STUDIES ON WISTAR RATS TO ASCERTAIN ANTI-DIABETIC POTENTIAL OF KODO AND KUTKI MILLET

[1]Nidhi Kaushik*, [2]Komal Chauhan, [3]Manjeet Aggarwal, [4]Rakesh Kumar Khandal

[1]Research Scholar, [2]Head of Department, [3]Head of Department, [4]President (Research and Development)
[1]Department, Basic & Applied Science,
[1]National Institute of Food Technology Entrepreneurship and Management, Sonepat, Haryana-131028, India.

Abstract
The present in vivo study deals with assessing the anti-diabetic potential of two millet varieties: Kodo and Kutki on wistar rats induced with diabetes by injecting (45 mg/kg body weight of rat) a freshly prepared solution of streptozotocin. Five different groups of rats were chosen: I) Normal control; fed with normal basal diet, II) Diabetic Control, diabetic rats but fed with normal diet, III) diabetic rats medicated with the anti-diabetic medicine: glibenclamide (5 mg/kg body weight) and fed with basal diet, IV) diabetic rats, fed with diet based on Kodo and V) diabetic rats, fed with diet based on Kutki, The diabetic rats (group III) were fed with anti-diabetic medicine while rats of the group (IV) and (V) were fed with a diet based on Kodo and Kutki respectively for 60 days. Glucose levels of rats of groups (IV) and (V) registered significant reduction, while certain biochemical changes indicated that rats were getting to towards their normal health parameters ($P < 0.05$). Examination of the pancreas, liver, stomach, heart, brain, and kidneys revealed that the rats (group IV and V) were as good as those of group (III). Both Kodo and Kutki exhibited effects similar or as good as anti-diabetic medicine. Rats of the group (IV) and (V) were found to be even healthier than rats (group, III), on basal diet. Thus, Kodo and Kutki showed an anti-hyperglycemic potential to control diabetes.
Index terms: **anti-diabetic, *in vivo*, Kodo, Kutki, Nutraceutical, Streptozotocin**

I. INTRODUCTION

Kodo millet (*Paspalum scrobiculatum*) and Kutki millet (*Panicum sumatrense)*, are two staple crops cultivated in tribal areas of many countries including India. They are akin to rice; their grains are consumed as a wholesome food, while their plant material is used as a fodder. These crops are drought-resistant, grow well in semi-arid conditions and hence, they are known to be the most environment-friendly with the lowest water footprint. Grains of these millets are small-sized, with a horny seed coat that must be removed before cooking (Kiran et al., 2014). Both Kodo and Kutki are a rich source of various micronutrients, needed for the body, e.g. potassium, phosphorous, magnesium, iron, zinc, copper, and manganese (National Dairy Board, 2012; NIN, 2003), meeting the mineral requirements of humans. These millets are also known for their nutraceutical properties, in regulating chronic diseases like diabetes, cardiovascular diseases, arthritis, etc. (Kiran et al., 2014) because of the presence of various nutrients like amino acids, vitamins, antioxidants, etc. The most important feature of these millets is that they are gluten-free. They are also considered as functional foods, with antioxidant potential (Rao et. al., 2017) and the preferred option for those allergic to gluten. Ayurvedic texts like Charak Samhita and Sushruta Samhita, have described them to be useful in managing diabetes mellitus (Sachan Kumar, 2004). Based on the experience of eating these millets for time immemorial, there exists also a strong traditional knowledge about health benefits associated with them. For example, as per an adage in Karnataka, India: "Those who eat rice will grow as a lightweight; their body being just like that of a bird; those who eat Jowar will become as strong as a wolf but those who eat Ragi (millets) will be the strongest of them all, plus they would remain free from any illness: remain 'nirogi' in Sanskrit!"

As per Murty and Subramanyam, 1989, consumption of these millets is good for those who are allergic to gluten and also because of presence of constituents like, polyphenols etc. they are also known as immunity enhancer. It is a known fact that dietary polyphenols and phytates present in these millets can lower down digestibility of carbohydrates thereby enhancing the transit time and thus maintaining blood sugar level or have a low glycemic index (Thompson et al., 1987). The risk of diabetes can be reduced through appropriate diet and pharmacological arbitration (Irondi et al., 2016). The incidences of type 2 diabetes can be reduced by half among individuals by modifications in their lifestyle and by including millet-based diet regularly in their food (Li et al. 2008). Presence of complex carbohydrates, dietary fiber,

polyphenols, phytates, phytoconstituents, etc. are ascribed (Shobana et al., 2010) toa reduced risk of diabetes. Consumption of these millets, daily, may help improve: immunity, eliminate malnutrition and serve as a preventive measure for cardiovascular disease because they also help in maintaining proper levels of blood pressure, good cholesterol and triglycerides. For well-being, Kodo and Kutki must be part of a daily diet, it is evident from literature (Anusha et al., 2018).

Besides all these virtues, these millets are also known for their potential as crops for sustainable agriculture. The aim of this *in vivo* study on wistar rats is to assess the anti-diabetic potential of Kodo and Kutki millets. Rats induced with diabetes were fed with diet based on these millets and health parameters of rats were monitored and compared with diabetic rats treated with anti-diabetic medicine. Rats given basal diet were taken as "control" for the study. An attempt is made to find out if Kodo and Kutki exhibit any anti-diabetic potential?

II. MATERIALS AND METHODS

2.1 Preparation of feed:

The grains of Kodo and Kutki sourced from Krishi Vigyan Kendra, Dindori (Madhya Pradesh), India, were finely ground in a lab-scale flour mill and their flours were packed in airtight containers. Compositions (as per Table 1) of feed based on Kodo and Kutki were prepared. Standard basal diet, in form of pellets, was procured from Amrut Food Limited, Chandigarh, India.

Chemical Reagents:

Streptozotocin was sourced from (Sigma chemical Company St. Louis U.S.A), glibenclamide and other chemicals and reagents used in the study were of analytical grade.

2.2 Experimental Animals

Healthy adult Wistar male rats, weighing 150g to 180g were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, India. The study was conducted after getting due approval from Institutional Animal Ethics Committee (IAEC), formed by the University of NIFTEM, following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). During the experiments, animals were housed individually in experimental cages, maintained at temperatures of 22 ± 3ºC, relative humidity of 45-55% and subjected to light and dark cycles of 12 hours each. Animals of all the groups were acclimatized for 7 days, before the start of experimental studies. During the acclimatization period, the rats were fed with a standard basal diet and water *ad libitum*.

Table 1. Composition of Diets in grams given to rats of different groups

Component	Basal Diet given to Group I, II, III	Diet based on Kodo for diabetics given to Group IV	Diet based on Kutki for diabetics given to Group V
Casein	200	200	200
Mineral mix	90	90	90
Vitamin mix	10	10	10
Cellulose	50	50	50
Corn starch	600	-	-
Oil	50	50	50
Kodo	--	600	--
Kutki	--	--	600
Total	1000	1000	1000

2.3 Determination of Proximate composition of Kodo and Kutki flour

The results of proximate analysis of flours of Kodo and Kutki carried out as per standard method (Association of Official Analytical Chemists, 2005) are presented in Table 2.

2.4 Induction of Diabetes Mellitus in Wistar Rats

Rats were injected intraperitoneally only once first with a dose of nicotinamide (230 mg/Kg body weight of rat) as a protectant of beta cells of Islets of Langerhans of the pancreas, followed immediately by a dose of freshly prepared

STZ (45 mg/ kg of body weight of rat) solution in 0.1M citrate buffer (pH 4.5) in a volume of 1 ml/kg body weight (Siddique et al., 1987), for inducing diabetes. Normal control group was injected with 1 ml citrate buffer as a vehicle. To avoid drug-induced hypoglycemic mortality, rats induced with diabetes were fed with 5% glucose solution instead of water to drink overnight. The induction of diabetes through hyperglycemia was confirmed by testing for fasting blood glucose levels, using a glucometer, after 48 h of injection. Only those rats which showed blood glucose more than 200 mg/dl were considered diabetic and selected for the study. The diabetic rats were then divided into different groups as stated above for further experiment. Glycosylated hemoglobin (HbA1c), a biomarker for ascertaining stress hyperglycemia and diabetic hyperglycemia (Kundu et al. 2013), was used as the parameter to establish Type 2 Diabetes.

2.5 Experimental design

The rats were divided into five different groups of ten animals each. The rats were fed with the basal diet (3600C) for 60 days: Group I, as a Normal control, fed with a basal diet; Group II, diabetic control, fed with a basal diet; Group III diabetic rats were given anti-diabetic medicine, glibenclamide (1.25 mg/Kg of body weight) and fed with a basal diet; Group IV, diabetic, fed with a diet based on Kodo flour; Group V, diabetic fed with a diet based on Kutki flour. During the study, the fasting blood glucose, from rats' tail vein, was measured at different time intervals. The average food intake was recorded daily and the change in body weight of rats was monitored weekly throughout the study. At the end of the study, the rats fasted overnight and only water was provided before they were sacrificed. Samples of blood and liver were collected for biochemical assays and tissues of liver, brain, stomach, pancreas, kidneys, heart were evaluated for histo-pathological evaluation.

2.6 Biochemical assays

Samples of blood were withdrawn from the orbital sinus under the light ether anesthesia. The blood samples were collected into heparinized capillary tubes into two pre-chilled vials, one containing Potassium EDTA as anticoagulant (1 to 2 mg/ml of blood) and the other vial without any addition. Vials containing samples of blood were properly mixed, to avoid clot formation. The blood samples collected within the Heparinised tubes were centrifuged at 800 rpm for 10 minutes to separate the plasma. All the animals were sacrificed after that, by cervical dislocation. The liver was taken out immediately after sacrifice, and perfused with ice-cold isotonic saline and weighed. A small portion of the tissue of the liver was minced for enzyme activity assay and other biochemical evaluations, coded, and stored under refrigerated conditions till further analysis.

Following biochemical parameters were studied: glucose, lipid, and an enzymatic assay of blood and hepatic tissue. Fasting blood glucose (FBG) levels were measured using a portable glucometer and the percentage change in FBG levels during the treatment period was calculated. Serum glucose was measured by the O-toluidine method (Sasaki et al., 1972). Insulin level was assayed by Enzyme-Linked Immunosorbent Assay (ELISA) kit (Anderson et al., 1993). Estimation of Hb1Ac was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan (1985). Free fatty acids (FFA) (Lowry et al., 1976), phospholipids (Chen et al., 1956), total cholesterol (TC), triglycerides (TG), and HDL-cholesterol were estimated by using diagnostic kits (Span Diagnostic). VLDL and LDL-cholesterol were calculated as per Friedewald's equation (Friedwald et al., 1972). Estimation of serum protein (Lowry et al., 1951) was done colorimetrically by using bromocresol green. Determination of serum glutamate pyruvate transaminases (SGPT) and serum glutamate oxaloacetate transaminases (SGOT) were done by spectrophotometer, following the method as described by Reitman and Frankel, 1957. For measurement of serum alkaline phosphatase (ALP), method of King and Armstrong (1934) was used.

For determination of enzyme activity assay of hepatic tissue, 0.8-1.0g of tissue was minced in its 10 times volume of 0.2M/L Tris HCl of pH 8 containing 0.5M/L $CaCl_2$ using Potter Elevehjem apparatus at 0-4^{o}C using motor-driven Teflon pestle rotated at 3000 rpm and homogenized. The homogenate was centrifuged at 10000x g for 30 minutes at4^{o}C and 3/4th of the volume was carefully drawn using Pasteur's pipette. Hepatic antioxidant enzyme assay evaluated; glutathione peroxidase (GSHPx) (EC 1.11.1.9) (Necheles et al., 1968); catalase (CAT) (EC 1.11.1.6) (Luck et al., 1971) and superoxide dismutase (SOD) (EC 1.15.1.1) (Kono et al., 1971). The liver supernatant was extracted and used for the estimation of liver glycogen (Montgomery et al., 1957) and an assay of Hexokinase (Brandstrup, 1957), Fructose-1,6-bisphosphatase (Baginsky, 1974), Glucose-6- phosphatase (Gancedo et al., 1971).

2.7 Histopathological studies

Immediately after sacrificing, liver, kidneys, pancreas, brain and stomach was preserved in a 10% aqueous (neutral buffer of pH 7) solution of formalin. These tissues were trimmed and routinely processed. Tissue processing was done

to dehydrate in ascending grades of alcohol, cleared in xylene and then embedded in paraffin wax, using standard method for the purpose. Paraffin wax-embedded tissue blocks were made into sections of 3-4 µm thickness with the Rotary Microtome. All the slides were stained with Hematoxylin & Eosin (H & E) stain. The prepared slides were examined under a microscope for histopathological lesions, if any. Severity of the observed lesions were recorded as NAD=No Abnormality detected, 1= Minimal (<1%), 2= Mild (1-25%), 3= Moderate (26-50%), 4= Moderately Severe (51-75%) and 5= Severe (76-100%) and distribution was recorded as focal, multifocal and diffused.

2.8 Statistical analysis

Results were expressed as mean ± SEM of 10 rats. Statistical analysis of results was evaluated by ANOVA; one way and Student's-'t test. The values with $p \leq 0.05$ were considered statistically significant.

III. RESULTS AND DISCUSSION

This study, undertaken to evaluate the antidiabetic potential of Kodo and Kutki on STZ-induced diabetic rats, has revealed that all the animals in the various groups survived for the entire length of study without any mortality. The results of the study have demonstrated, not only the anti-diabetic effect of Kodo and Kutki, but they also provided significant insight into various health-related factors due to diet based on these millets.

3.1 Proximate Analysis:

From the results of proximate analysis of Kodo and Kutki presented in Table 2, it may be noted that both these millets are high in carbohydrates: Kodo (~67% by weight) and Kutki (~62% by weight) and low in proteins: (~ 9.1% and 8.3% by weight for Kodo and Kutki respectively). The content of carbohydrates in both the millets is lower than that of wheat (~71%) and rice (~76%). The protein content is also lower in both the millets in comparison to wheat (11.6% by weight) but it is higher than that of rice (~7.9 by weight). The ash content of Kodo is ~ 3.1% by weight and of Kutki it is ~ 5.4% by weight. The ash content of both the millets is much higher than that of wheat (~1.6%) and rice (~1.3%). This suggests that both the millets are a rich source of minerals in comparison to wheat and rice. The other important notable aspect of proximate analysis pertains to the fiber content; both Kodo (~5.4% by weight) and Kutki (~7.3% by weight) contain a significantly higher content of fiber than wheat (~2% by weight) and rice (~1% by weight). This comparison is made only to highlight the differences between these two varieties of millets and other food grains consumed as a staple food.

Table 2: Proximate Composition of Kodo and Kutki

Nutrients	Kodo	Kutki
Moisture %	10.9	10.7
Protein %	9.1	8.3
Fat %	3.4	5.2
Ash %	3.1	5.4
Fiber %	5.4	7.3
Total Carbohydrate %	67.1	61.7
Calcium (mg/g)	39.42	39.70
Iron (mg/100g)	14.7	1.56
Phenolic compounds	40.42	42.51

All the values are mean of triplicate readings.

3.2 Food Intake and Body Weight

The results of the average food intake of the rats are presented in Table 3. As evident from the data, the rats of the normal control group consumed the diet as much as a normal rat does for its growth. But the relative food consumption is significantly high in the case of a diabetic control group, indicating that the metabolic system after the induction of diabetes had got disordered. The changes in the metabolic system of rats can be understood also from the observations of the feed-intake in the case of diabetic rats treated with an anti-diabetic medicine. The relative food consumption increased from 5.8 g in the case of a diabetic group to 3.6 g in the case of rats treated with medicine showing that the metabolism was reversing from being disordered to be in order. The rats fed with a diet based on Kodo and Kutki reverted to the consumption levels similar to that of the normal group. These results clearly show that

Kodo and Kutki behaved as well as an anti-diabetic medicine. Based on these findings, it can be said that both Kodo and Kutki have the characteristics of a functional food with nutraceutical effects.

Table 3: Average Intake of Food by Rats of Different Groups

Group	Classification	Average Food Consumption (g/100 g body weight)	Remarks
Group I	Normal Control	3.1± 0.46	Normal Growth
Group II	Diabetic Control	5.8±0.42[a]	Disturbed metabolism
Group III	Diabetic+ Glibenclamide	3.6±0.88[b]	Reverting to Normal
Group IV	Diabetic+Kodo	3.7±0.81[b]	Reversing metabolism
Group V	Diabetic+Kutki	3.9 ±0.77[b]	Reversing metabolism

Groups II are compared with Group I; Groups III, IV, and V are compared with Group II

[a] $p \leq 0.05$: Significantly different from Normal Control (I)

[b] $p \leq 0.05$: Significantly different DiabeticControl (II)

NS: Non-Significant ($p \geq 0.05$)

The changes in average body weights (g) of the rats are presented in Table 4. It may be seen that the normal control group registered an increase in body weight by ~40%. This much gain in body weight throughout the study matched well with the normal growth of rats. This shows that the rats were maintaining the desired growth rate as far as the bodyweight is concerned. The Diabetic control group on the other hand suffered a decrease in body weight to the extent of ~13%. The loss in body weight, in spite of the fact that average feed intake of diabetic rats was much higher than the normal control provides a lot of insight to the metabolic changes taking place on induction of diabetes. In other words, this validated the assumption about the disorderliness of the metabolic system in diabetic rats. In the case of diabetic rats treated with anti-diabetic medicine, the weight gain was found to be ~ 22%. This showed that the medicine worked well in bringing metabolism in order. What is encouraging is the observation about the weight gain of the diabetic rats fed with Kodo and Kutki to the extent of ~ 20% over the period of study. This confirms the anti-diabetic effect of Kodo and Kutki. Looking at the content of nutrients in both these millets, it can be said that the presence of considerable content of vitamins, minerals, and anti-oxidants must have played their role in this.

Table 4.Average Body Weight (g) of Rats

Group	Classification	Initial weight	Final weight	Weight gain(+)/loss(-) (%)
Group I	Normal Control	165.40±3.06	232.80±3.19	40.74
Group II	Diabetic Control	165.00±2.26	142.30±1.95	-13.74[a]
Group III	Diabetic+ Glibenclamide	163.30±2.26	200.40±1.35	22.71[b]
Group IV	Diabetic+ Kodo	165.10±2.85	199.30±2.75	20.71[b]
Group V	Diabetic+ Kutki	163.90±2.42	196.20±2.78	19.70[b]

Groups II are compared with Group I; Groups III, IV, and V are compared with Group II

[a] $p \leq 0.05$: Significantly different from Normal Control (I)

[b] $p \leq 0.05$: Significantly different DiabeticControl (II)

NS: Non-Significant ($p \geq 0.05$)

The diets designed by incorporating Kodo and Kutki fed to diabetic rats to check if Kodo and Kutki could help in curing the animals from diabetes after the same was induced. By feeding the rats with basal diet, the rats were maintained to diabetes by raising sugar levels in the blood leading to a condition of hyperinsulinemia. The subsequent injection of STZ, which is a β- cell toxin as also a well-known diabetogenic substance, showed a harmful effect on functional β- cells in the pancreas (Lenzen, 2008 and Marianna et al. 2006), thereby leading to a large-scale reduction

in the release of insulin. As a result, the deficiency of insulin caused high glucose levels in the blood (Grover et al. 2002) and other metabolic aberrations associated with T2D (Kaur et al. 2011).

Thus, rats induced with diabetes exhibited hyperglycemia and insulin resistance, akin to the natural history and metabolic characteristics of T2 diabetes in humans (Srinivasan et al. 2005). The results (Table 5) of biochemical parameters of diabetic rats corroborate all the above-reported findings in the literature. The sugar levels of diabetic control were 358 on day zero and the same increased to 372 by the end of the study, indicating that the tendency of hyperglycemia did not decline throughout the study period; rats remained diabetic till the end of the study. There was an increase in sugar levels from 358 on day zero to 372 on day 60. Sugar levels in diabetic control rats treated with medicine on the other hand registered a steady decrease from the day the medicine was started. The sugar level after seven days came down from 362 to 140.2. The values of sugar levels come down further to levels of 110 in 30 days and after that maintained at that level throughout studies as evident from the results of 60th day which was 118.6. The decrease in sugar levels of diabetic rats (group III) was attributed to the anti-hyperglycemic activities (of the drug): increase in sensitivity of insulin, leading to uptake of peripheral glucose, and thus oxidation of fatty acids and production of glucose by liver and hence absorption of glucose also gets affected from the digestive tract (Collier et al. 2006). This explained the mechanism by why the anti-diabetic medicine was effective in controlling the sugar levels, observed here. Now, looking at the data of sugar levels of diabetic rats fed with a diet based on Kodo and Kutki, it may be said that both Kodo and Kutki brought about an effect similar to what was rendered by the anti-diabetic medicine. However, the effect of diet was gradual rather than instant like in the case of medicine. The values of sugar levels registered a regular and gradual decrease with time; on day 7^{th}, the sugar levels came down from 357.4 on day zero to 234.6 in the case of Kodo and from 356.6 on day zero to 236. On day 30^{th}, the values came down further to 165.2 in the case of Kodo and 166.8 in the case of Kutki. On the 60^{th} day, in the case of Kodo, it was 130.8 and 131.2 in the case of Kutki. Thus, for both Kodo and Kutki, the rats were found to have almost come out of diabetes. This shows that diets do play a role in controlling diabetes and an extraordinary virtue i.e. anti-diabetic potential of Kodo and Kutki was established and the same must be exploited.

Table 5. Fasting Blood Sugar Levels (mg/dl) of rats throughout the study

Group	Classification	Day 0	Day 7	Day 30	Day 60	% Change
Group I	Normal Control	89.9±5.22	91.7±4.97	91.4±5.46	91.2±4.67	1.45 (Increase)
Group II	Diabetic Control	358±7.84	353.6±8.29	369.2±7.40	372.6±7.73	4.08 (Increase)[a]
Group III	Diabetic+ Glibenclamide	362±20.29	207.2±16.42	182.2±9.09	145.8±8.17	59.72 (Decrease)[b]
Group IV	Diabetic control+Kodo	353.2±18.45	234.6±9.42	165.2±6.22	141.2±7.89	60.02 (Decrease)[b]
Group V	Diabetic control+Kutki	356.6±15.52	223±9.38	163.8±5.93	145.0±6.82	59.33 (Decrease)[b]

Groups II are compared with Group I; Groups III, IV, and V are compared with Group II

[a] $p \leq 0.05$: Significantly different from Normal Control (I)

[b] $p \leq 0.05$: Significantly different DiabeticControl (II)

NS: Non-Significant ($p \geq 0.05$)

It is desired at this juncture to refer to the studies already reported, in the literature, with similar objectives as this study, to provide reasons for such effects of Kodo and Kutki; why and how they behave as hyper-glycemic? Irondi et al. 2014 reported similar results on diabetic rats and ascribed them to the presence of phenolic compounds in the diet. The anti-diabetic activity of flavonoids, polyphenols, nutrients, etc. could be explained through several mechanisms including the inhibition of carbohydrate-hydrolyzing enzymes (Iwai et al. 2006). The increase in blood sugar levels in diabetic rats was accompanied by a reduction in their hepatic glycogen concentration, relative to the normal control group, which is not possible under normal metabolic conditions due to the presence of insulin. Under normal conditions, insulin enhanced the intracellular glycogen deposition by stimulating activities of glycogen synthase and inhibiting glycogen phosphorylase (Shivanna et al. 2013). The functioning of insulin got impeded in the case of hypoinsulinemic conditions caused by the injection of STZ. The induction of diabetes, therefore, was manifested by depletion of hepatic glycogen levels, as reported by Ahmed et al. (2010). The notable aspect of the present study is that the damaging effect of STZ was found to have got reversed when the diabetic rats were fed with diets based on

Kodo and Kutki. It may be inferred, therefore, that Kodo and Kutki caused an increase in the release of insulin, which may be by the generation of glycogen synthase (Selvan et al. 2008). It is almost like getting back to the normal metabolic order. Thus, the hyperglycemic conditions of diabetic rats fed with a diet based on Kodo and Kutki, as observed in this study may be attributed to the presence of high contents of flavonoids and phenolic compounds in both these millets.

The results of parameters such as insulin, glycosylated hemoglobin (Hb1Ac), hemoglobin, and glycogen are presented in Table 6. It may be noted that the insulin content in diabetic rats (~ 4.35 g/dl) was found to be significantly lower than that of the normal control (~ 19.6 g/dl). In the case of diabetic rats fed with a diet based on both Kodo and Kutki, the insulin levels almost matched with those of the normal control. This again validates the assumption that both these millets have the potential to reverse the malady of diabetes. In the case of diabetic rats treated with the anti-diabetic medicine, the insulin level was found to be more than the diabetic control but lower than that of the normal control as well as those of rats fed with millet-based diet. Results of hemoglobin and Hb1Ac, provide a clear picture of the metabolic condition of rats of different groups. In the case of diabetic rats, a reduction in the value of hemoglobin can be ascribed to the increase in Hb1Ac. In all other cases, HbA1C values are not much different from the normal control. Reduction of glycogen content in diabetic rats also indicates that the metabolic system after induction of diabetes got disturbed. The effect of Kodo and Kutki in bringing normalcy of metabolism is evident from these results.

Table 6: Results of parameters: Insulin, Glycosylated Haemoglobin (Hb1Ac), Haemoglobin, Glycogen.
(Values are mean ± SEM of 10 rats in each group)

Group	Classification	Insulin (g/dl)	HbA_1C (%)	Haemoglobin (g/dl)	Glycogen (mg/g wet tissue)
Group I	Normal Control	19.6±0.22	3.5±0.42	13.6±0.23	46.7±0.21
Group II	Diabetic Control	4.35±0.24[a]	12.9±0.49[a]	8.4±0.34[a]	22.5±4.40[a]
Group III	Diabetic+ Glibenclamide	14.5±0.29[b]	5.10±0.18[b]	12.0±0.5[b]	34.5±2.09[b]
Group IV	Diabetic Control+Kodo	20.7±0.45[b]	5.37±0.24[b]	11.5±0.57[b]	42.2±6.22[b]
Group V	Diabetic Control+Kutki	19.2±0.52[b]	6.01±0.48[b]	10.9±0.42[b]	39.8±5.90[b]

Groups II are compared with Group I; Groups III, IV and V are compared with Group II

[a] $p \leq 0.05$: Significantly different from Normal Control (I)

[b] $p \leq 0.05$: Significantly different DiabeticControl (II)

NS : Non Significant ($p \geq 0.05$)

To explain why and how millets could bring such changes in rats, it would be worthwhile to look at studies reported in literature on similar aspects. A study (Ojewole 2005; Bhowmik et al. 2009) demonstrated anti-hyperglycemic effect of extracts of different barks when they were fed to diabetic rats. Whether the same is the case in the present study is the question that can be answered from the observations of Hb1Ac values. Higher values (12.9%) of HbA1c observed in diabetic rats (diabetic control, Group II) than those (3.5%) in the normal control (group I) may be attributed to the enhanced rate of glycosylation of hemoglobin under T2 diabetic conditions induced by administration of STZ. Similar results were reported by Bernadette et al. 2008. The HbA1C values of diabetic rats (group III) treated with anti-diabetic medicine were found to be lower than the diabetic control (group II) which is an obvious observation. The mechanism of action explained in the case of effect of medicine on sugar levels in blood is applicable here as well. Reduction in HbA1c levels in diabetic rats (group IV and V) fed with diet based on Kodo and Kutki, compared with diabetic control (group II) validated the anti-hyperglycemic effect of Kodo and Kutki. The diabetic groups were found to have a significantly higher level of HbA1c than the normal control group. But compared with the diabetic control group, diabetic groups fed with a diet based on Kodo and Kutki registered significantly lower HbA1c levels; indicating that the Kodo and Kutki inhibited the process of glycosylation of hemoglobin which occurred due to the hyperglycemic effect brought about on injecting STZ. The HbA1c level of diabetic rats fed with a diet based on Kodo and Kutki was comparable with that of the normal control group. What is worth remarkable here is that the sugar levels in diabetic rats fed with a diet based on Kodo and Kutki were found to be lower than even in diabetic rats treated with glibenclamide. Determination of Hb1Ac is self-monitoring of blood

glucose, therefore, plays important complementary roles for the management of diabetes mellitus (Maruthupandian et al., 2011).

3.3 Lipid Profile

The effects of a diet based on Kodo and Kutki on the lipid profile: total cholesterol, triglycerides, HDL, LDL-C, and VLDL of the rats are presented in Table 7. In comparison to the normal control group, the diabetic rats had a significant increase in their plasma total cholesterol, triglycerides, LDL, and VLDL with a concomitant significant decrease in their plasma HDL concentration. However, the diabetic rats fed with a diet based on Kodo and Kutki were found to have significantly lower levels of plasma, total cholesterol, triglycerides, LDL, and VLDL while showing a significant increase in HDL. The diet based on both the Kodo and Kutki was found highly effective in improving the lipid profile of the diabetic rats; even better than the case where the rats were treated with glibenclamide. A similar trend was observed in hepatic lipid profile, indicating hyperlipidemia in STZ induced diabetes. Kodo and Kutki enriched diets restored the levels (Table7). Raised lipid profiles are usually seen in diabetics and such elevation represents a risk factor for coronary heart diseases (Mironova et al., 2000). The hypolipidemic effect may be due to inhibition of fatty acid synthesis (Chi et al., 1982) enhanced excretion, or lowered absorption of cholesterol (Vinson et al., 1998). Insulin activates the enzyme lipoprotein lipase and hydrolyses triglycerides in normal metabolism while the deficiency of it results in their inactivation and thus leading to hypertriglyceridemic condition. The significant reduction of serum lipid levels in diabetic rats after feeding Kodo and Kutki in diet may be directly attributed to improvements in insulin levels. Results are as per a previous study conducted by Maruthupandian and Mohan, 2011.

3.3.1 Lipid Profile of Serum and Hepatic Tissues

The results of parameters of lipid profile of serum and hepatic tissues are presented in Table 7. From the results of parameters such as: total cholesterol, HDL, LDL, VLDL, triglycerides, it is evident that the diabetic control (group II) registered higher values of all these parameters except HDL compared with the diabetic rats (group III) treated with anti-diabetic medicine as well as the diabetic rats (group IV and V) fed with diet based on Kodo and Kutki. As stated above, under the normal metabolic conditions, release of insulin is sufficient enough to be able to modulate lipid metabolism by activating lipoprotein lipase to hydrolyze triglycerides to form fatty acids and glycerol. The fatty acids that are produced from hydrolysis of triglycerides get either oxidized, to be used as energy for growth etc., or they get re-esterified and get stored, as a fat, in body tissues. But in the case of T2 diabetes, mainly because of insulin resistance and/or insulin deficiency, levels of total cholesterol as well as LDL raised causing dyslipidemia (Chahil and Ginsberg 2006). Furthermore, in the case of diabetes due to abnormalities in lipid metabolism cause of the release of free fatty acid from insulin-resistant fat cells and hence lipid profile get affected (Chehade et al. 2013). Contrary to this, the lipoprotein lipase gets inactivated due to deficiency of insulin and/or resistance, resulting in a condition of hyper-triglyceridemia as there was the changed plasma lipid profile (elevated triglycerides, total cholesterol, LDL and VLDL; and reduced HDL) of the diabetic rats in this study are in agreement with the alterations in lipid profiles of diabetic rats reported by other researchers (Zhang et al. 2010). It is a well-known fact that LDL helps in transporting cholesterol from the liver to body tissues (Pedersen, 2001). Thus, increased LDL values caused deposition of cholesterol in the arteries and aorta and that is why, such a condition is considered as a precursor of CVD, in diabetic patients (Ormazabal et al.,2018).On the other hand, HDL, is known as a transporter of endogenous cholesterol from other parts of body tissues to the liver and on the other hand for their metabolism and excretion. Thus, HDL lipoprotein is known for a chraracteristic of preventing cholesterol deposition in the arteries, thereby preventing plaque formation or atherosclerosis (Xu et al. 2005). The observed decrease in triglyceride, total cholesterol, LDL, and VLDL; and a concomitant increase in the HDL levels of diabetic rats fed with diet based on Kodo and Kutki is the direct indication of modification or improvement in metabolism because body system is regaining its normal functioning because of improvement in insulin secretion by the pancreas.

Table7: Results of Lipid Profile of, Serum and Hepatic Tissue, of Rats (Values are mean ± SEM of 10 rats in each group)

Group	FFA (mg/100g)	TG (mg/100g)	TC (mg/100g)	PL (mg/100g)	FFA (mg/100ml)	TG (mg/100ml)	PL(mg/100ml)	TC (mg/dl)	LDL–C (mg/dl)	VLDL–C (mg/dl)	HDL-C (mg/dl)
	Hepatic				Blood/Serum						
Group I	0.73 ± 0.012	428.4 ± 1.05	363.2 ± 1.58	1829.7 ± 0.62	11.7 ± 0.19	85.3 ± 2.50	142.2 ± 4.08	95.0 ± 2.40	23.2 ± 3.97	17.1 ± 0.50	54.7 ± 2.67
Group II	0.94 ± 0.00[a]	524.8 ± 1.83[a]	47.2 ± 1.54[a]	2234.0 ± 0.84[a]	21.1 ± 0.68[a]	178.3 ± 4.22[a]	165.8 ± 3.19[a]	168.4 ± 2.63[a]	103.5 ± 3.30[a]	35.7 ± 0.84[a]	29.2 .± 3.01[a]
Group III	0.80 ± 0.003[b]	487.5 ± 2.46[b]	395.3 ± 1.37[b]	2012.2 ± 1.6[b]	14.5 ± 0.35[b]	95.4 ± 4.20[b]	147.2 ± 5.01[b]	99.5 ± 2.72[b]	29.5 ± 6.73[b]	19.1 ± 0.84[b]	50.9 ± 5.17[b]
Group IV	0.64 ± 0.002[b]	431.7 ± 2.65[b]	374.5 ± 2.11[b]	1880.5 ± 0.80[b]	12.9 ± 0.63[b]	88.0 ± 3.02[b]	144.3 ± 3.30[b]	101.5 ± 4.77[b]	30.5 ± 7.18[b]	17.6 ± 0.60[b]	53.4 ± 3.27[b]
Group V	0.71 ± 0.007[b]	443.2 ± 2.43[b]	381.1 ± 1.35[b]	1910.2 ± 1.97[b]	15.6 ± 0.36[b]	90.5 ± 3.66[b]	146.1 ± 2.92[b]	106.1 ± 2.77[b]	37.2 ± 4.11[b]	18.1 ± 0.73[b]	50.8 ± 3.65[b]

FFA: Free Fatty Acids, TG: Triglycerides, TC: Total Cholesterol, PL: Phospholipids
Groups II are compared with Group I; Groups III, IV and V are compared with Group II

[a] $p \leq 0.05$: Significantly different from Normal Control (I)

[b] $p \leq 0.05$: Significantly different Diabetic Control (II)

NS : Non Significant ($p \geq 0.05$)

From the results of various studies presented above, it may be stated that diet can play a vital role in controlling diabetes, etc. (Oboh et al. 2010). For example, studies reported on the consumption of polyphenol-rich diets had demonstrated the anti-diabetic effects (Arts and Hollman, 2005). The proximate analysis of Kodo and Kutki presented in Table 2, show that both these varieties of millets are rich in polyphenols, nutrients, minerals, and fiber. Because of the presence of high content of nutrients etc., both of these millet varieties are considered to be superior to other commonly consumed cereals like wheat and rice (Chandra Prabha and Selvi, 2016).

Liver function Profile and Protein content in serum are presented in Table 8. It may be noted that the values of AST and ALP are consistently also, significantly, higher in the diabetic group than in the normal control group. In comparison to the diabetic control group, there was a significant decrease in the activities of these three enzymes when the diabetic rats were fed with a diet based on Kodo and Kutki. Based on these observations, it may be said that the presence of Kodo and Kutki in the diet was responsible for minimizing the level of hepatocellular damage caused by the injection of a dose of STZ. In comparison to the normal control group, a significant reduction in total protein in the blood of the diabetic groups was observed. But with the intake of a diet based on Kodo and Kutki, a significant increase in total protein levels in plasma was recorded, in comparison to the diabetic control group.

A significant reduction of protein content, in serum, of rats, were observed in STZ induced diabetic rats (Group II) when compared to control (Group I) and glibenclamide-treated rats (Group III). This could be ascribed, probably to the presence of an insufficient amount of insulin which led to an increase in degradation of protein alongside the decrease in synthesis protein in the liver. A feeding diet based on both Kodo and Kutki might have restored the levels of insulin because of which the protein in serum reverted to near normal (Table 8).

Results of activity of the hepatic marker enzymes in serum revealed that the levels of SGPT and SGOT were elevated in Group II. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of STZ. Kodo and Kutki regulated the activity of SGPT and SGOT in the liver of rats intoxicated with STZ. The effect of glibenclamide on the recovery of hepatic enzyme activity in serum was very similar to that of the earlier study (Preethi et al., 2009). The restorations of SGPT and SGOT to the irrespective normal levels after treatment with both glibenclamide and Kodo and Kutki further strengthen its anti-diabetogenic effects. Moreover, SGPT and SGOT levels also act as indicators of liver function and restoration of normal levels of these parameters indicate normal functioning of the liver. Since STZ can also affect the liver by a free radical mechanism. In addition to the assessment of SGPT and SGOT levels during diabetes, the assessment of enzymatic activities of phosphatases like alkaline phosphatase (ALP) is very much required clinically because any changes in their levels is a sign indicating about damage of tissues (Singh et

al., 2001). Serum ALP increased considerably ($p<0.05$) in diabetic rats, which may be, due to extensive damage to the liver by STZ. Treatment with glibenclamide and feeding diet based on Kodo and Kutki recorded a significant ($p<0.05$) decline in ALP levels.

The reduced plasma protein levels of the diabetic rats, in comparison with that of the normal control rats, could be attributed to decreased protein synthesis coupled with increased muscle proteolysis (Gray and Cooper 2011). Insulin regulates protein metabolism by stimulating protein synthesis and retarding protein degradation (Murray et al. 2000). Thus, diabetic hypoinsulinemia results in decreased protein synthesis in all tissues due to decreased production of alkaline phosphatase in an absolute or relative deficiency of insulin (Murugan and Pari 2007). The decreased plasma protein was, however, restored toward normal in diabetic rats fed with a diet based on Kodo and Kutki, probably due to the diet which could improve the secretion, sensitivity, and action of insulin in the diabetic rats. The overall antidiabetic activity of the Kodo and Kutki may be attributed to its polyphenols, including the flavonoids and phenolic acids, as earlier reported (Irondi et al. 2014). In addition to modulating carbohydrate metabolism by inhibiting α- glucosidase and α-amylase, the polyphenols are known to exhibit anti-diabetic effect through other mechanisms such as reduction in intestinal absorption of dietary carbohydrate; improvement of β-cell function and insulin action; stimulation of insulin secretion; and anti-oxidative and anti-inflammatory properties (Cabrera et al. 2006; Iwai et al. 2006). All these explanations seem true for both the millets which are rich in polyphenols, flavonoids anti-oxidants, etc.

Table 8. Results of Liver Function markers and Protein content in serum of Rats.

Group	Classification	ALT/SGPT (U/L)	AST/SGOT (U/L)	ALP (U/L)	Protein (g/dl)
Group	Normal Control	15.2±2.20	20.8±0.79	155.5±4.38	7.2±0.30
Group I	Diabetic Control	35.40±2.41[a]	32.9±2.33[a]	271.0±5.42[a]	4.9±0.40[a]
Group II	Diabetic+Glibenclamide	16.80±2.39[b]	17.6±2.22[b]	165.9±4.75[b]	7.4±0.22[b]
Group III	Diabetic control+Kodo	18.0±1.83[b]	21.0±2.05[b]	186.9±4.41[b]	7.4±0.42[b]
Group IV	Diabetic control+Kutki	20.9±2.08[b]	24.1±2.73[b]	191.90±2.88[b]	6.7±0.44[b]

Groups II are compared with Group I; Groups III, IV and V are compared with Group II

[a] $p \leq 0.05$: Significantly different from Normal Control (I)

[b] $p \leq 0.05$: Significantly different Diabetic Control (II)

NS : Non Significant ($p \geq 0.05$)

The Results of enzymatic activity are presented in Table 9. In comparison to normal control, the values of SOD, CAT, and GPx were found to be much lower values. The diabetic rats when treated with anti-diabetic medicine were found to register much higher values than diabetic control. The results of the value of these three enzymes in the case of diabetic rats fed with Kodo as well as Kutki were found to be encouraging. It is well-established fact that CAT, SOD, and GSHPx are very significant enzymes in protecting and quenching free radicals formed in various tissues (Oberly et al., 1974). This clearly shows that Kodo and Kutki when fed to diabetic rats could reverse the reduction in the values of all antioxidant enzymes due to induction of diabetes. It clearly shows that the fact of feeding diabetic rats with Kodo and Kutki is more pronounced and beneficial than that of anti-diabetic medicine.

Table 9. Results of Hepatic Antioxidant Enzymatic Activity of Rats (Values are mean ± SEM of 10 rats in each group)

Group	**Classification**	**Glutathione peroxidase (g of GSH utilized/min/mg protein)**	**Catalase (values x 10^{-3} units/mg protein)**	**Superoxide dismutase (units/mg protein)**
Group I	Normal Control	7.4±0.45	66.4±0.91	2.9±0.27
Group II	Diabetic Control	5.2±0.42[a]	33.6±1.30[a]	1.1.±0.24[a]
Group	Diabetic	6.32±0.28[b]	52.5±0.43[b]	2.2±0.21[b]

III	Control+Glibenclamide			
Group IV	Diabetic control+Kodo	8.0±0.32[b]	69.5±0.87[b]	3.3±0.16[b]
Group V	Diabetic control+Kutki	8.7±0.36[b]	62.2±0.71[b]	3.0±0.23[b]

Groups IIare compared with Group I; Groups III, IV and Vare compared with Group II

[a]p ≤ 0.05 : Significantly different from Normal Control (I)

[b]p ≤ 0.05 : Significantly different DiabeticControl (II)

NS : Non Significant (p ≥0.05)

The results of the effect of feeding Kodo and Kutki to diabetic rats could be seen by a change in the activities of hepatic enzymes hexokinase, glucose 6-phosphatase, and fructose-1, 6- bisphosphatase of experimental animals are presented in Table 10. It may be noted here that in the case of diabetic control the values of Hexokinase were found to be significantly lower and fructose- 1, 6- bisphosphatase and glucose 6-phosphatase were higher than normal control. This explains the effect of STZ on all three enzymes and the results are in order as far as the damaging action of STZ is concerned. As reported earlier (Jayanthi et al., 2010) injection of STZ reduces the hexokinase while it increases the fructose- 1, 6-bisphosphatase, and glucose 6-phosphatase activity. The results of these three enzymes for diabetic rats fed with Kodo and Kutki were on a similar line as recorded for diabetic rats. There was an increase in the hexokinase enzyme activity on feeding on a diet based on Kodo and Kutki showing the values much higher than the diabetic control and somewhat closer to normal control. The values of hexokinase for diabetic rats fed with Kodo and Kutki were found to be higher than that of diabetic control treated with the anti-diabetic medicine. This again shows that the diabetic rats were experiencing metabolic transformations from being diabetic to being normal. Thanks to the antidiabetic effect of Kodo and Kutki. Similar observations were made in the case of fructose- 1, 6- bisphosphatase, and glucose 6-phosphatase enzymes. Here again, the values obtained for diabetic rats fed with Kodo and Kutki were found to be as good as that of Normal control.

Table10: Results of Enzymatic Activities of Hexokinase, Fructose 1, 6–bis-phosphatase, Glucose 6–Phosphatase. Hepatic Tissues of Rats (Values are mean ± SEM of 10 rats in each group)

Group	Classification	Hexokinase (ì moles of glucose - 6 – phosphate formed/h/ mg protein)	Fructose 1 , 6 – bisphosphatase (nmoles of phosphorous liberated/h/ mg protein)	Glucose 6– phosphatase (n moles of phosphorous liberated/h/mg protein)
Group I	Normal Control	186.1±6.51	489.2±4.61	1041.2± 9.64
Group II	Diabetic Control	130.9±1.46[a]	770.1±6.5[a]	1244.5 ± 2.17[a]
Group III	Diabetic Control+ Glibenclamide	165.2±5.20[b]	576.3 ±2.57[b]	1120.8±4.84[b]
Group IV	Diabetic control+ Kodo	176.1±4.65[b]	514.3±4.17[b]	1095.5±3.11[b]
Group V	Diabetic control+ Kutki	170.3±8.23[b]	552.6±1.14[b]	1161.5 ± 4.46[b]

Groups II are compared with Group I; Groups III, IV and V are compared with Group II

[a]p ≤ 0.05 : Significantly different from Normal Control (I)

[b]p ≤ 0.05 : Significantly different Diabetic Control (II)

NS : Non Significant (p ≥0.05)

The results of the histo-pathological evaluation are presented in Table 11 and Figure 1. As evident from the observations recorded for various groups under study, the following notable points are worth considering:

In the case of diabetic control rats, there were prominent changes in the case of liver, kidney, pancreas, and heart in comparison to normal control. This clearly explains the biochemical changes in parameters as well as the values of

various enzymes observed for the diabetic control group. The detrimental effect of injection of STZ, due to which various health parameters showed deterioration, can easily be explained from the histo-pathological observation of key organs of the body.

The diabetic rats when treated with the anti-diabetic drug were found to minimize the damaging effect of STZ. It was evident from the improvement seen in histo-pathological evaluation of liver, pancreas, and Kidney due to anti-diabetic drug.

In the case of Kodo and Kutki based diet fed to the diabetic rats, the damaging effect due to diabetes seemed eliminated. The histo-pathological conditions of key organs of the rats were found to be as good as the Normal control. Thanks to a diet based on Kodo. The condition is much better than even the rats treated with the anti-diabetic drug. The histopathological evaluation has helped in providing evidence for the changes in various health parameters of the rats. Hence, the antidiabetic potential of Kodo and Kutki is evident from the results.

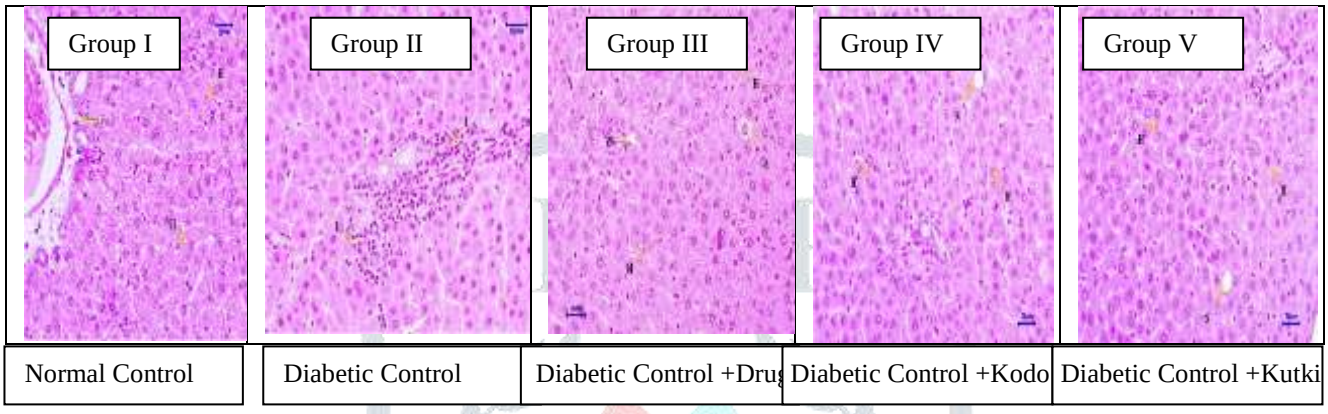

Figure 1: Photo-Micrograph (400 X) of Histopathological Slides of Rats of different groups

Table 11: Histopathological Observations on Slide of Rats

Groups	Organs					
	Liver	**Kidney**	**Pancreas**	**Heart**	**Stomach**	**Brain**
Normal Control	No abnormality found	No abnormality found	No abnormality found	No abnormality found	No abnormality found	No abnormality found
Diabetic Control	focal mild hepatic steatosis and focal to multifocal mild perivascular lymphocytic infiltration in liver	focal mild to multifocal moderate tubular dilation and multifocal mild to moderate lymphocytic infiltration at tubules	focal mild adipose tissue deposition at acini and multifocal mild to moderate atrophy of Islet of Langerhans	focal mild peri-aortic lymphocytic infiltration in heart	No abnormality found	No abnormality found
Diabetic Control+ Kodo	focal mild hepatic steatosis and multifocal mild perivascular lymphocytic infiltration in liver	focal mild tubular dilation and focal to multifocal mild lymphocytic infiltration at tubules	focal minimal to mild cytoplasmic vacuolation at Islet of Langerhans and focal minimal to mild atrophy of Islet of Langerhans	No abnormality found	No abnormality found	No abnormality found
Diabetic Control+ Kutki	focal mild hepatic steatosis and focal mild perivascular lymphocytic infiltration in liver	focal mild tubular dilation and focal mild to moderate lymphocytic infiltration at tubules	focal minimal to mild cytoplasmic vacuolation at Islet of Langerhans and focal minimal atrophy of Islet of Langerhans	No abnormality found	No abnormality found	No abnormality found
Diabetic Control+ Glibanclamide	focal mild hepatic steatosis	multifocal mild lymphocytic infiltration at tubules in kidneys	focal minimal cytoplasmic vacuolation at Islet of Langerhans and focal minimal atrophy of Islet of Langerhans	No abnormality found	No abnormality found	No abnormality found

IV. CONCLUSION

The study was conducted to ascertain the anti-diabetic effect on diabetes induced wistar rats. The results revealed that feeding of Kodo and Kutki based diets could change in the levels of fasting blood glucose, hepatic glycogen, Hb1Ac, lipid profile, and the liver function biomarkers of diabetic rats. Thus, based on these results it may be said that incorporating diet-based on Kodo and Kutki, one could help in the management of diabetes or if taken regularly in daily meal it could help in preventing the degenerative disorders like diabetes. The future research area could be to identify the pathways responsible for controlling sugar level and to elucidate a proper mechanism to explain the role played by Kodo and Kutki need to be investigated. Even though sufficient evidences of anti-diabetic potential of Kodo and Kutki are

presented here, for understanding the exact mechanism by which Kodo and Kutki could reverse the diabetic effect of STZ, detailed study would have to be conducted to understand the role that Kodo and Kutki played on beta cells or any other metabolic system at the cellular as well as molecular level. It must be mentioned here that Kodo and Kutki or for that matter any other variety of millets are known to bring such health benefits. But for those who are looking for more scientific evidences to further substantiate the traditional wisdom, about these grains, there exists a vast field of research. It may be worthwhile to mention here that there are certain regions in Rajasthan, India where not a single case of diabetes is reported, even though all over India and in fact in other parts of Rajasthan diabetes is prevalent. This is often ascribed to the eating habits of the population in those regions where millets are the staple diet throughout the year and for the whole life. Such experiences of daily life would help validate the findings of the present study; diets do play a role in treating diabetes.

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VI. Declaration of Interest

None.

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