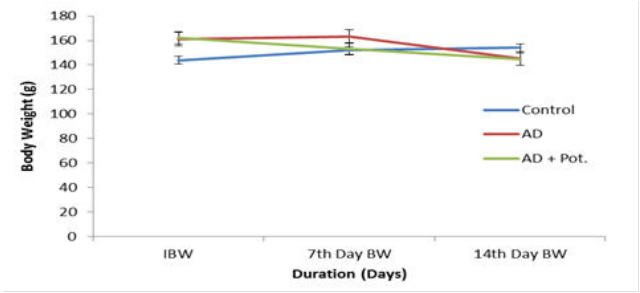



**Figure I:**Showing changes in Body Weights due to P. Oleracea Administration to Alloxan Diabetic Rats

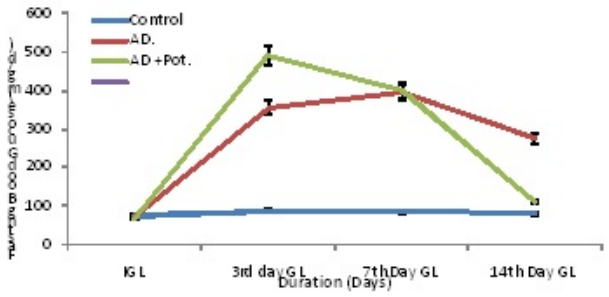
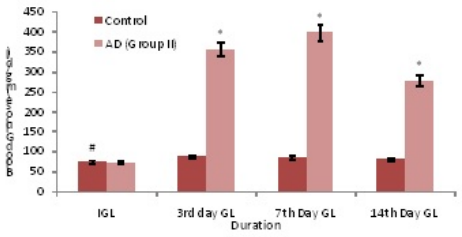
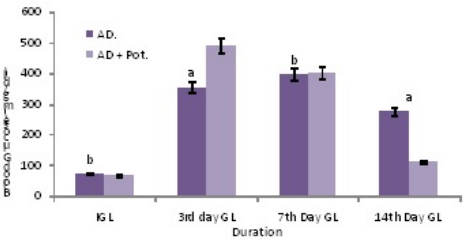



**Figure II:** Showing changes in Fasting Blood Glucose levels due to P. Oleracea Administration




*: Statistically significant increase (at $p < 0.05$) compare with control, #: Insignificant increase GL = Glucose Level. IGL = Initial Glucose Level

**Figure III:** Durational Changes in Blood Glucose Level of Alloxan Treated Rats




a: Statistically significant increase (at $p < 0.05$), b: Statistically insignificant decrease compared to AD group. GL = Glucose Level. IGL = Initial Glucose Level, AD = Alloxan Diabetic and Pot. = Portulacaoleracea treatment

**Figure IV:** Comparative Changes in Blood Glucose Levels of Portulacaoleracea Treated, Alloxan Diabetic Rats

## Discussion

This study was aimed at investigating the possible effects of Portulacaoleracea aqueous extract on selected enzymes of carbohydrate metabolism in Wistar rats with the view to explaining its hypoglycemic actions.

Table I of the result from this study showed a maintained, steady growth (figure I) throughout a period of 14 days of experimentation. There was a significant reduction in the growth of the diabetic untreated rats; which was consistent with the earlier report of Lanka et al., 2006. However, the diabetic treated rats also showed a reduction in weight over the priod. This observation was inconsistent with previous reports of Sabeha and Nahida (2018). A possible reason for this may be that most other researches experimented with ethanolic extract,

which is lipid soluble. This lipid solubility of the extract may contain more nutritious ingredients than the aqueous extract, thus accounting for the differences in findings as such. Zhou et al., (2015) had also reported that aqueous extract of P. oleracea caused a reduction in high fat diet induced oxidative stress; suggestive of its ability to improve lipid metabolism and reduce fat accumulation, hence, it is possible that this could be the reason for reduced weight.

Results from table II showed an insignificant change in blood glucose of normoglycemic rats during the 14 days period of experiment. As shown from the table, the alloxan diabetic group also had a statistically significant increase. This observation is consistent with diabetes induced by alloxan; which destroys the beta cells of the islet of langherans (Szkudelski, 2001). P. oleracea treated diabetic rats produced a statistically significant decrease in blood glucose levels over the period of experiment. This is in agreement with the earlier report of Naiho, (2012).

Figure II of the result presentation revealed that alloxan diabetic rats maintained a statistically significant higher blood glucose level compared to control on the third, seventh and fourteenth days of experimentation. For figure IV however, alloxan diabetic rats treated with Portulacaoleracea extract showed a statistically significant reduction in blood glucose levels when compared to diabetic untreated rats on the 14th day of experimentation.

Table IV of obtained result shows some carbohydrate metabolic enzymes assessed from the liver tissue. The table indicates a statistically significant increase in pyruvate kinase of alloxan diabetic rats compared to control. However, there was an insignificant change in Hexokinase and Fructose 1, 6, Bisphosphatase of alloxan Diabetic, compared to control group. This suggests that alloxan diabetes may increase the rate of glycolysis as the enzymes (pyruvate kinase) is one of the rate limiting enzymes of glycolytic pathway. The results of table V show a significant reduction in pyruvate kinase and hexokinase levels, but non-significant change in fructose 1, 6, Bisphosphate level. This suggests that the extract did not reverse the reduction rate in glycolytic pathway. Table IV shows a non-significant change in lactate dehydrogenase, malate dehydrogenase (MDH) but a significant decrease in succinate dehydrogenase (SDH) of Alloxan diabetes (AD) + Potulacaoleracea compared to Alloxan diabetic group. This is suggestive that alloxan induced diabetes reduced the rate of TCA (tricarboxylic acid) cycle. From table V, there was a significant increase in MDH and a significant decrease in SDH levels of AD + Potulacaoleracea group compared to AD group. It appeared that the hypoglycaemic effect of aqueous extract of Potulacaoleracea may not be mediated via upregulation of glycolytic and TCA pathways.

## CONCLUSION

This study has revealed that aqueous extract of Potulacaoleracea does not have the capacity to reverse weight loss in alloxan diabetic rats. The study also confirms the hypoglycaemic effect of the extract. The mechanism of this hypoglycaemic effect may not be explained by changes in glycolytic pathway or TCA cycle and the extract could not upregulate the enzymes of these pathways.

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