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Original article

In-vivo hyperglycemic, antioxidant, histopathological changes, and simultaneous measurement of kaempferol verified by high-performance thin layer chromatography of *Setaria italica* in streptozotocin -induced diabetic rats

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ABSTRACT

Background: *Setaria italica* (common name- foxtail, kangni) is one of the major food crops which is prominently cultivated in southern regions of India and in certain regions of Uttar Pradesh. Besides the crop's consumption as a general source of carbohydrate rich cereal, the seeds of the crop are comprised of more fiber. So, it is recommended to add in the dietary supplementation of the diabetic people across the country.

Objective: In this paper, it intends to investigate the antidiabetic activity and antioxidant activity of *S. italica (foxtail millet)* seeds in diabetic rats.

Methods: The six genotypes of foxtail millets (*S. italica)* namely Kangni-1, Kangni-4, Kangni-5, Kangni-6, Kangni-7 & Kangni-10 respectively were subjected to in vitro investigations via. comprehensive metabolic panel (CMP) involving blood glucose study, Kidney & Liver function test, and antioxidant study (*Catalase test; Glutathione S-transferase (GST); Superoxide Dismutase (SOD); glutathione (GSH); hiobarbituric acid reactive substances (TBARS) & Glutathione peroxidase (GPx)* and were performed in vivo animal investigations in Wistar rats. The STZ induced diabetic rats were fed with doses of different S. italica seed aqueous extract to evaluate its anti-hyperglycemic activity by oral administration of SISAE. Further, it was compared with Glibenclamide which acts as one of the standard oral hypoglycemic agents.

Results: From achieved outcomes, a significant fall of blood glucose level (70%) produced 300 mg SISAE/kg b.w. after 6 h of extract administration. However, no change could be produced by these doses of the SISAE in normal rats' blood glucose levels. A significant fall in glucose level along with significant glycemic control by lower HbA1c levels was observed in diabetic treated rats after 3 weeks of treatment with 300 mg of SISAE/kg b.w./day when comparing to untreated diabetic rats. Among these five genotypes of *S. italica*, the differences in the glycemic index were found. a significant fall could be found in blood glucose levels of Wistar rats, when every experimental rat was incorporating with the extract of different genotypes of *Setaria italica L. Beauv* than the rats treated with Glibenclamide in every 7 days of interval. The level of catalase, SOD, GST, GPx, GSH and TBARS showed variation while the rats were fed with the extract

Abbreviations: SI, *Setaria italica*; K-1, Kangni-1; K-4, Kangni-4; K-5, Kangni-5; K-6, Kangni-6; K-7, Kangni-7; K-10, Kangni-10; A.U., Astronomical unit; D.M., Diabetes mellitus; HbA1c, Hemoglobin A1 C test; S.T.Z., Streptozotocin; B.W., Body weight; I.P., Inter peritoneal; IDDM, Insulin dependent diabetes mellites; NIDDM, Non-insulin dependent diabetes mellites; H.P.T.L.C., High-performance thin-layer chromatography; Rf, Retention factor; SOD, Supper oxide dismutase; GST, Glutathione S, transferases; GPx, Glutathione Peroxidase; GSH, Glutathione; TBARS, Thiobarbituric acid reactive substances.

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of *S. italica* in the liver test of rats. In kidney function test, the result shows that there is significant relationship between foxtail extract and kidney function of STZ induced diabetes rats. They show the change in their serum creatinine level, serum urea and serum uric acid.

Conclusion: The result obtained from the study shows that the extract of *S. italica* seeds is capable for the hypolipidemic and antihyperglycemic activities, thereby, they serve as one of the good sources for herbal medicinal items.

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1. Introduction

Globally, type 2 diabetes (T2D) attributes as one of the common diabetic types which is accounting for about 90% of diabetes prevalence. The metabolic disease is characterized via hyperglycemia followed by abnormal carbohydrate metabolism that developed insulin resistance & dysfunctionalities in pancreatic β-cell (DeFronzo & Tripathy, 2009). As a result of long-term exposure of higher level of glycemic levels implicated in resultant overdevelopment of ROS. With buildup in ROS-induced oxidative stress turns out to be one key reason that triggers the diabetes progression resulting in cellular damage, and dysregulation of genetic expression attributing to further impairment of insulin secretion & disruption of insulin signaling pathways drastically (Gallagher et al., 2010). Even though hyperlipidemia plays a pivotal role, hyperglycemia is considered as one of the main factors in the onset of diabetes. Upon considering some of the major organs that responsible for blood glucose and its regulation, pancreas, liver and also the influence of kidney, skeletal muscle, intestine and adipose tissue poses a significant role (Migdal & Serres, 2011).

The clinical presentation of diabetes involves with hyperglycemia, hypertriglyceridemia & hypercholesterolemia, which collectively contribute to the impaired secretory functioning in insulin alongside with reduced tissue sensitivity of the insulin, which subsequently results in insulin resistance and/or combination of both. Characteristically, diabetes results in serious endocrine syndrome followed by poor metabolic control, thereby poses to be a key responsible factor for increased likelihood of acquiring the risks of cardiovascular diseases namely, atherosclerosis, diabetic cataract or blindness, renal failure, (Kang et al., 2020).

From the clinical standpoint, the overall diabetic prevalence has skyrocketed in recent times which has forced the global health community to search for alternative plan involving with the development of natural remedies as well as dietary interventions that could serve as a safer alternative for synthetic drugs for diabetic management. With growing evidences of utilizing phytochemicals like- polyphenols rendered from cereal grains as well as from other medicinal plants tend to provide a potential therapeutic benefit like alleviating the overall complications that arises from diabetes & obesity complications, and also counteracts with the inhibitory effects against α-glucosidase & α-amylase (Pradeep & Sreerama, 2017). Dietary intervention involving millet-based diet has gained popularity in recent times. Millets in general, presents a collective term to refer several of the cereal-based small-seeded edible grasses that belong to the Poaceae family. It can be observed that the cultivation of these millet-based food crops is highly in the regions of semiarid and arid & semiarid all over the world (Chandrasekara et al., 2010).

When considering the nutritional values of millets, the food crop is well-known for its considerable level of health benefits when consumed as multigrain & also as a gluten-free cereals (Shahidi & Chandrasekara, 2013) Apart from their nutritional benefits, millets comprise of numerous phytochemicals and endowed highly with phenolic compounds that aids in controlling the diabetes, cardiovascular complications and cancer (Chandrasekara & Shahidi, 2011). Due to the presence of synergistic and additive effects of many compounds in the grains, the beneficial health outcomes can be observed. Therefore, identifying the health benefits of the grains is very significant. Yet, the phytochemical & nutrient compositions of cereals are affected by many of the factors namely, climatic & environmental conditions, genotype and soil (Upadhyaya et al., 2011).

One among six small-grained cereals is *Setaria italica*, which is cultivated highly in Asia as a food crop. In US and Europe, these millets were cultivated as animal feed. The millets have panicles like as the fox's tail that is, the appearance of panicle is long with erect, soft & long hairs, thus the name of this millet was evolved. The main foxtail millet growing countries are Japan, China and India. It is believed that in the Central region of China, foxtail millet has been first domesticated. In general, foxtail millet is the most significant underutilized small millet which is grown across the country. This millet is mainly grown under well agro-climatic conditions. Foxtail millet is a good source of dietary fiber & ß carotene. Globally, in the total millets production, foxtail millet has been ranked second. In Asia and Southern European, it is the primary staple food for millions of people. Mostly, foxtail millets are self-pollinated averaging of about 4% with cross pollination. It is known for its drought to nee, tolerance to diseases & pest and appropriate nutrient deficient soils.

Whilst, It is reported that the presence of health-functional properties and phytochemicals present in the millet are involved in reducing the insulin resistance and maintain the blood glucose level. Also, if we consume it in everyday diets, the risk of developing chronic diseases such as type 2-diabetes and cholesterol metabolism is reduced. A dose-dependent decrease was exhibited by foxtail millet on diabetic rats' fasting blood glucose up to the body weight of about 300 mg kg-1. At 41%, the fasting blood glucose level stayed approximately constant. Mainly, the foxtail millets' health benefits are attributed to phytochemicals, minerals, vitamins, antioxidants & other bioactive compounds in the millet make this millet as one of the promising functional foods. Potentially, this foxtail millet can be used for preparing many different foods with low-glycemic index which then lead to a higher development in the control of cholesterol and diabetes for a longer period. It is important for any of the intervention studies with foxtail millet for establishing its impacts on hyperlipidemia and hyperglycemia. Its medicinal uses involving with appetizer, digestive, astringent, emollient & diuretic. However, the plants' hypolipidemic and anti- hyperglycemic activities have not been systemically determined so far. Therefore, the current investigation involves with a detailed overview on hyperglycemic & antioxidant activity of *S. italica* extract in STZ induced diabetic rats.

2. Materials & methods

2.1. Collection of plant material

Firstly, the collection involved *S. italica* dry seeds were done based on genotypes selection in year of 2018 with 50 genotypes

and 2019 with 10, accession were collected from the I.C.R.I.S.A.T. and N.B.P.G.R., New Delhi, during Kharif –2018 and grown in the genetics and plant breeding research experimental field of S.H.U.A.T.S. The following genotypes identified by the Directorate of research S.H.U.A.T.S., Prayagraj, namely, Kangni-1, Kangni-4, Kangni-5, Kangni-6, Kangni-7 & Kangni-10 were selected after 2 year 2018 & 2019 field experiment based on qualitative and quantitative parameters (Singh D. et al. 2021) further, the collected seeds were powdered, and the powdered seeds were used to extract the antidiabetic active principle(s) with the use of a protease while sulfur dioxide wet-milling process. In Uttar Pradesh, the seed of Foxtail millet was made into flour (Philips HL1632, India).

2.2. Preparation of aqueous extract

Foxtail millet's aqueous extract was prepared and further seed grains were soaked in alkaline solution(w/v) of about 0.25% in 1:5 ratio at 4 °C for 24 h. Then, the soaked seeds were washed with distilled water, grained with a blender & finally filtered with 70 mesh sieves (Paraginski et al, 2019). The slurry was further centrifuged & resuspension was done in 0.25 % alkali solution (w/v) in 1:5 ratio at 4 °C for 24 h. It is repeated for 4x times. It was again washed with distilled water till it would reach its pH 7.0 and also drying it for 24 h at 45 °C. after incubation, starch was collected. Therefore, obtained pellet was then dried at 50 °C for 24 h. using mortar and pestle, the dried starch was ground well and then it was undergone to the process of 70 s mesh sieve for 100 times fine sample and packed in airtight container for further use showing in Fig. 1.

2.3. Antidiabetic activity in vivo model

2.3.1. Experimental animals

The Antidiabetic activity was determined via employing in vivo model protocol. From IAEC (Institutional Animal Ethical Committee), researcher undertook prior approval for animals & the necessary care for the laboratory. For the study, the young male Wistar healthy rats of 7–8 weeks old, weighing approximately in ranges between 150 and 200 g were acquired from inbred animal house of CDRI, Lucknow. The animal house was well ventilating and maintained at 27°c with relative humidity, maintaining an optimal 12 h dark and 12 h light cycle. The rats selected for the study were kept in hygienic polypropylene or stainless-steel cages in standard environmental conditions. In fact, animals were acclimatized for 15 days before the experiment to laboratory conditions. Doses were selected based on acute oral toxicity test (AOTT) as per Organization for Economic co-operation and Development (OECD) guideline 423 LD_{50} dose determination was carried out in experimental animals. Experimental sample were kept under ventilated animal house. During and even before the experiments, every group of rats were nourished with water, laboratory pellet diet & libitum.

2.3.2. ***Experimental design –*** *(Seed Extracts of foxtail millet)*

The 54- Wistar albino rats will be classified into nine groups. Every group has six animals. Through the gavage, drugs will be administrated orally as showcased.

Group I: Normal control

Group II: Diabetic treated with Glibenclamide

Group III: Diabetic / Positive control (STZ)

Group IV: This diabetic control group was treated with seed Extracts of K1

Group V: This diabetic control group was treated with Extracts of K4

Group VI: In this group, they treated with seed Extracts of K5

Group VII: Here, they were treated with seed Extracts of K6

Group VIII: In this group, diabetic control rats were treated with seed Extracts of K7

Group IX: Diabetic control group were treated with seed Extracts of *K*10

2.3.3. Experimental induction of diabetes

By using the freshly prepared HFD (High fat dight) and STZ (35 mg/kg body weight) in Na-citrate buffer (0.1 M) with the 4.5 pH, diabetes induced in the experimental animals to overnight fasted rats. HFD made By adding fat commonest in the pellets. The control group will be injected with citrate buffer alone while other groups will be injecting with streptozotocin. The induction of diabetes will confirm by estimating the blood glucose level after 72hrs of streptozotocin injection. Those rats with 200 mg/dl of blood glucose level were used for further diabetic studies (Sireesha *et al.*, 2011)

2.3.4. Assessment of body weight

Using weighing machine, the body weight of all the experimental rats will be measured. From the start of the study to the end, the

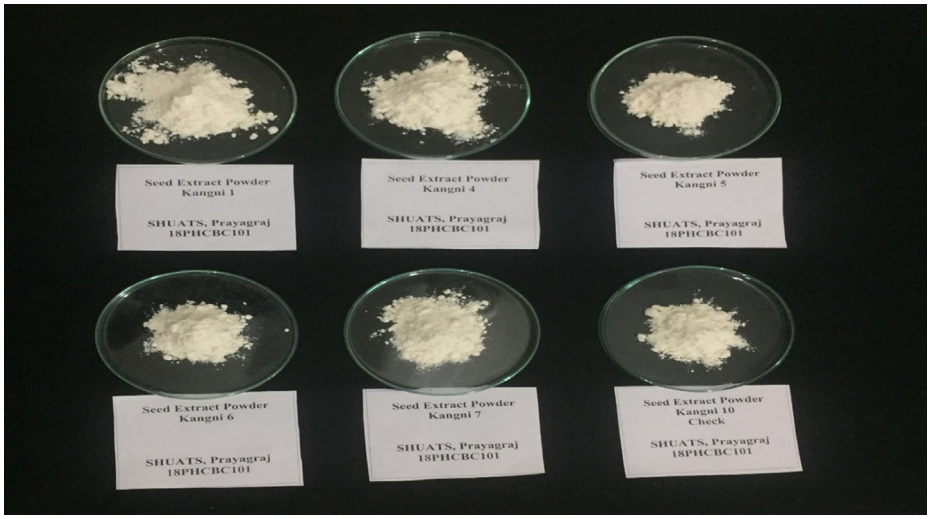


Fig. 1. Aqueous extract after isolation of starch Seed Extract powder of *Setaria italica.*

weight of the animals was monitored regularly at 7 days interval (1st, 7th, 14th, and 21st day). (Sireesha *et al.,* 2011)

2.3.5. Blood sugar level

The collection of blood samples was done by using "the *retro*-orbital venous plexus with heparinized capillary tubes" in the experimental animals after making the experimental animals fast for 12–16 h. The millet based diet lowered HbA1c (19.14%), fasting glucose (13.5 %), insulin (1.9%) concentrations Using One-touch glucometer (LifeScan, Inc., CA), the blood glucose level was estimated. Subsequently, the glucose levels were monitored for every seven days (1–21 days) and also oral glucose tolerance test was carried out (Sireesha et al., 2011).

2.4. Biochemical analysis and histopathological analysis

2.4.1. Biochemical analysis

Through via intraorbital sinus, blood retrieved for biochemical analysis were collected and preparation of serum was carried out by the process of centrifugation (Sigma-Aldrich, USA) for 10 min under 4000 rpm. At − 80 °C, the serum sample were kept prior to biochemical analysis.

2.4.2. Determination of in vivo antioxidant parameters

The dissection of liver and kidney of liver was done and they were washed with ice cold saline and kept it at the temperature of about at 40 °C (Mishra *et al.,* 2011).

2.4.3. Measurement of Superoxide dismutase activity (SOD)

As per Murugan *et al.,* (2006) method, SOD was reported. The mixture of chloroform & ethanol was shaken well for a minute at the temperature of 40 °C and centrifuged the mixture. After centrifugation, it is observed that the supernatant has enzyme activity. The mixture of assay is made of sodium pyrophosphate buffer of 186 mM phenazine methosulfate (PMS), 780 mM nicotinamide adenine dinucleotide (NADH) & 30 mM nitro blue tetrazolium (NBT); diluted enzyme preparation & water in a total volume. The reaction was stopped by adding the NADH. In a water bath, the mixture was incubated at 370 °C. Also, the reaction was stopped by adding glacial acetic acid (17.4 M). Then, the appearance of violet color was observed. The solution was then extracted in an n-butanol reagent blank at 560 nm, the activity was performed, and the result obtained was denoted as SOD activity/mg protein. To inhibit the production of chromogen, the enzyme's concentration required by 50% in conditions of defined assay which is termed as one unit of enzyme activity.

2.4.4. Measurement of catalase activity

In the presence of catalase, the degradation of hydrogen peroxide (H_2O_2) was followed at 240 nm. In order to prepare a total volume of 3 mL, 17 A 50 mM sample & buffered substrate (pH 7.0 containing 10 mM H_2O_2, 50 mM phosphate buffer) were mixed and also there was a fall observed in the absorbance at the temperature of 370°. By utilizing extinction coefficient of H_2O_2 (0.041/ μmole.cm^2) at 240 nm, the activity was calculated. The results obtained for catalase activity (CAT) are denoted as catalase activity/mg protein (Murugan et al, 2006).

2.4.5. Determination of reduced glutathione (GSH)

In 0.1 mM EDTA, the homogenates of kidney & liver were assorted with 5% trichloroacetic acid. Every sample underwent for the process of centrifugation. Further, 0.1 M phos. buffer (at pH 8) & its supernatant were mixed. The color appearance could be observed by adding 0.01% DTNB. Spectrophotometer recorded its absorbance (412 nm) (Murugan et al., 2006).

2.4.6. Determination of GPx

Sample was mixed with 10 mM sodium azide, H_2O_2 and reduced glutathione, 0.8 mM EDTA; enzyme (homogenates of kidney & liver) & phosphate buffer. The sample is kept for incubation. The rxn were arrested via adding 10% TCA & was centrifuged. the supernatant of sodium hydrogen phosphate & DTNB were mixed which showed the colour & was absorbed at 412 nm in UV–visible spectrophotometer. Graded conc. of the std. was similarly treated. millimoles per milligram (mmol/mg) is the unit by which Glutathione peroxidase activity in the liver homogenate are denoted (Murugan et al., 2006). Using the method of Rotruck et al., (1973), Glutathione-S-transferase (GST) was estimated. By using the method of Ohkawa et al., (1979), thiobarbituric acid reactive substances (TBRAS) were estimated.

2.4.7. Determination of liver function

alanine aminotransferase (ALT; SGPT), Alkaline phosphatase (ALP), Bilirubin (direct & indirect method), Aspartate aminotransferase (AST; SGOT), globulin, bilirubin (BIL), albumin (ALB) & total protein (TP) were measured with the help of ALP Flex® Reagent Cartridge (Siemens Healthcare Diagnostics Inc., Newark, USA), ALTI Flex® Reagent Cartridge, AST Flex® Reagent Cartridge, ALB Flex® Reagent Cartridge, TP Flex® Reagent Cartridge & BIL Flex® Reagent Cartridge respectively. As per the instruction of the manufacturer, all experiments were performed.

2.4.8. Determination of kidney function

By using CREA Flex® Reagent Cartridge, URCA Flex® Reagent Cartridge (Siemens Healthcare Diagnostics Inc., Newark, USA) and BUN Flex® Reagent Cartridge, serum urea, Creatinine (CREA) & uric acid (URCA) were estimated correspondingly. According to the manufacturer's instruction, all experiments were performed.

2.4.9. Histopathological studies

Using cervical dislocation, the control as well as treated rats were sacrificed and the collection of entire tissue specimens were collected from the right lobe of the pancreas, liver & right kidney that were fixed in buffered formalin (10%) and further processed to embed in paraffin wax with the help of routine protocols and microtome cut 5 μm-thick sections. The routine protocol helps to stain the sections with haematoxylin/eosin dye and an Olympus BX50 photomicroscope at 45X helps to examine the stained section. Using light microscopy, the pathological findings of examination were recorded (Attalla et.al., 2010).

2.4.10. H.P.T.L.C. of total extract

Sample was mix in CH_3OH and utilized for the H.P.T.L.C. estimation. The plates were developed in methanol: ethyl acetate: formic acid: toluene in 0.4:6:1.8:6 proportion. The cover was practical under colorimetry beam after derivatizing with observed the developed plate under U.V. radiation at 254 nm, visible light. The plate was then scanned densitometric at various wavelengths viz.254 the profile was recorded also the Rf values and the R.P (relative percentage) of the area in every peak in drug extract applied were calculated. Choice of the solvent system depends on the feature of the main compounds to be split. The system was selected based on the trial-and-error method allotropic series. Test solution of dissolved S.I. drug ratio of 10 mg per ml in methanol was used for H.P.T.L.C. Qualitative and quantitative estimation of Kaempferol, quercetin was considered to be the in-situ evaluation of U. V. absorbance, U.V.- visible and fluorescence quenching directly. The scanner covers the spots into a chromatogram which consists of the peaks parallel in appearance to that H.P.T.L.C. chromatogram. On the order chart, the portion of the scanned image is in relation to the spot's retention factor value on the layer & the peak area/height was in relation to the substance concentra-

tion on the spot. Densitometer scanning was done by using the CAMAG HPTLC scanner (Nagendran, 2013).

2.5. Statistics

With the help of SPSS version 26.0 software (SPSS Inc., Chicago, IL, USA), the data rendered from the corresponding tests were analyzed. In addition, the collected data were denoted as mean ± standard deviation (SD). By comparing the importance of control as well as experimental groups, Student's *t*-test will analyze different data statistically. As a significant difference could be observed between different experimental groups in the analysis statistically, *P*-value of 0.05 was considered.

3. Results

Foxtail millet has moderate glycemic index and low starch digestibility. In this study, diabetes was first induced in the young male Wistar rats in 150 and 200 kg weight by using streptozotocin. The main symptom of the diabetes disease is water intake. In diabetic group, there could be seen that water consumption was increased. Incorporation of extract of foxtail millet in dietary sup-

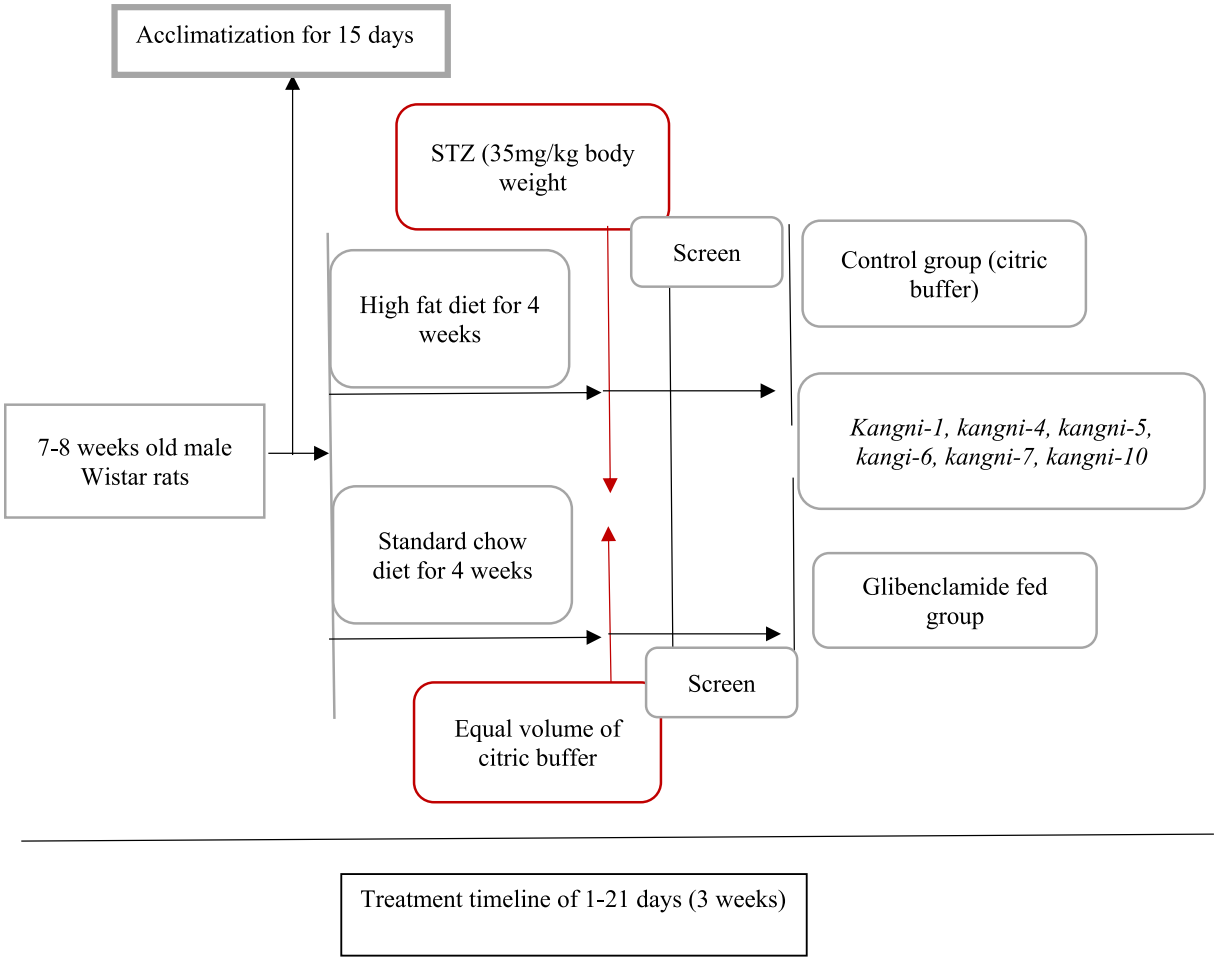


Fig. 2. Experimental design.

Table 1
Effects of Foxtail millet seed extract (*Setaria italica L. Beauv.*) on blood glucose levels in streptozotocin – induced diabetic rats (Means ± SD).

Treatment (group)	0 day (mg/dL)	7th day (mg/dL)	14th day (mg/dL)	21st day (mg/dL)
Normal control	80 ± 10.17	79.6 ± 10.23	97.16 ± 10.68	79.16 ± 10.68
Diabetic control	238.52 ± 61.32	227.15 ± 17.12	220.32 ± 16.40	216.50 ± 14.52
Glibenclamide	246.15 ± 13.06	182.50 ± 10.57	125.65 ± 16.85	82.15 ± 15.95
Kangni-1	341.33 ± 82.08	270.33 ± 27.43	148.66 ± 20.89	80.16 ± 12.43
Kangni-4	302.16 ± 112.31	220.5 ± 67.71	125.33 ± 15.51	80.6 ± 21.82
Kangni-5	324.5 ± 59.11	249.16 ± 46.52	159.33 ± 18.14	63 ± 5.29
Kangni-6	279.85 ± 40.59	219.83 ± 45.51	147.33 ± 39.74	77 ± 15.04
Kangni-7	362.16 ± 83.73	293.33 ± 83.40	122.33 ± 24.06	72.33 ± 10.78
Kangni-10(Check)	336.66 ± 32.66	307.5 ± 21.07	128.5 ± 11.76	62.84 ± 6.86
F	9.054	7.420	8.867	75.082
Kruskal-Wallis H	29.264315	25.443	31.679	40.573
Sig.	0.000	0.000	0.000	0.000

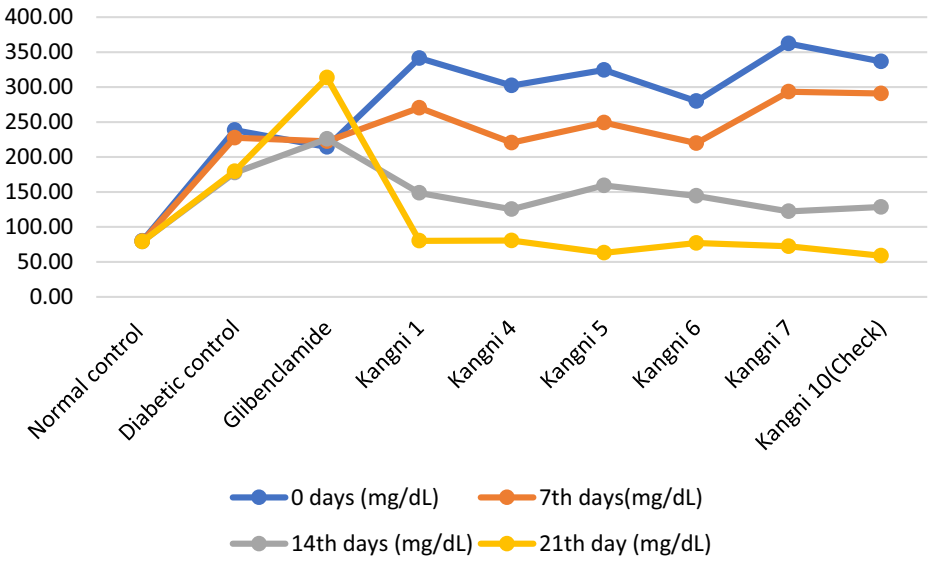


Fig. 3. Graph indicating glucose levels in STZ – induced diabetic group.

Table 2
Effects of *Setaria italica L. Beauv.* on Free radical scavenging activity of liver of Albino Wistar rat (Mean ± SEM) (%).

S. no	Treatment group	Catalase	SOD	GPx	GST	GSH	TBARS
1	Normal control	83.33 ± 2.93	9.20 ± 0.42	9.43 ± 0.43	7.12 ± 0.43	49.57 ± 1.44	0.97 ± 0.27
2	Diabetic control	42.20 ± 2.75	3.7 ± 0.19	5.44 ± 0.28	3.25 ± 0.19	25.57 ± 1.49	2.03 ± 0.19
3	Glibenclamide	66.20 ± 2.13	5.08 ± 0.25	7.08 ± 0.29	5.52 ± 0.29	39.65 ± 1.84	1.47 ± 0.22
4	Kangni-1	43.23 ± 2.22	4.72 ± 0.18	7.02 ± 1.01	3.41 ± 0.15	33.26 ± 2.08	1.86 ± 0.57
5	Kangni-4	57.67 ± 3.49	5.81 ± 0.21	8.51 ± 1.05	3.25 ± 0.12	39.89 ± 2.11	1.51 ± 0.52
6	Kangni-5	69.13 ± 3.04	6.41 ± 0.17	09.71 ± 1.02	4.52 ± 0.52	45.36 ± 2.81	1.17 ± 0.51
7	Kangni-6	71.30 ± 3.60	6.43 ± 0.42	8.52 ± 0.43	6.05 ± 0.19	43.77 ± 3.85	0.96 ± 0.007
8	Kangni-7	89.35 ± 2.92	7.72 ± 0.22	10.51 ± 1.06	6.52 ± 0.38	48.71 ± 2.70	0.91 ± 0.32
9	Kangni-10	83.72 ± 3.42	3.72 ± 0.15	4.02 ± 1.01	3.12 ± 0.19	35.62 ± 1.81	1.52 ± 0.25

plement showed reduction significantly in intake of water during diabetes. During the study, body weight of all groups was monitored. The anti-hyperglycemic activity of different genotypes of *S. italica* on diabetes rats was observed as well as foxtail millets' free radical scavenging activity in liver & kidney were observed in the study which are in the following sections explained in Fig. 2.

3.1. Effects of foxtail millet seed extract (Setaria italica L. Beauv.) on blood glucose levels in streptozotocin – Induced diabetic rats

In the below Table 1, Fig. 3 the effect of SISAE on the normal & diabetic rats are given. To compare many groups of the same design, Kruskal–Wallis test was used. At every time duration, there could be seen a significant difference statistically (p-value < 0.001) among the experimental groups. The below Table 1 shows that a significant fall could be observed in Wistar rats' blood glucose levels, when rats were incorporating with the extract of different genotypes of *S. italica* such as Kangni-1, Kangni-4, Kangni-5, Kangni-6, Kangni-7 and Kangni-10 when compared to rats treated with Glibenclamide in every 7 days of interval (1th day, 7th day, 14th day and 21th day). This shows that foxtail millet has the significant property of anti-hyperglycaemic activity.

3.2. Effects of foxtail millet seed extract (Setaria italica L. Beauv.) on Free radical scavenging activity of liver of Albino Wistar rats

The below Table 2 and Fig. 4 depicts that the effect of seed extract of *S. italica* on the antioxidant enzymes. In the below table, it shows that the activity of these antioxidant enzymes reported lower in control group and Glibenclamide fed group than normal group. In Kangni-1, Kangni-4, Kangni-5 and Kangni-6 fed diabetic rats, there could be seen the decreased level of catalase, SOD, GPx, GST & GSH and increased level of TBARS than normal control. In Kangni-7 and Kangni-10 fed diabetes rats, it can be observed that the level of catalase is increased than normal control & the GPx level is increased in Kangni-7 fed diabetes rats than normal control group. Otherwise, SOD, GST, GSH & TBARS levels are lower while comparing to normal control group and the GPx, GST, GSH and TBARS levels are lower when compared to normal group. It indicates that supplementation of foxtail millet keeps the liver's antioxidant enzymes in control. The data represent as mean ± standard deviation of six rats in each group. $^{a}p < 0.05$, $^{b}p < 0.01$ and $^{c}p < 0.001$ compared to diabetic control group. $^{z}p < 0.001$ as compared to control group.

3.3. Effects of Setaria italica L. Beauv. On Free radical scavenging activity of kidney of Albino Wistar rats

The below Table 3, Fig. 5 showcased effects of seed extracts of *S. Italica* on the antioxidant enzymes. The table shows that antioxidant enzymes in diabetic control and Glibenclamide fed diabetes rats are lower when compared to normal control group and TBARS present in both diabetic control and Glibenclamide fed diabetes rats are higher in level when comparing to normal group. Subsequently, catalase in Kangni-1, Kangni-4, Kangni-5, Kangni-7 and Kangni-10 are higher and GPx in Kangni-1, Kangni-5, Kangni-7

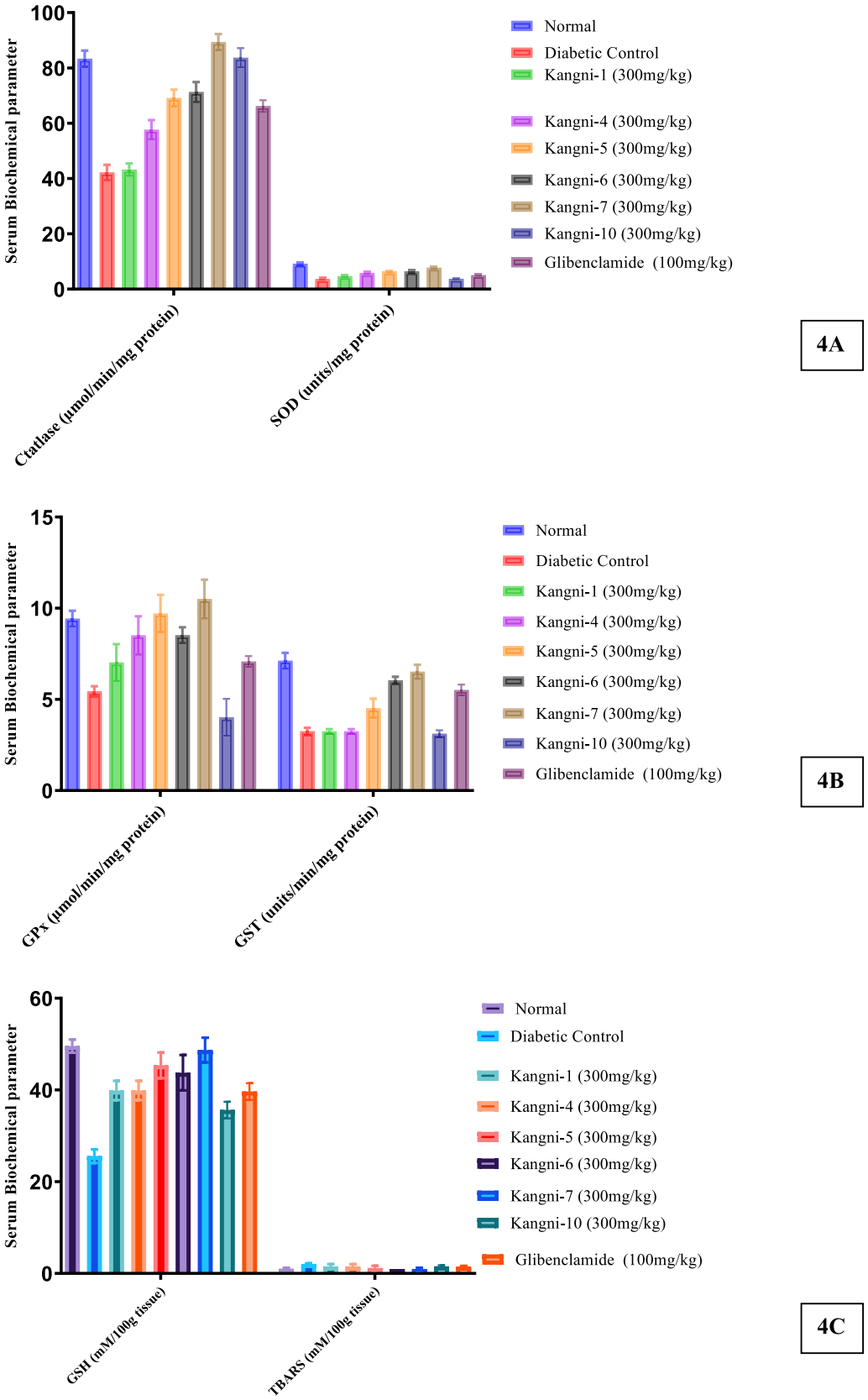


Fig. 4. 3A, 3B, 3C represent effect of S.I. seeds extract on Free radical scavenging activity of liver of Albino Wistar rat (Mean ± SEM) (%).

Table 3
Effects of Foxtail millet extract *(Setaria italica L. Beauv.)* on Free radical scavenging activity of kidney of Albino Wistar rat (Mean ± SEM) (%).

S. no	Treatment group	Catalase	SOD	GPx	GST	GSH	TBARS
1	Normal control	43.28 ± 2.66	14.32 ± 0.43	7.37 ± 0.09	6.03 ± 0.43	34.79 ± 1.99	1.52 ± 0.21
2	Diabetic control	26.20 ± 1.89	8.23 ± 0.29	4.50 ± 0.28	2.42 ± 0.15	21.67 ± 1.27	2.40 ± 0.27
3	Glibenclamide	31.58 ± 2.13	10.70 ± 0.54	6.23 ± 0.15	4.75 ± 0.46	27.57 ± 1.20	1.62 ± 0.31
4	Kangni-1	72.29 ± 2.9	14.72 ± 0.31	13.05 ± 1.01	4.03 ± 0.40	36.71 ± 1.02	1.53 ± 0.22
5	Kangni-4	51.72 ± 3.0	8.51 ± 0.22	7.22 ± 0.99	2.03 ± 0.13	29.27 ± 1.12	2.45 ± 0.21
6	Kangni-5	64.74 ± 3.66	11.09 ± 0.67	10.52 ± 1.54	3.02 ± 0.21	32.79 ± 1.43	1.43 ± 0.92
7	Kangni-6	33.63 ± 2.92	12.17 ± 0.57	6.83 ± 0.43	4.30 ± 0.28	31.40 ± 1.57	1.40 ± 0.02
8	Kangni-7	73.39 ± 3.53	13.10 ± 0.50	12.48 ± 1.10	5.99 ± 0.43	34.42 ± 1.17	1.58 ± 0.79
9	Kangni-10	69.34 ± 3.26	09.99 ± 0.51	8.92 ± 1.10	3.41 ± 0.25	30.90 ± 1.30	2.07 ± 0.52

and Kangni-10 are higher than normal control. SOD in Kangni-1 is higher in level than normal control rats. This shows that supplementation of foxtail millet keeps the antioxidant enzymes in control. The data represent as mean ± standard deviation of six rats in each group. $^{a}p < 0.05$, $^{b}p < 0.01$ and $^{c}p < 0.001$ compared to diabetic control group. $^{z}p < 0.001$ as compared to control group.

3.4. Effects of seed extract prepared by seed of foxtail millet (Setaria italica L. Beauv.) on liver function test of serum biochemical analysis in streptozotocin – Induced diabetic rats (Means ± SD)

The interaction shows Fig. 6 that the total variation is about 35.73% and its p-value obtained (**<0.0001**) is less than p-value significantly in mean ± standard deviation of six rats in each group. In addition, its row factor shows that the total variation is about 57.73% and p-value obtained (<0.001) is less than significant p-value. Subsequently, Column factor shows the total variation of about 6.550% and p-value obtained (<0.001) is less than significant p-value. Therefore, it shows that there is a significant relationship between liver function in streptozotocin induced diabetic rats & S.I. seed extract effect.

In liver function test, 5A graph obtained from graph pad prism shows that the level of serum bilirubin, S. Bilirubin direct and S. Bilirubin Indirect, when the rat is normal and in diabetic control and also when rats were fed with Glibenclamide (100 mg/ kg), all the six genotypes of Kangni-1, Kangni-4, Kangni-5, Kangni-6, Kangni-7 & Kangni-10 in 300 mg/kg. S. bilirubin level in these group of rats shows that it lies in the range of 0.5 and 1.2 mg/dl correspondingly. The level of S. Bilirubin Direct lies in the range of 0.2 and 0.8 mg/dl. And the level of S. Bilirubin Indirect of these group rats lies in the range of 0.2 and 0.6 correspondingly.

5B shows that the level of serum glutamic-pyruvic transaminase (S.G.P.T), Serum Glutamic Oxaloacetic Transaminase (SGOT) and S. Alkaline Phosphatase in normal, diabetic control rats, rats fed with Glibenclamide (100 mg/ kg), all the six genotypes of Kangni-1, Kangni-4, Kangni-5, Kangni-6, Kangni-7 & Kangni-10 in 300 mg/kg. Subsequently, the level of SGPT, SGOT and S. Alkaline Phosphatase in Glibenclamide (100 mg/kg) fed rat lies at 1001 mg/dl, 2500 mg/dl and 400 mg/dl. The level of these three enzymes in Kangni-10 (300 mg/dl) fed rats lies at 200 mg/dl, 1900 mg/dl and it shows that the level of S. Alkaline phosphatase is 0 mg/kg. In Kangni-6 fed rats, the level of SGPT, SGOT and S. Alkaline Phosphatase lies at 500 mg/dl, 800 mg/dl and the level of S. Alkaline phosphatase is 0. In diabetic control rats, these enzymes lie at the points of 200 mg/dl, 0 mg/dl & 1000 mg/dl correspondingly. In Kangni-4 fed rats, the level of these enzymes shows that they lie in the range of 100 mg/dl, 800 mg/dl and 0 mg/dl. In Kangni-1 fed rats, the level of SGPT is at 100 mg/dl, SGOT at 300 mg/dl and S. Alkaline phosphatase at 0. In normal rats, the level of SGPT is at 100 mg/dl, SGOT at 300 mg/dl and S. Alkaline phosphatase is at 100 mg/dl. In kangni-7 fed rats, the level of these three enzymes is at 0 mg/dl, 200 mg/dl and 700 mg/dl correspondingly. In kangni-5 fed rats, the level of these three enzymes is at 900 mg/dl, 200 mg/dl and 100 mg/dl respectively.

In 5C, it shows that in Glibenclamide fed rats, the level of total protein, albumin and globulin lies at 7.8 mg/dl, 6.8 mg/dl and 1 mg/dl respectively. In addition, the level of globulin, total protein & albumin in normal, diabetic control rats & Kangni-1, Kangni-4, Kangni-5, Kangni-6, Kangni-7 & Kangni-10 fed diabetic rats lies in the range of 4.6 and 8.4 mg/dl, 2.8 mg/dl and 5.6 mg/dl & 0.4 and 4 mg/dl respectively.

3.5. Effects of seed extract prepared by seed of foxtail millet (Setaria italica L. Beauv.) on kidney function test of serum biochemical analysis in streptozotocin – Induced diabetic rats (Means ± SD)

In mean ± standard deviation of six rats in each group, interaction shows in Fig. 7 that the total variation is about 2.89% and its p-value obtained (<0.001) is less than significant p-value (0.05). In addition, its row factor shows that the total variation is about 94.37 % and p-value obtained (<0.001) is less than significant p-value. Subsequently, Column factor shows the total variation is about 1. 753% and p-value obtained (<0.001) is less than significant p-value. Therefore, it shows that there is a significant relationship between kidney function of STZ induced rats and *S. italica* seed extract effect.

In Fig. 5, it is represented that the level of serum creatinine in normal, diabetic control rats and all the six genotypes of *S. italica* and glibenclamide fed rats is at 0. The level of serum urea in these groups is in the range of 40 mg/dl and 70 mg/dl respectively. Subsequently, the level of serum uric acid is in the range of 4 mg/dl and 10 mg/dl.

3.6. Histopathological studies

By the investigation of effects on tissues, a more in-depth study of disease' toxic effects are provided by histopathological gross since the architecture of the tissues is preserved by the preparation process. To detect the histological changes SISAE treated rates are treatments with six groups affected STZ-induced diabetic organs study in experimental rats. No major changes are shown by gross studies in the alteration of the tissues of vital organs namely, pancreas, liver and kidney. Yet, in all six groups of rats, minor lymphocytic infiltrations & cholesterol hemorrhage were stopped in the organs namely, kidney, pancreas & liver respectively. The minor changes observed in these organs show that the extract might be non-toxic to those parts. In normal, histological structure of liver were observed in every group that shows in L.A., L.D., L.H. group. A liver with severe hepatocytes destruction is showed by L.B., L. C. group with congested hepatic inflammations, nuclear condensation & loss of hectic lobules in the S.T.Z. induced diabetic rats. A major part of pancreatic tissue that located nearer to the spleen

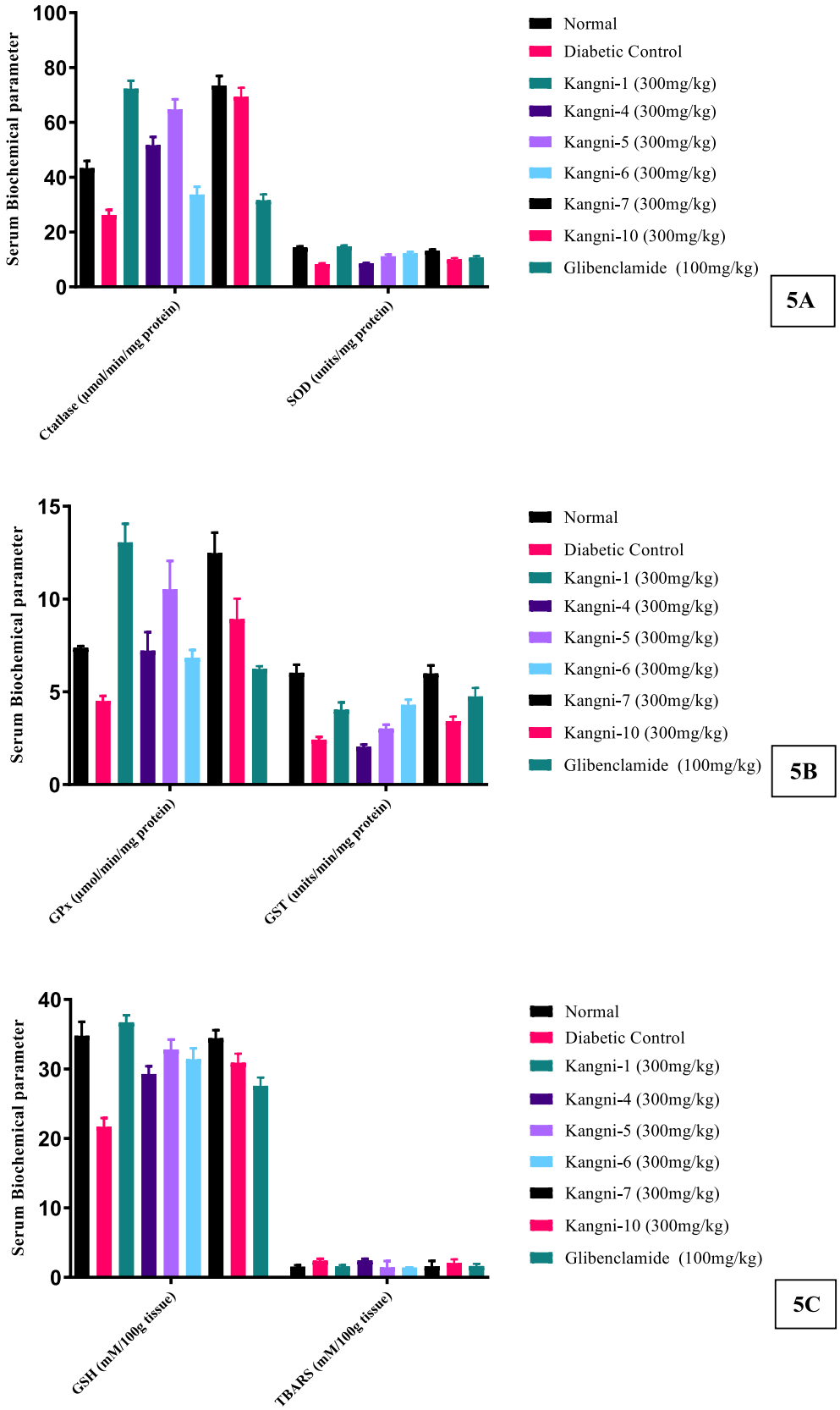
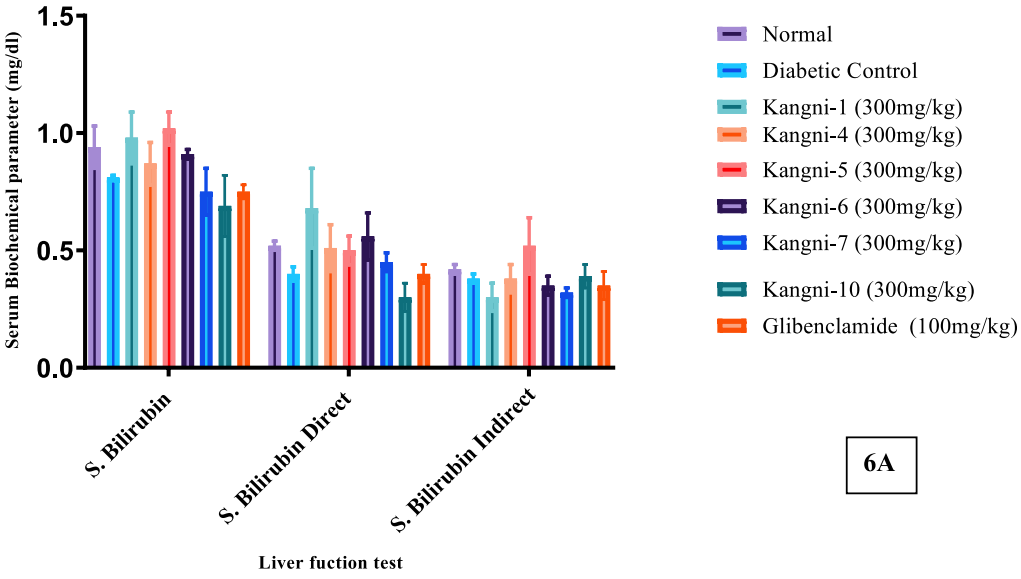
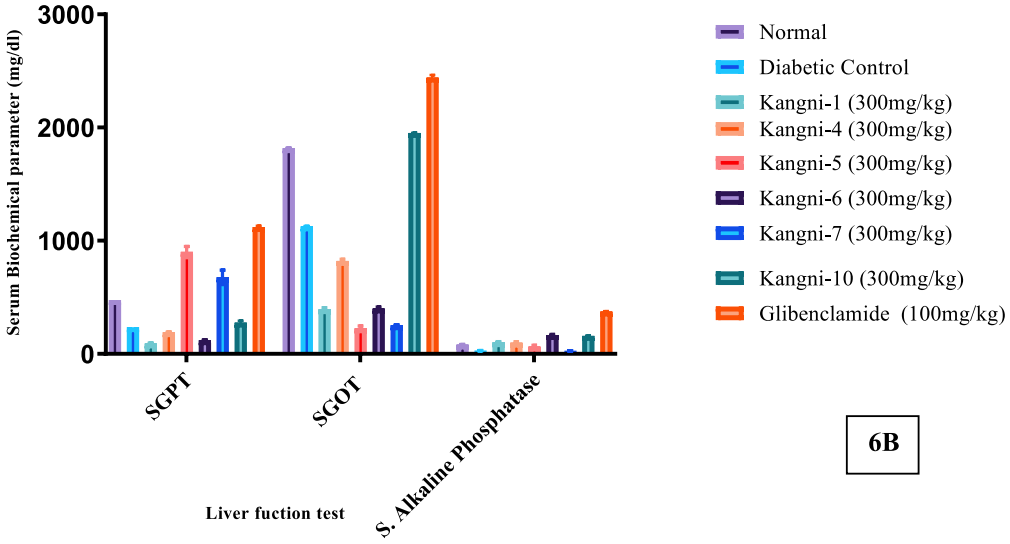


Fig. 5. 4A, 4B, 4C represent effect of *S. italica* seeds extract on Free radical scavenging activity of liver of Albino Wistar rat (Mean ± SEM) (%).





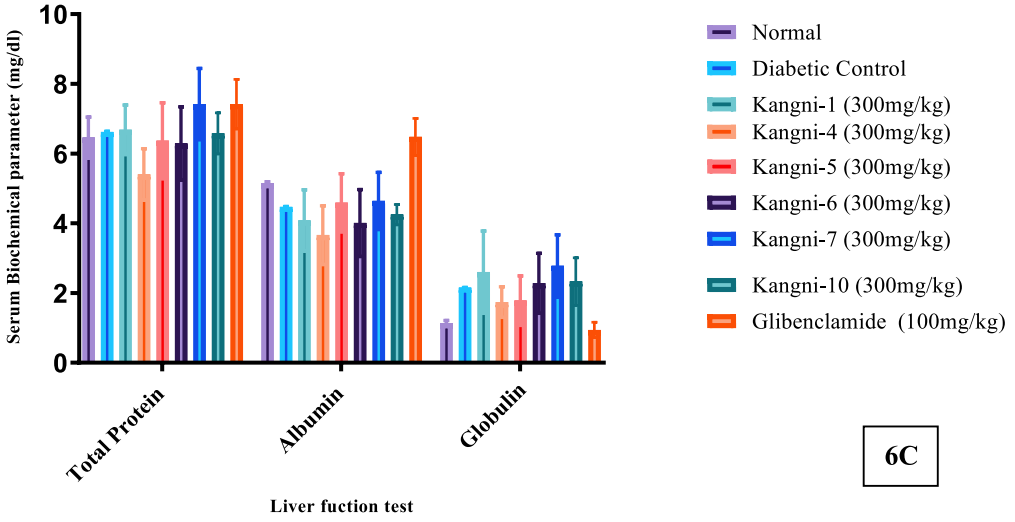


Fig. 6. 5A, 5B, 5C represent effect of *S. italica* seeds extract on Free radical scavenging activity of liver of Albino Wistar rat (Mean ± SEM) (%).

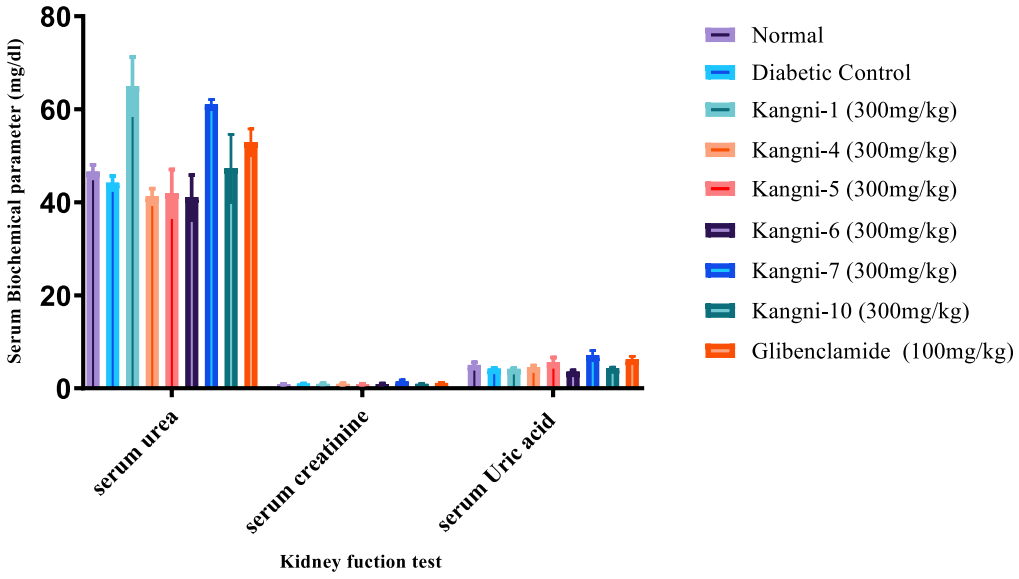


Fig. 7. Graph represents the effect of *S. italica* seeds extract on Free radical scavenging activity of kidney of Albino Wistar rat (Mean ± SEM) (%).

is represented by the pancreas in the greater momentum. Necrosis is nothing but the occurrence of morphological changes in reversible & irreversible cell injury. Normal kidney tubules were observed in epithelial cells of group K.A., K.D., K.H., K.I. on the surface of the blebs, it can be observed that the early or reversible ischemic injury was appeared by which the increase of occasional cell swelling & eosinophilia of cytoplasm were observed in group K.B., K.C swing blow in Fig. 8. Pancreases divided into lobules by connective tissue septae are composed largely of grape like clusters of acini which help in digestion. P.A., P.D., P.H. and P.I. group relatively pathological changes of both exocrine and endocrine part of cells represented by vacuolation also marked as decrease of β-cells, normal histological changes where P.B., P.C., P.E., P.F., P.G. group found major changes.

Hence, the very first report of any toxic effects of this scientific unexplored mangrove standing crop will be provided by this present study that is used highly by the local healers to cure the diseases. The result obtained after conducting a preliminary DPPH test showed the R.O.S. scavenging activity that is helpful to determine the chemical compound present in the extract that leads to their quantitative estimation and also leads to active Phyto-constituents pharmacologically. Acute oral glucose tolerance test considered as one of the initial studies by which the basis of drug dose is provided for the particular rat. Due to drugs & chemicals, indicators of early signs in the rat of toxicity are general behavioral changes in the experimental rat regarding body weight, mortality rate. No mortality was produced by investigations of sub-acute SISAE. in rats as well as very minimal behavioral changes were produced in these nine groups. When comparing to control group, bodyweight was increased in experimental rats which remained most significant and was considered normal. Therefore, it can be concluded that any major clinical signs were not produced and the normal growth pattern of the experimental rats was not affected by SISAE. oral administration throughout the research treatment period (21 days).

The prepared sample was analyzed by the validated H.P.T.L.C. method. The amounts of the referred standard were calculated from the Kaempferol standard calibration curve. Evaluation of a band corresponding to marker compound Kaempferol was visible in both reference solution and test solution tracks. 10 μl of the test soln. was applied on each precoated H.P.T.L.C. plates along with silica gel 60F 254. Further, the plate is made in the solvent system of toluene: ethyl acetate: formic acid: methanol in the ration of 6:6:1. 8:0.4. For Kaempferol, the plate was developed in the solvent system to 8 cm height, then dried & scanned densitometric ally at the wavelength of 254 nm. By plotting the peak against the concentration oof Kaempferol applied, the peak area was recorded & the calibration curve was prepared.

The generated fingerprinting data of various samples K-1, K-4, K-5, K-6, K7, *K*10. gives the good result and standards were visualized under visible and U.V. light (Fig. 10) and the % area of Kaempferol in K-1, K-4, K-5, K-6, K-7, K-10 extracts. K-7 kaempferol baseline correction was slope was five, peak threshold min., low slope, peak threshold min., area was fifty, peak threshold max., height was 10 A.U., peak threshold min., height was 990 AU, track start position was 27.3 mm, and track end position was 33.0 mm for quercetin. kaempferol integration of baseline correction was slope was five min., peak threshold min., low Slope, peak threshold min., height was 10 A.U., peak threshold min. area was 50, peak threshold max. height was 990 AU, track start position was 41.3 mm, and track end position was 51.5 mm. K-10 kaempferol Rf value was found 0.36 mm, K-6 Rf value was 0.39 mm, K-5 Rf value was 0.37 mm, K-4 Rf value was 0.37, K-1 Rf value 35 mm on HPTLC plate. Plate size was 20.0 × 10.0 cm, Temperature was 120 °C and Time was 20 Minutes in oven for drying the plate. Number of tracks was 7, Scan start pos. 5.0 mm, Position of first track was 15.0 mm, Micro Optimize optical system Light, Distance between tracks 28.3 mm, Scan end pos. 85.0 mm, slit dimensions 6.00 × 0.30 mm, Data resolution: 100 μm/step & Scanning speed: 20 mm/s (Fig. 9).

4. Discussion

In many developing countries, the use of medicinal plants & traditional medicines for maintaining the good health as a normative basis is observed (Tiwari & Madhusudanarao, 2002). Globally, DM remains the fastest growing metabolic diseases. As there can be observing a continuous rise in the knowledge of the disease nature, more suitable & challenging therapies need to be adapted. Now-a-days, as many people are considering the side effects of allopathy medicines, they are changing to take herbal medicines etc. In fact, while treating diabetes, remedies of traditional plants have been highly used for many centuries (Akhtar & Ali, 1984; Rai et al.,

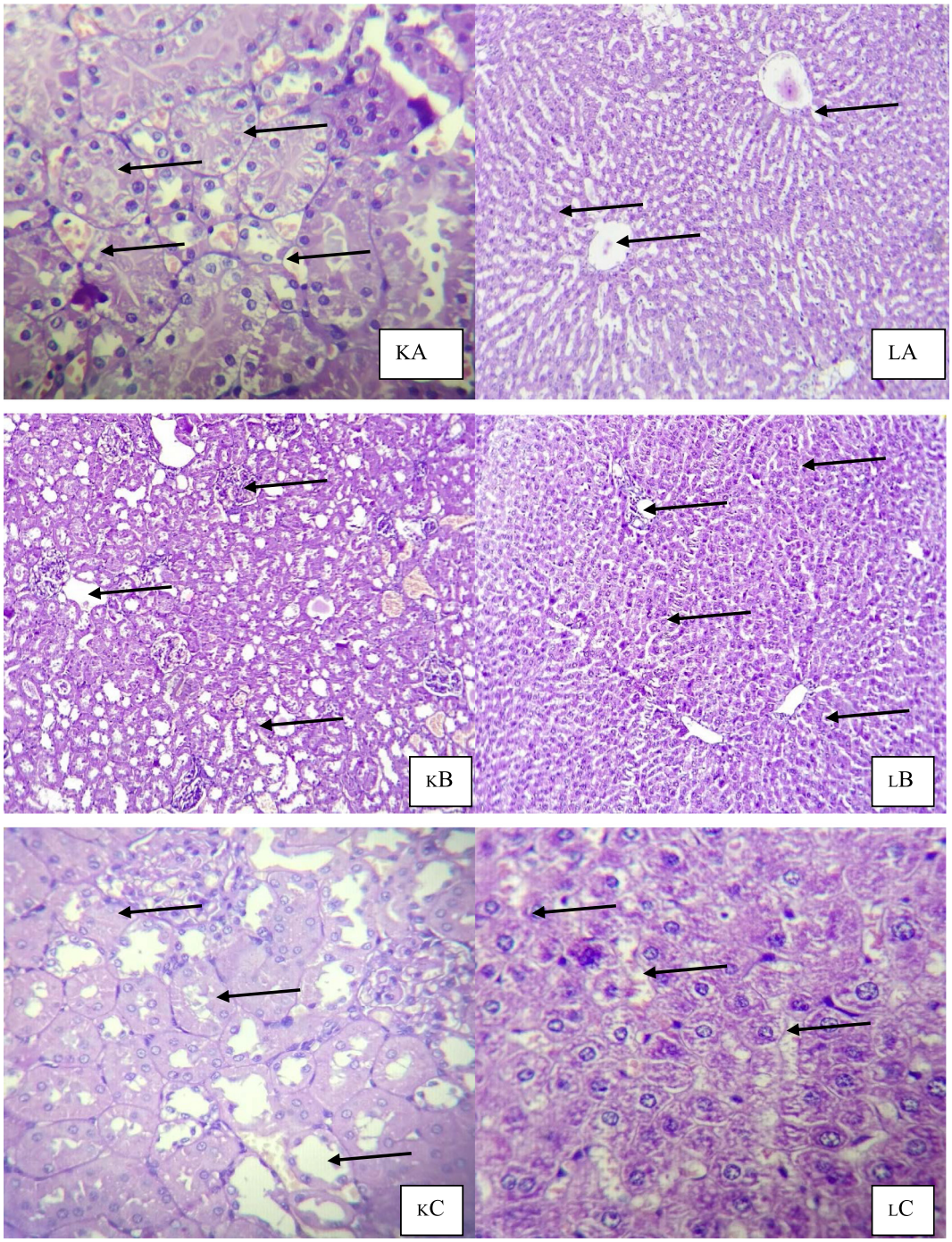


Fig. 8. Histopathology study of vital organs from Wistar Albino rats. Photographs of histological findings of different organs from rat treated with normal control vehicle and S.I. seeds extract (300 mg/kg. bwt.) for the period of 21 days (H&E staining, 50X magnification) diabetes was induced at high fat diet with low dose S.T.Z. of experimental rats. K.A.- kidney of group A, LA- liver of group A, K.B.- Kidney of group B, L.B.- liver of group B, K.C.- Kidney of group C, L.C.- liver of group C, K.D.- Kidney of group D, L.D.- liver of group D, K.E.- Kidney of group E, L.E.- Liver of group E, K.F.- Kidney of group F, L.F.- Liver of group F, K.G.- Kidney of group G, L.G.- Liver of group G, K.H.- Kidney of group H, L. H.- Liver of group H, K.I.- Kidney of group I, L.I.- Liver of group I, PA- Pancreas of group A, P.B.- Pancreas of group B, P.C.- Pancreas of group C, P.D.- Pancreas of group D, PE- Pancreas of group E, PF- Pancreas of group F, PG- Pancreas of group G, PH- Pancreas of group H, PI- Pancreas of group I and Values are given as mean ± SD.

2007; Kesari et al., 2007 Kes; Kesari et al, 2005). However, few remedies had been proved and evaluated scientifically. Hence, the effect of *S. italica* on anti-hyperglycemic control & its antioxidant activity in STZ-induced diabetic rats has been investigated in this paper.

A dose-dependent effect was showed by aqueous extract of five different genotypes of S. italica seeds in diabetic rats after 12–16 h of fasting. Probably, the expected higher hypoglycemic effect cannot be produced by the higher doses, as there are certain substances present in the extract that gets interfered with the hypoglycemic effect of foxtail millet. So, the hyperglycemic action is exerted by the possible mechanism in diabetic rats due to that it can be potentiating the release of insulin. Body weight of rats, tissue proteins (Chatterjea & Shinde, 1976) and muscle wasting were

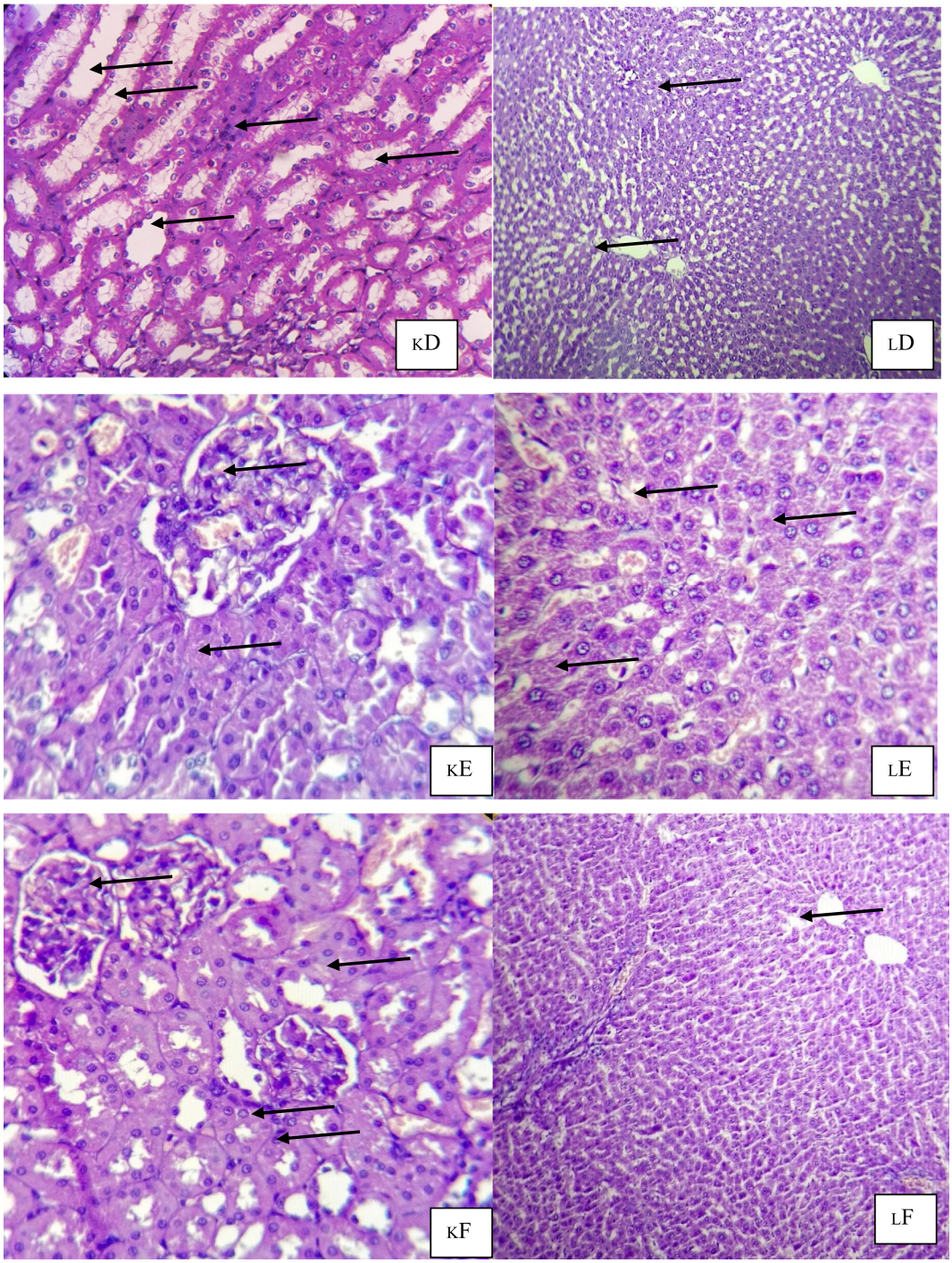
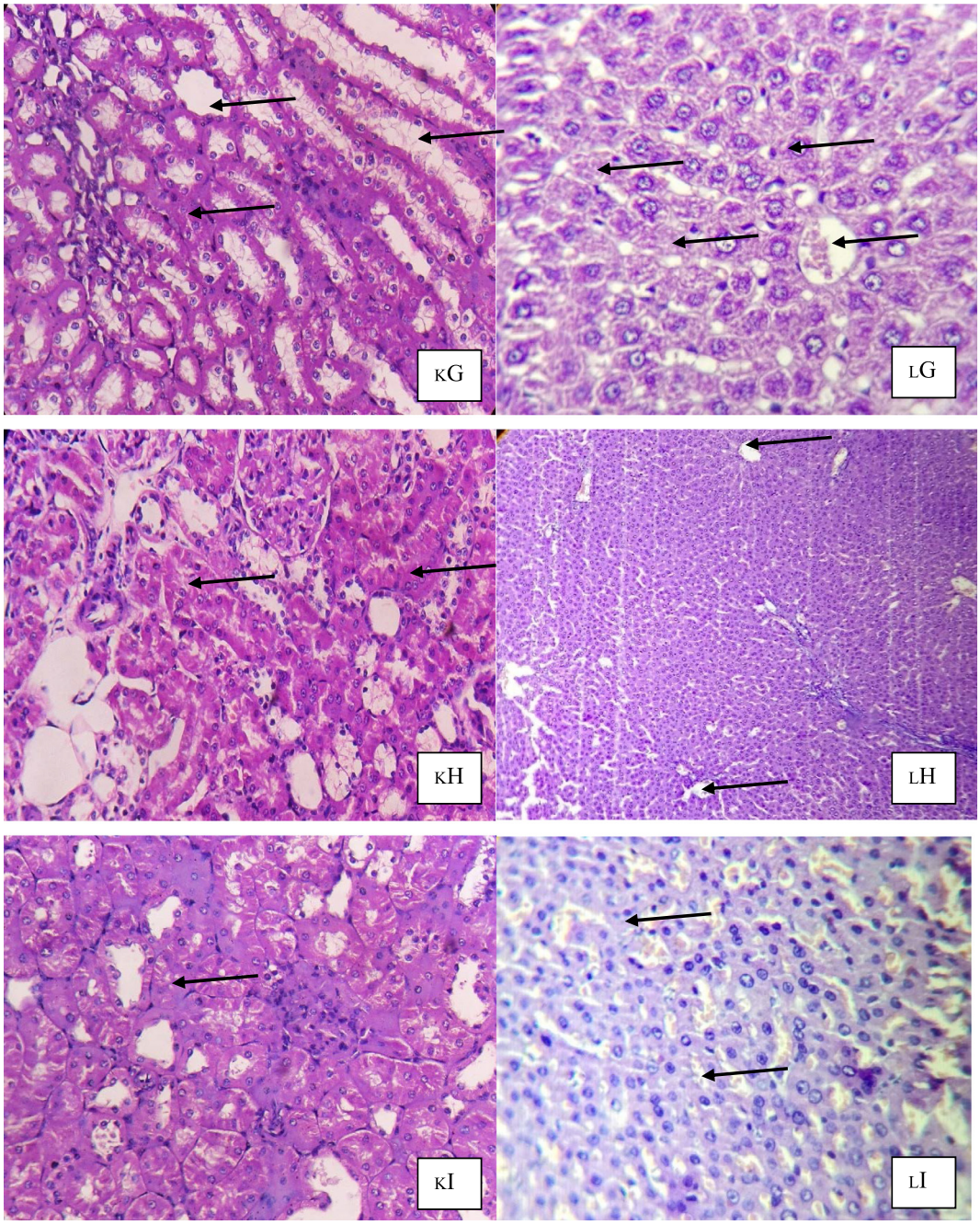


Fig. 8 (*continued*)

decreased because of diabetes induced with STZ (Swanston-Flatt et al., 1990). When comparing with untreated diabetic rats, there was an increase in the rats' body weight with the SISAE due to the control observed in blood glucose level.

In liver function test, Serum urea is higher in Glibenclamide fed diabetic rats, Kangni-1, Kangni-7 and Kangni-10. The level of serum creatinine in diabetic control, Glibenclamide, Kangni-1, Kangni-4, Kangni-5, Kangni-6, Kangni-7 and Kangni-10 is higher when compared to normal group. There can be seen the fluctuations in the level of S. bilirubin direct, serum uric acid, S. bilirubin, S. bilirubin indirect, S. G. P. T, S. G. O. T, S. Alkaline phosphatase, S. Albumin, total protein and Globulin in diabetic control, Glibenclamide, Kangni-1, Kangni-4, Kangni-5, Kangni-6, Kangni-7 and Kangni-10 groups while comparing to normal control.

Therefore, it is observed that the changes in the effects of *S. italica* on blood glucose levels and its antioxidant levels in liver and kidney are beneficial to prevent diabetic complications and also it helps to improve lipid metabolism in diabetes patients (Gupta et al., 2005). It is clear that foxtail millet (S. italica) has many benefits to control the diabetes status. From the rendered study outcome showcased higher blood glucose level among diabetic control ($p < 0.05$) when compared to that of normal control group during the experimental time period (i.e. 21 days). By comparing the values of the six genotypes of foxtail millet extract with dia-



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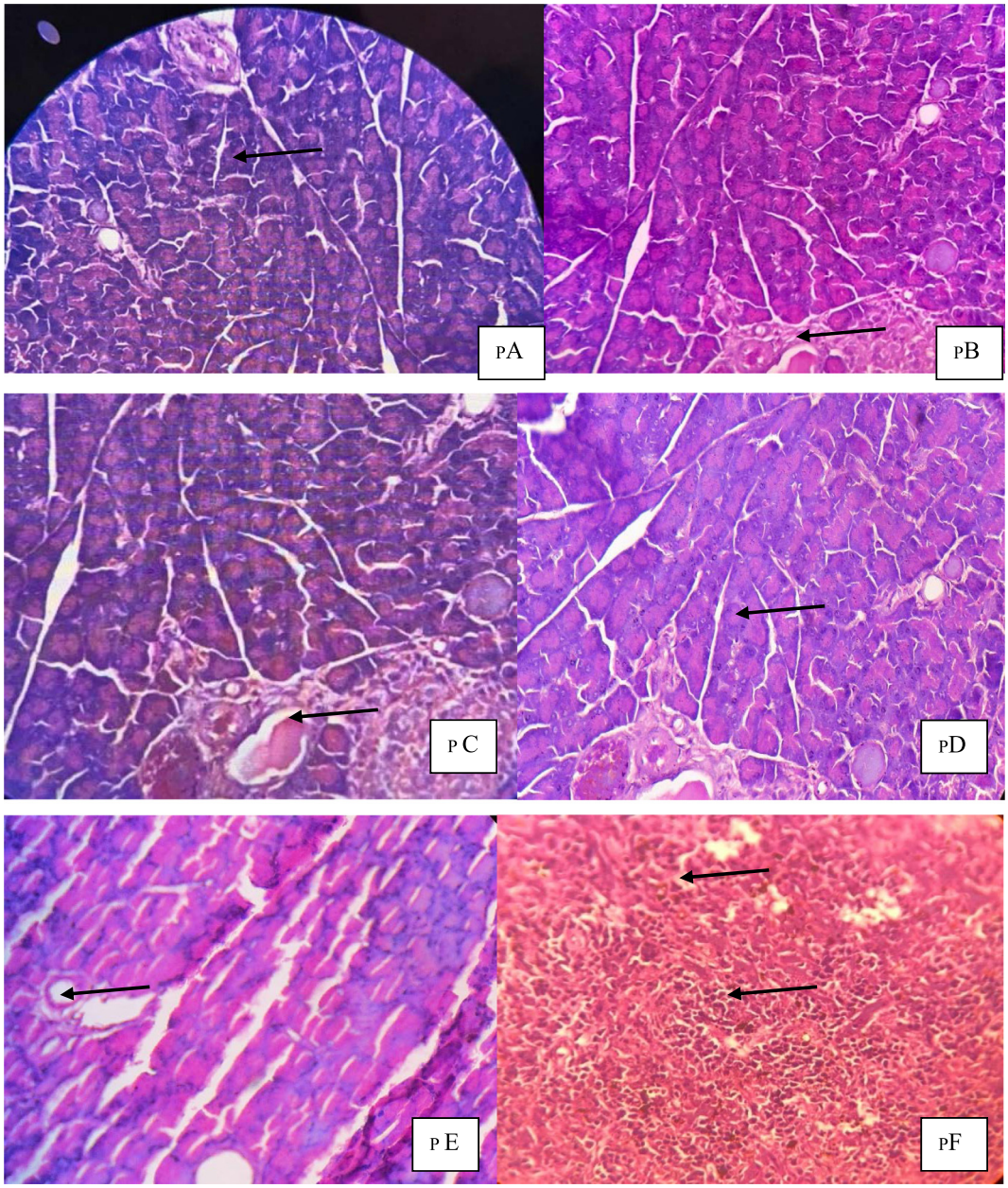
betic control group, the effect of foxtail millet on blood glucose level was found. It is observed that the extract of foxtail millet can possibly be able to lower the blood glucose level within these 21 days of administering foxtail millet (dose dependent) which appeared to have greatly improved when compared to diabetic Control group. When comparing to the standard drug glibenclamide, the effect of foxtail millet appeared to be very significant and potent in terms of their reducing blood glucose levels. Glibenclamide which is one of the oral hypoglycaemic drugs that remains as 'first-generation drug' for diabetes. The underlying phenomenon concerned with the development hypoglycaemic effect was elicited via acting on the channel of ATP sensitive K^+ ions in pancreatic β-cells. Thus the potent hypoglycemic activity observed in the case of foxtail millet (Thathola et al., 2011) and its possible mechanism involved in their mode of action can be due to their stimulating effect on insulin secretion via pancreas followed by their role towards enhanced insulin sensitivity which could be attributed due to their presence of higher amount of selenium & magnesium in many organs (Sada et al., 2016). It can be observed that magnesium has the ability to improve cells for responding to insulin through rising the adiponectin hormone level. By increasing the fat metabolism in tissues, the glucose accumulation in the blood is prevented by adiponectin (Nishizara, 2009). Also, selenium shows that it has hypoglycaemic property which can be compared with glibenclamide (Mohammed et al., 2015). Additionally, foxtail millet's lipid profile shows that it is one of the good indicators of if a person is prone for developing heart attack or stroke which is caused by atherosclerosis. One of the risks is hyperlipidemia that



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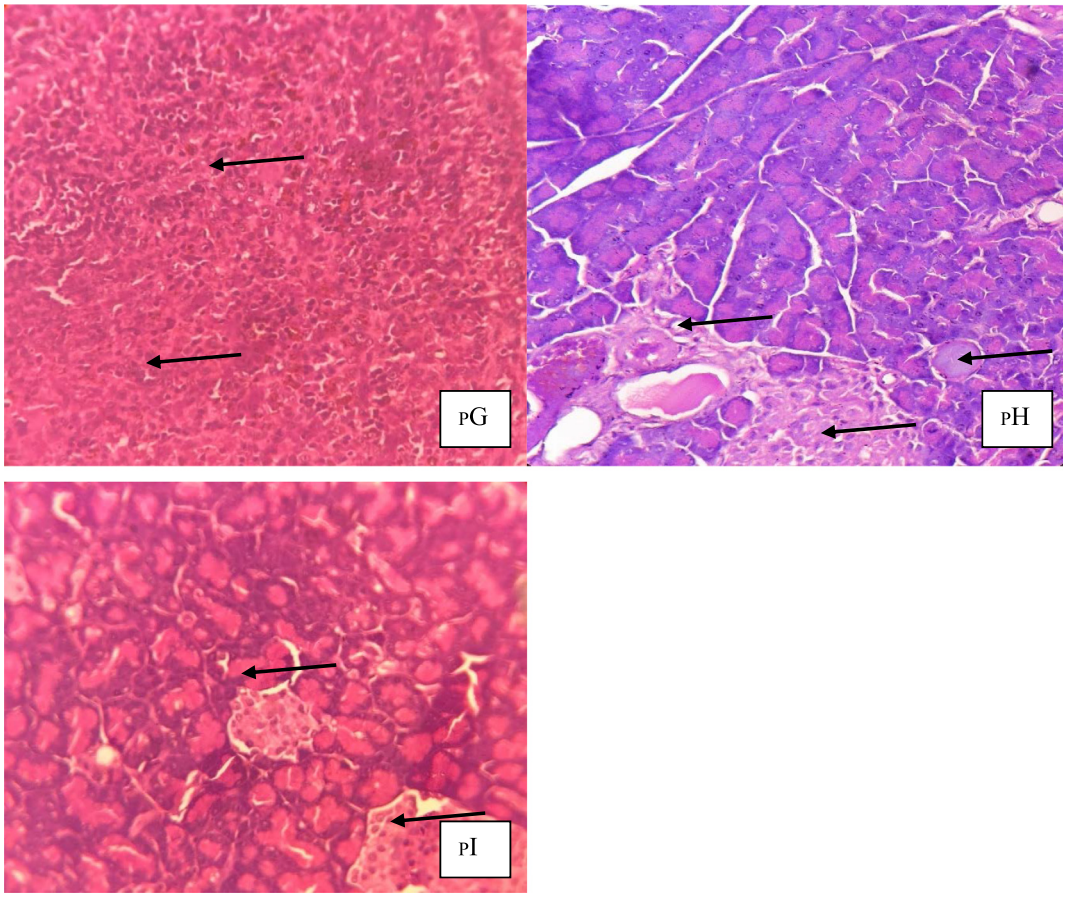
can be seen frequently among many of the diabetic patients. Levels of serum lipid are commonly increased in DM & a risk factor for coronary heart disease is represented by this elevation (Daniel *et al*., 2012). And foxtail millet's cholesterol lowering effect may be mainly due to the flavonoids' availability in it. Flavonoids which are antioxidants by which the oxidation of cholesterol may be inhibited (prevents atherosclerotic plaque development) (Middleton et al., 2000; Duarte-Alameida, et al., 2007). Suppression of ROS formation can be included in the antioxidant mechanism through chelation and upregulation of trace elements that involved in production of free radical, scavenging reactive species or else protecting the natural defenses of antioxidant (Van Acker et al., 1998). It can be observed that the role of oxidative stress in the hyperglycemia pathogenesis has been supported by many reports and antioxidant activity of flavonoids presents in foxtail millets possess advantageous effect in hyperglycemia treatment. Thus, the antidiabetic & antioxidant activities of foxtail millets were performed in the study.

In histopathological study, the degeneration and necrosis of pancreatic tissue were observed in the study. In the tissues of the organs, there could be observed cell damage mainly in the empty space on islets of Langerhans. In group A rats, islets of Langerhans & nuclei were found. In the meantime, many empty spaces which showed the severe damage in Groups B & C. Still, Groups D & E were observed that they had β cells in pancreas even though they were not like as Group A (Pricilla & EOGHN, 2020). Investigation done to know effects on tissues with the extract, histopathological gross provided in-depth study of diseases toxic effects, since the architecture of the tissues is preserved by the preparation process of gross. A liver with severe hepatocytes destruction is showed by L.B., L.C. group with congested hepatic inflammations, nuclear condensation & loss of hectic lobules in the diabetic rats. it can be



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observed that the early or reversible ischemic injury was appeared by which the increase of occasional cell swelling & eosinophilia of cytoplasm were observed in group K.B., K.C. In this study, the result obtained for histopathological study is similar to Noman *et al.*, (2009) who mentioned that the liver of mice induced with STZ has a direct effect on the liver hepatocytes or it will affect indirectly via the induced diabetes mellitus. It is confirmed by Nivin, et al., (2012) that the liver histopathology is improved by millet diet as well as the liver can be protected against toxicity by enhancing the level of antioxidant enzymes (Afaf et al., 2018).

By the determination of dissolution, content of uniformity, assay value, drugs bioavailability and drug-drug interaction, HPTLC technique of versatile molecules separation in most of Pharmacopoeia were intended. For these reasons, every research laboratory has been equipped with HPTLC system now-a-days. In fact, the number of samples are handled at a time even in divergent nature as well as the composition of sample supports in many experimental analyses. Considering that if time machine is used, it will speed up the work that finally makes the experiment impractical with some other analytical techniques. Sample extract crude drug was used for the standardization, first step is towards of standardization in herbal drugs. Different tests of overall standardization were well described as in Indian Herbal Pharmacopoeia. Standardization tests are limited for HPTLC for the characterization of components like phytochemicals, flavanols, amino acids etc., which is use for purpose of routine quality control in the experimental research. The data from the samples can compared with "total extract" is described as "fingerprint" technique to know the large amount of chromatographic data of "standard". Characterization data were obtained which is applicable by multi wavelength of scanning in instruments UV, in-situ UV spectra, fluorescence scan and image comparison where applicable, data after post chromatographic derivatization. HPTLC fingerprint data comparison of such a "standard" with that of a sample can be accepted as the rapid, reliable and modern procedure for routine quality control as shown in the literature with various examples. Modern HPTLC is a powerful and reliable method for qualitative and quantitative analysis. Also, it is referred as instrumental thin layer or planer chromatography. Conventional TLC has been modified as the instrumental technique for utilizing the methods with full potential. Also, the principle of HPTLC and TLC is similar. Without any damage to the layer, gently, the samples to be chromatographed are applied to the pre-coated plates in the form of a band/spot. Disposable sample applicators/glass capillaries help to notice the volume. Like as TLC, the exact positions & volume precision are done in the similar way. The migration of solvent that predetermined distance into fractions. The fractions remain stored on the layer after the solvents are evaporated from the mobile phase. The tracts are scanned in the visible or ultraviolet range of the spectrum with a light beam mainly for evaluating the chromatogram. Diffused reflectance measures absorbance or fluorescence. In fact, the HPTLC technique is accepted widely for quantifying and separating the natural compounds namely, simple phenols, flavonoids, antraquinones, phenolic acids, ginenosides & lignans (Goudar, G., & Sathisha, 2016). Yet, no reported methods were found for HPTLC separation & determination of phenolic acids present in foxtail millet. This method helps in the identification of the required adsorbents that have health beneficial effects on reducing the hyperglycemia (Kaur & Gupta, 2018). An integral part of correct identity is evaluating the crude extract of *Setaria italica*. In this pro-

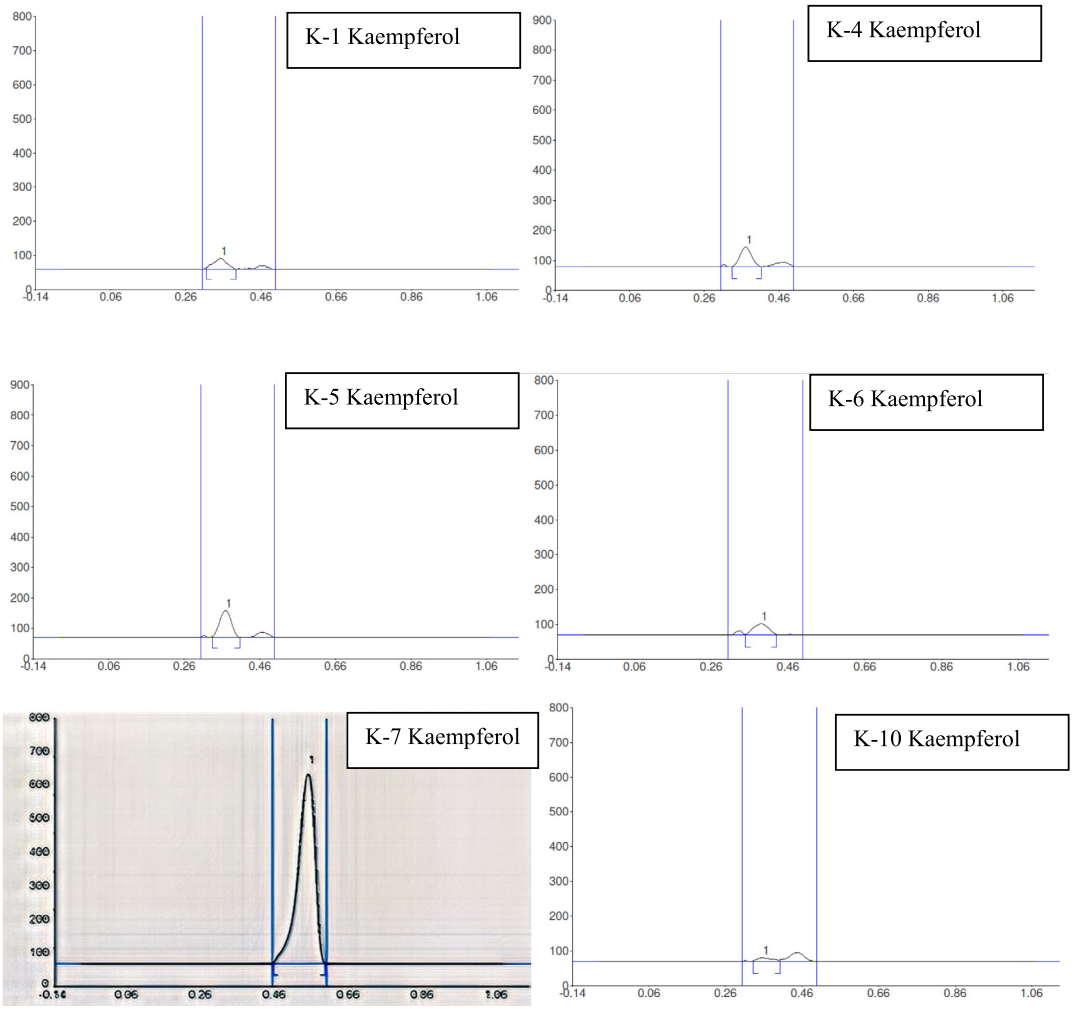

Fig. 9. Kaempferol H.P.T.L.C. spectra of ethanolic extract of *S. italica* selected best performed genotype K-1, K-4, K-5, K-6, K7, *K*10.

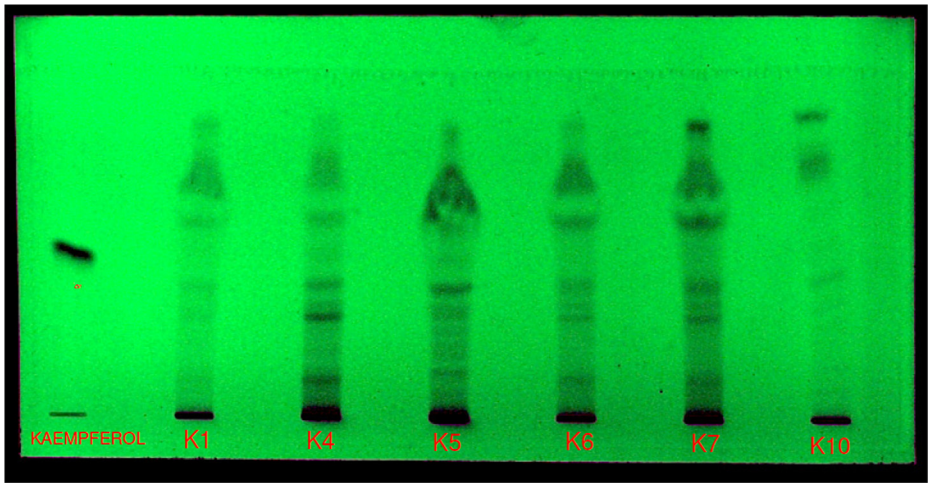

Fig. 10. Kaempferol H.P.T.L.C. spectra of ethanolic extract of *S. italica* selected best performed genotype K-1, K-4, K-5, K-6, K7, *K*10.

cess, HPTLC technique acts as one of the phytochemical markers (Attimarad, et al., 2011; Misra et al., 2014) and it is very efficiently using in the field of plant taxonomy to identify every plant through its secondary metabolites (Saleem *et al.*, 2021). Thus, to identify herbal components, HPTLC fingerprinting is an accurate, linear & precise (Cortés et al., 2014). In fact, this finger printing remains beneficial in herbal medicines or products quality control and also checking for the substances which adulterate the products (Teo et al., 2013). Thus, to evaluate the different marketed pharmaceutical preparations, this HPTLC technique remains very helpful

(Gandhi et al., 2012; Meena & Sandhya, 2013; Patel et al., 2013). Through qualitative assessment using HPTLC indicated that the methanolic roots and leaves extract from *H. radicata* exhibited secondary metabolites such as- flavonoids, saponins, terpenoids & glycosides. In addition to this, the occurrence of medicinal importance acquired metabolites were showed by well-resolved profiles of HPTLC. Furthermore, the traditional practices concerning with therapeutic application is evident from the achieved HPTLC profiles (Senguttuvan & Subramaniam, 2016).

5. Conclusion

The effect of foxtail millet on diabetes has been studied by using diabetes induced rats. It shows that foxtail millet has great impact in controlling blood glucose level. The glycemic control can be improved significantly by intaking certain amount of foxtail millet per day. The interaction of reduced inflammation decreased insulin resistance & increased leptin concentrations might be resulted by the anti-hyperglycemic effect of foxtail millet. Also, free radical scavenging activity of foxtail millet on liver and kidney has been studied. It shows that there can be seen the moderate antioxidant activity of foxtail millets in diabetes rats. Also, other whole grains have the beneficial nutrients in reducing the blood glucose level. Hence, people are suggested to intake the appropriate number of whole grains on their everyday basis. The result of this animal experiment has proved that the obtained results demonstrate that excellent antihyperglycemic and antioxidant activities can be seen in the S. italica seeds extract. In this study, the histological changes in liver are observed only in the group of L.B., L.C. group with congested hepatic inflammations, nuclear condensation & loss of hectic lobules in the S.T.Z. induced diabetic rats and only very minor changes are observed in other groups namely, L.A., L.D., L.E. group. In K. A, K.D., K.E groups, normal kidney tubules were only observed and reversible ischemic injury appeared in K.B, K.C increases the occasional cell swelling and cytoplasm eosinophilia. Presently, the selected HPTLC technique is validated & most accurate to identifying and quantifying the kaempferol & quercetin which present in foxtail millet has important medicinal value. Flavonols in all six genotypes of *S. italica* show the maximum quantity of referred markers in the extract of *Setaria italica*. This developed HPTLC method is found to be accurate, precise, sensitive, specific, accurate & robust to screen and quantify flavonols. When comparing to GC & HPLC, HPTLC technique has few limitations such as lower plate efficiency & limited developing distance. Still, HPTLC serves as one of the effective tools for evaluating the quality of medicinal plants because of its low requirements, simplicity & low cost. In addition, kaempferol & quercetin are used for reducing the significant pathologies of airway hyperactivity, asthma including neutrophil & eosinophil recruitment, collagen & mucus production and bronchial epithelial cell activation. Therefore, extract of *S. italica* are enriched with flavonoids that can be used in the neutraceutical & pharmaceutical industry.

Ethical approval

The Antidiabetic activity in vivo model protocol was approved by the Institutional Animal Ethical Committee (I.A.E.C.) animals and the care of the laboratory was taken as per the C.P.C.S.E.A. regulation (Reg. No. UIP/ IAEC/ Nov.-2020/12.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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