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SOME UNTOWARD EFFECTS ASSOCIATED WITH THE USE OF THE BIOPREPARATION FROM *Picralima nitida* SEEDS EXTRACT AS ANTIDIABETIC AGENT

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The study was aimed to investigate some untoward effects that could be associated with the use of *P. nitida* as hypoglycemic agent using some biochemical and histological evidences.

The antioxidant property of the plant was determined by using 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity. Biochemical studies in plasma using determining the testes such as blood glucose, alanine and aspartate aminotransferases, gamma glutamyl transferase activities, electrolytes (sodium, potassium and bicarbonate, lipid peroxidation levels, haematological parameters (red blood cell and whole blood cell, platelets, and lymphocyte counts), blood glucose level, lipid profile, and also liver and kidney function tests were performed. Histopathological examinations of the liver, kidney and pancreas were done following the standard Heamatoxylin and Eosin staining method.

Methanol extract of the seeds has the highest antioxidant level (36.73%), indicating higher free radical scavenging activity; followed by aqueous extract (19.36%) and coconut water extract (4.09%). There was significant reduction ($P < 0.05$) in blood glucose of all the treated rats at the end of the experiment (ranging from 55.59% to 41.66%). Significant increase ($P < 0.05$) in body weights of the treated rats were also observed at the end of the treatment (ranging from 9.26% to 38.89%). There was a significant ($P < 0.05$) increase in the hematological parameters in all the extract treated groups. There was also significant decrease ($P < 0.05$) in the lipid profiles of the treated groups. Plasma studied enzymes activities decreased in all treated groups. Ionoregulatory disturbances observed included hyperkalemia and hypernatremia in all the treated groups but were reduced significantly ($P < 0.05$) at the end of the treatment. Urea and bicarbonate concentrations and also of lipid peroxidation level decreased significantly in all the groups. The histopathological studies revealed that the extracts were unable to ameliorate some observable pathologic conditions associated with induced diabetic tissues. Although, diabetes mellitus have been reported to be associated with varied histological changes in different organs, in this study, histological examinations of the pancreas of the treated and untreated groups showed varying degree of degenerations but the extent of severity in the lesions were more pronounced in the extract treated groups. In this relation the obtained results of this study which revealed the hypoglycemic and antioxidant potentials of *Picralima nitida* seed extracts for the treatment of diabetes mellitus should be taken with caution in administering the *P. nitida* seed extract as an hypoglycemic agent.

Key words: *Picralima nitida*, diabetes mellitus, biochemical, histological evidences.

Diabetes is becoming one of the prevalent disease conditions worldwide. Diabetes mellitus, often simply referred to as diabetes, is a group of metabolic disease in which a person has high blood sugar, either because the body does not produce enough insulin, or as a result of insensitivity of target cells to the insulin that is produced [1]. Clinically, there are three main types of diabetes: Type 1 diabetes mellitus, also known as Insulin Dependent Diabetes Mellitus (IDDM) or juvenile onset diabetes is caused by an autoimmune destruction of the insulin secreting β-cells of the pancreas [2]. It usually develops during childhood, adolescence or during early adulthood [3], since the insulin producing β cells are partially or completely loss, the patient requires daily injection of insulin [4]. It is also characterized by a condition in which the pancreas does not produce insulin at all, which could be as a result of damage on

the pancreatic cells. Type 2 is often referred to as Insulin Independent Diabetic which is characterized by a condition in which the pancreas does not produce enough insulin or the insulin produced is not working properly. Gestational diabetes is when pregnant women who have never had diabetes before have high blood glucose level during pregnancy, in some cases it may precede development of diabetes type 2 [2]. Long term diabetes mellitus is commonly accompanied by other cardiovascular risk factors such as dyslipidemia, hypertension, prothrombic factors and microvascular problems involving eyes, kidney and peripheral nerves [5].

Treatment of diabetes is very important because it is a life threatening ailment and presently there is no known cure [6]. Management of diabetes often concentrates on keeping blood sugar levels close to normal (euglycemia) as possible without presenting undue danger to the patient. Dietary management, regular exercise, and use of appropriate medications are some other management regime. Long term diabetes mellitus is commonly accompanied by other cardiovascular risk factors such as dyslipidemia, hypertension, prothrombic factors, ketoacidosis, and non-ketotic hypersosmolar coma and microvascular problems involving eyes, kidney and peripheral nerves failure, poor wound healing, gangrene on the feet which may lead to amputation and erectile dysfunction or impotence [5, 7].

Picralima nitida is a member of the family *Apocynaceae* and order *gentianales* and is widely distributed in the tropical rainforests of Africa, as homesteads or bushes [8, 9]. It can measure up to 20–35 m high when fully grown, having white flower in terminal inflorescence and very large paired fruits (pods). Its leaves are opposite, simple, and entire. The fruit (pod) has many seeds which are usually embedded in the pulpy material known as pulp. The pericarp of the fruit which contains latex is known as the rind [10]. *P. nitida* seed is known as Akuamma seed in Ghana, Osi-igwe seed in Igboland (Eastern Nigeria), Eso Abere in Yorubaland (Western Nigeria) [11].

Picralima nitida (Abere) has been used as antimalarial, antifungal, analgesic and antidiabetic agent. Traditionally, the seeds are crushed or grinded into powdery form and taken orally, for the treatment of malaria, diarrhoea, diabetic, as a painkiller and stimulant. The antimicrobial, antipyretic and antifungal activities of this plant have been reported [12–14]. Pronounced inhibitory activities against asexual erythrocytic forms of *Plasmodium falciparum* was reported to be highest in the root, stem, bark, and fruit rind extracts while the leaf and seed extracts yielded much lower activity or were completely inactive [15]. The antimalarial activity of the plant has been attributed to its alkaloid components [16–18]. The trypanocidal and antileshmanial activities of the plant have been reported [19, 20]. The plant also possesses opium analgesic [21, 22] and anticholinesterase properties [23]. The seeds of *Picralima nitida* extract have also been reported to possess hypoglycaemic, with the coconut water extract of the seed having higher hypoglycaemic effect than the aqueous extract [24, 25].

This study was designed to evaluate some possible untoward effects the *P. nitida* seed extracts could have while using it as an hypoglycemic or antidiabetic agent using both biochemical and histological tests.

Materials and methods

Materials

Fresh seeds of *Picralima nitida* were purchased from Itoku market in Abeokuta, Ogun State, Nigeria and were authenticated by Dr. Aworinde (Plant taxonomist) of the Department of Biological Sciences, Federal University of Agriculture Abeokuta. Technology of the seeds obtaining is contain washing and rinsing with distilled water, mopping with clean tissue paper, cutting into pieces and allowed to air dried after which they were grinded into powdery form.

Animals

42 adult Wistar rats purchased from the College of Veterinary Medicine, University of Agriculture Abeokuta were used for this study. The rats were allowed to acclimatize for seven days in cages at room temperature. They were given free access to commercial feed and water *ad libitum*.

A Technology of Extract Preparation

Twenty-five grams (25 g) each of the dried powdery form of the *Picralima nitida* seed was soaked into 500 ml of distilled water, coconut water and methanol separately. The mixture was shaken vigorously and stirred at regular interval for 48 hrs to allow for equilibration of the mixture. The mixture was then filtered using sterile muslin bag, and the filtrate obtained was concentrated to constant residual weight which was used to calculate percentage yield. The extract obtained was kept inside air

tight container and stored at 4 °C prior to use.

The percentage yield for methanolic, distilled water and coconut water extracts were 74.6%, 25.04% and 35.8% respectively.

Determination of antiradical activity of Picralima nitida

The antiradical activity of the plant was determined by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity as described by Ayoub et al., [26]. The DPPH radical scavenging activity was calculated using the formula:

DPPH radical scavenging activity (%) = $[(A_0 - A_1/A_0) \times 100]$.

Where A_0 is the absorbance of the control, A_1 is the absorbance of extract or standard sample.

Induction of Diabetes Mellitus

Diabetes mellitus was induced in the rats following the method described in [26]. The rats were given a single intraperitoneal injection of 150 mg/kg of freshly prepared alloxan monohydrate dissolved in normal saline after an overnight fasting. Rats that survived after the seventh day with blood glucose concentrations more than 150 mg/100ml were considered diabetic.

Animal grouping and administration of extract

The rats were divided into seven groups of six rats per group and treated as follows:

Group 1: served as control; non-induced rats and were given free access to normal feed and water *ad libitum*. *Group 2:* untreated diabetes-induced rats. *Group 3:* non-induced rats, received coconut water only. *Group 4:* diabetes-induced rats, received coconut water extract (100 mg/kg body weight). *Group 5:* diabetes-induced rats, received distilled water extract (100 mg/kg body weight). *Group 6:* diabetes-induced rats, received methanol extract (100 mg/kg body weight). *Group7:* diabetes induced rats, received 10 mg/kg body weight of glibenclamide (standard drug).

Blood Glucose Determination

A drop of blood was collected by cutting the tip of the tail of conscious rat and placed on the strip of ACCU-CHEK glucometer for the determination of blood glucose.

Periodic Weighing

Body weights of each rats in all the groups was measured every three days throughout the period of the treatment.

Blood collection and dissection

At the end of the experiment, the rats were anesthesized under light diethyl ether, blood samples was collected from each rat by cardiac puncture into heparinised tubes. The tubes were rocked gently to allow proper mixing of the blood. Thereafter, the rats were dissected and the liver and kidneys of each rat excised, mopped, weighed and stored in 10% formalin for histopathological studies.

Preparation of Plasma

Test tubes containing whole blood samples collected were centrifuged at 4000 rpm for five minutes, the supernatant (plasma) was decanted into another tube and labeled accordingly.

Biochemical Analysis

Plasma Aspartate Aminotransferase (AST) and Plasma Alanine Aminotransferase (ALT) assays: AST and ALT was measured spectrophotometrically by the method of Reitman and Frankel [27] as described in Randox kit manual.

Plasma Urea

Urea in the plasma was determined using Urease-Berthelot method as described in Randox kit manual [28].

Plasma Cholesterol

Plasma cholesterol was determined after enzymatic hydrolysis and oxidation according to the method described by Trinder [29] as described in Randox diagnostic kit manual.

Plasma Triglycerides

Plasma triglycerides are determined after enzymatic hydrolysis with lipases according to the method described in Randox kit manual.

Estimation of vLDL-Cholesterol

The concentration of Very Low Density Lipoprotein (vLDL) cholesterol was calculated by modification of the Friedewald formular [30]. vLDL-Cholesterol was calculated as Triglycerol concentration obtained divided by five.

Plasma Gamma-glutamyltransferase (GGT)

Plasma GGT was measured spectrophotometrically by the method described by Szasz [31] as described in Randox Diagnostic kit manual.

Lipid peroxidation

The extent of lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS) by measuring the amount of malondialdehyde (MDA) formed according to the method described by Drapper et al. [32], with slight modifications.

Determination of plasma sodium and potassium

Plasma sodium and potassium concentration were determined using the flame photometry method.

Determination of Bicarbonate concentration

The plasma bicarbonate concentration was determined by titration.

Packed Cell Volume (PCV) Estimation

PCV was determined by the microhaematocrit method [33] as described by Omotainse and Anosa [34].

Haemoglobin (Hb) Concentration

The haemoglobin concentration was determined colorimetrically using Cypress diagnostic kit.

Red Blood Cell (RBC) and Whole Blood Cell (WBC) *count*

The RBC and total WBC counts were carried out by the use of the Neubauer haemocytometer according to the method of Schalm et al. [33] as described by [34].

Histopathological examinations

Histopathological examinations of the liver, kidney and pancreas was done following the standard Heamatoxylin and Eosin (H&E) staining method as described by Krause [35].

Statistical Analysis

Results obtained were expressed as mean ± S.D. The levels of homogeneity among the groups were tested using one-way Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT). Analysis was done using statistical package for Social Science (SPSS) version 16.0. Difference at $P < 0.05$ were considered statistically significant.

Results and discussion

The antiradical activity of the different extracts of Picralima nitida seeds

The antiradical activity of all the studied extract of *P. nitida* seed are summarized in Table 1. The methanolic extraction of the seed has the lowest absorbance among the three when compared with the standard, indicating higher free radical scavenging activity.

Table 1. **The antiradical activity of the different extracts of *Picralima nitida* seeds**

EXTRACT	ABSORBANCE	% INHIBITION
AQUEOUS EXTRACT	0.444	19.36
METHANOLIC EXTRACT	0.348	36.73
COCONUT WATER EXTRACT	0.528	4.09
STANDARD (VIT C)	0.0015	100

Body weight of Animals

Table 2 shows the summary of the effect of the plant seed extracts on body weights of the animals. After the intraperitoneal injection of 150 mg/kg alloxan monohydrate, mean body weight of all the alloxan-induced groups were significantly reduced ($P < 0.05$). During treatment, all diabetic-induced but treated rats showed increase ($P < 0.05$) in body weight when compared with their weights after induction prior treatment. The result showed higher weight gain in the rats treated with methanolic and coconut water extract of *P. nitida* seeds when compared to other treated groups. There was consistent increase in weight of the normal control groups while the diabetes induced but not treated group showed a consistent decrease in weight throughout the experiment.

The summary of the blood glucose levels of the rats is presented in Table 3. Before induction of diabetes the mean blood glucose level of the rats ranged from 81.67 to 91.33 mg/dl. There was increase in the mean blood glucose of all the induced groups, having values between 223.33 and 265.33 mg/dl which confirmed them diabetic. All the groups treated with the extracts had decreased mean glucose values respectively. Glibenclamide treated rats also showed decrease in mean blood glucose from 245.00 to 110.67 mg/dl at the end of the experiment. However, the diabetic untreated rats maintained high plasma glucose.

Effects of treatment on the haematological parameters are presented in Table 4. The Packed Cell Volume (PCV) and Hb contents of the alloxan-induced but untreated group and the induced but distilled water extract treated group were significantly lower ($P < 0.05$) than the control. Only the induced but methanol extract treated group had no significant difference in the RBC content when compared with the control. The WBC and lymphocyte contents of all the alloxan induced groups were significantly lower ($P < 0.05$) than that of the control, with the alloxan-induced untreated group having the lowest value. Comparison the lymphocyte contents of the induced groups with the non-induced group, only the alloxan-induced and the glibenclamide-treated groups had a significant increase ($P < 0.05$). Alloxan induced-methanolic extract-treated group had the least lymphocyte level.

Table 5 depicts the effect of treatments on some lipid profile parameters. The result showed that alloxan caused significant ($P < 0.05$) increase in the lipid profile (total

Table 2. **Effect of treatments of *Picralima nitida* seeds extract on body weight (g) of rats**

GRP	TREAT-MENT	DAY 1	DAY 7	DAY 10	DAY 13	DAY 16	DAY 19
1	Control	80 ±11.18^{a}	99 ±13.42^{a}	107±10.37^{b}	117±10.37^{b}	122 ±7.58^{bc}	132±10.37^{bc}
2	Alloxan only	129 ±4.18^{b}	118±4.48^{b}	115 ±4.08^{b}	111 ±7.50^{b}	109 ±9.02^{a}	108±11.68^{ab}
3	CW only	80 ±11.18^{a}	103 ±13.0^{ab}	115±11.18^{b}	128±13.51^{bc}	143±11.51^{cd}	151 ±15.58^{c}
4	A + CW	120±20.92^{b}	104±11.94^{ab}	104±20.43^{ab}	111 ±22.75^{b}	123±16.47^{bc}	134 ±13.77^{c}
5	A + DS	105±20.92^{b}	92 ±16.05^{a}	90 ±13.69^{a}	88±9.75^{a}	97 ±10.41^{a}	98 ±17.56^{a}
6	A + MET	124±6.88^{b}	115±10.61^{b}	123±18.37^{b}	142±5.77^{c}	147±10.41^{d}	150±10.00^{c}
7	A + GLIB	126±29.88^{b}	109±20.43^{ab}	123±16.05^{b}	127±23.63^{bc}	132±27.54^{bcd}	142±27.54^{c}

Notes: hereinafter – values are mean ± SD. Values within the same row with different superscripts are significantly different at $P < 0.05$, ($n = 6$); CW only: Coconut water only; A + CW: Alloxan + Coconut water extract of *P. nitida* seed; A + DS: Alloxan + Distilled water extract of *P. nitida* seed; A + MET: Alloxan + Methanol extract of *P. nitida* seed; A + GLIB: Alloxan + Glibenclamide.

Table 3. **Effect of treatments of *Picralima nitida* seeds on the plasma glucose level (mg/dL) of rats**

GR	TREAT-MENT	DAY 1	DAY 7	DAY 10	DAY 13	DAY 16	DAY 19
1	Control	90.60±5.18^{a}	84.80±3.96^{a}	85.20±5.26^{a}	81.6±6.27^{a}	84.6±4.22^{a}	81.4±5.94^{ab}
2	Alloxan only	91.33±6.51^{a}	265.33±41.59^{b}	292±66.73^{c}	250.67±35.02^{c}	249.67±40^{e}	247.67±46.2^{d}
3	CW only	83.60±5.59^{a}	82.80±5.07^{a}	83±4.47^{a}	79.6±8.68^{a}	91.8±1.3^{a}	75±9.03^{a}
4	A+ CW	86.75±10.99^{a}	234.25±32.09^{b}	227.75±31.17^{b}	204±9.35^{b}	194.75±8.66^{d}	144.5±45.2^{c}
5	A + DS	81.67±7.57^{a}	223.33±29.19^{b}	287±62.64^{c}	223.33±34.5^{bc}	174±3.46^{cd}	128.67±21^{c}
6	A+MET	88.67±3.06^{a}	249.33±39.53^{b}	214.67±11.72^{b}	196.33±29.02^{b}	150.67±5.13^{bc}	122.67±6.11^{bc}
7	A+GLIB	79.33±14.01^{a}	245.00±33.45^{b}	203.33±39.07^{b}	193±29.51^{b}	134.67±22.37^{b}	110.6±4.58^{abc}

Table 4. **Effect of treatments of *Picralima nitida* on some hematological parameters of rats**

GRP	TREAT-MENT	PCV (%)	Hb (g/dl)	RBC ($\times 10^6$/mm^3)	WBC ($\times 10^3$ mm^3)	PLAT ($\times 10^3$ mm^3)	LYMP (%)
1	**Control**	45.08±3.03^{cd}	15.05± 0.89^{c}	7.66±0.48^{d}	10.10±0.69^{d}	638.20±7.86^{e}	64.00±2.55^{ab}
2	**Alloxan only**	31.53±1.67^{a}	10.34±0.43^{a}	5.43±0.31^{a}	5.13±0.21^{a}	217.00±6.25^{a}	77.67±3.51^{d}
3	**CW only**	41.40±2.69^{bc}	13.99±0.78^{bc}	7.30±0.29^{cd}	9.04±0.27^{d}	637.40±7.89^{e}	65.20±2.28^{b}
4	**A + CW**	41.08±0.72^{bc}	13.69±0.59^{bc}	6.88±0.21^{bc}	7.68±0.33^{c}	244.67±8.60^{c}	62.75±2.87^{ab}
5	**A + DS**	38.40±6.27^{b}	12.74±2.08^{b}	6.43±0.47^{b}	6.47±0.40^{c}	294.67±8.33^{c}	63.00±3.61^{ab}
6	**A + MET**	46.70±1.45^{d}	15.24±0.52^{c}	7.80±0.26^{d}	8.33±0.45^{c}	419.33±6.03^{d}	60.00±3.46^{a}
7	**A + GLIB**	42.73±2.18^{bc}	14.20±0.63^{bc}	7.03±0.47^{bc}	5.80±0.36^{ab}	228.00±11.14^{a}	71.33±1.53^{c}

Table 5. **Effect of treatments on some lipid profile parameters of rats**

GRP	TREATMENT	Total CHOL (mg/dl)	TRIG (mg/dl)	vLDL (mg/dl)
1	Control	87.20±3.69^{a}	62.40±3.49^{ab}	12.48±0.69^{ab}
2	Alloxan only	120.47±2.99^{e}	96.70±2.21^{d}	19.34±0.44^{d}
3	CW only	87.72±3.05^{a}	64.80±2.89^{ab}	12.88±0.52^{ab}
4	A + CW	97.88±1.42^{b}	67.03±7.55b^{c}	13.41±1.51^{bc}
5	A + DS	110.67±3.16^{d}	74.40±4.79^{c}	14.88±0.96^{c}
6	A + MET	103.63±4.30^{c}	67.23±5.39^{bc}	13.45±1.08^{bc}
7	A + GLIB	98.03±2.85^{b}	58.50±5.99^{a}	11.70±1.19^{a}

cholesterol, triglyceride and vLDL) of the treated and untreated groups when compared with the control. However, treatments with the *P nitida* seed extracts showed significant ($P < 0.05$) reduction of the lipid profile levels. The cholesterol, triglycerol, and very Low Density Lipoprotein values of the alloxan-induced glibenclamide treated group were significantly lowered ($P < 0.005$) when compared with the control. The alloxan-induced but distilled water treated group showed a significant increase in the lipid profile when compared with all other groups.

The influence of treatments on some liver function test parameters is presented in Table 6. The alloxan- induced but untreated group showed a significant increase in the activities of all marker enzymes namely; GGT, AST, and ALT, when compared with the control. None of the treatments was able to bring the activities of these enzymes to the level of the control.

Electrolytes and urea plasma levels of the different groups of rats were presented in Table 7. There was no significant different ($P > 0.05$) between distilled water extract treated group and glibenclamide treated group as well as between the control and group administered coconut water only. All the renal function parameters (urea, sodium, potassium and bicarbonate) of the glibenclamide group reduced significantly ($P < 0.05$) when compared with the control group.

Table 8 shows the summary of values of MDA concentration in the plasma of different groups. The MDA content of the extract treated groups decreased ($P < 0.05$) when compared to the control. However, the group administered alloxan only had a significant increase ($P < 0.05$) in the MDA content when compared to the control.

HISTOLOGICAL STUDIES

The liver

Histopathological examination of the liver of the rats in groups 1 and 3 showed normal appearance/architecture of the hepatocytes, bile ducts and blood vessels (Fig. 1).

Examination of the liver of untreated alloxan induced diabetic group showed lesions which include mononuclear cell infiltration of the portal triads particularly around the bile duct (Fig. 2), necrosis of the hepatocyte, diffuse disorganisation of the hepatocyte cords and diffuse distribution of mononuclear cells in the sinusoids. Photomicrograph of the treated groups also showed varying degree of lesions (Fig. 2–7). The degree of lesions in the treated groups are however milder compared to the untreated group.

Table 6. **Effect of treatments on activities of some markers enzymes of hepatotoxicity in rats**

GRP	TREATMENT	GGT (U/L)	AST (U/l)	ALT(U/l)
1	Control	23.82 ± 2.10^{a}	22.65 ± 2.90^{a}	37.02±2.20^{a}
2	Alloxan only	67.10 ± 3.30^{e}	79.01 ± 0.72^{f}	54.38±4.45^{d}
3	CW only	25.00 ± 1.49^{a}	35.93 ± 1.82^{b}	37.76±1.19^{a}
4	A + CW	34.38 ± 2.11^{bc}	45.16 ± 4.83^{c}	38.78±1.75^{ab}
5	A + DS	38.90 ± 1.92^{d}	55.17 ± 4.81^{d}	40.63±1.31^{ab}
6	A + MET	35.40 ± 3.48^{cd}	37.80 ± 0.70^{b}	42.00±1.76^{bc}
7	A + GLIB	30.97 ± 3.29^{b}	60.69 ± 1.54^{e}	45.67±3.79^{c}

Table 7. **Effect of treatments of *Picralima nitida* seeds on renal function parameters in rats**

GRP	TRTMENT	UREA (mg/dl)	SODIUM (mmol/L)	POTASSIUM (mmol/L)	BICARBONATE (mmol/L)
1	Control	19.58±1.86^{a}	73.00±7.42^{a}	3.56±0.61^{a}	12.00±1.23^{a}
2	Alloxan only	54.43±4.15^{e}	131.67±3.50^{d}	9.53±0.31^{d}	22.33±1.16^{d}
3	CW only	19.64±2.29^{a}	86.40±1.67^{b}	7.02±0.33^{bc}	16.00±1.00^{b}
4	A + CW	44.05±1.82^{d}	94.75±12.37^{bc}	7.13±0.61^{bc}	16.50±2.08^{bc}
5	A + DS	35.43±2.45^{c}	98.67±9.29^{c}	7.83±0.72^{c}	18.33±1.16^{c}
6	A + MET	26.97±1.06^{b}	104.33±3.22^{c}	8.07±0.59^{c}	18.00±1.00^{bc}
7	A + GLIB	34.93±1.59^{c}	85.00±2.00^{b}	6.10±2.23^{b}	13.67±0.58^{a}

Table 8. **Effect of treatments of P*icralima nitida* seeds on lipid peroxidation level in rats**

GRP	TREATMENT	MDA (µmol/L)
1	Control	0.48±0.33^{a}
2	Alloxan only	3.20±0.76^{c}
3	CW only	0.49±0.36^{a}
4	A + CW	1.24±0.38^{b}
5	A + DS	1.25±0.27^{b}
6	A + MET	1.27±0.21^{b}
7	A + GLIB	1.19±1.91^{b}

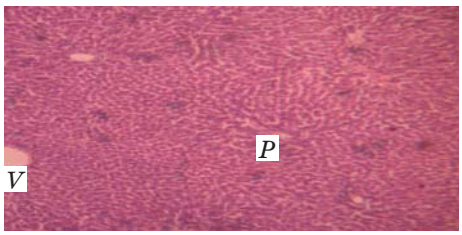

Fig. 1. **Photomicrograph of the liver section showing normal appearance of the perivascular spaces of the hepatocytes (*P*) and blood vessel (*V*) in the liver:**
of the normal control groups (1 and 3). ×200 H&E

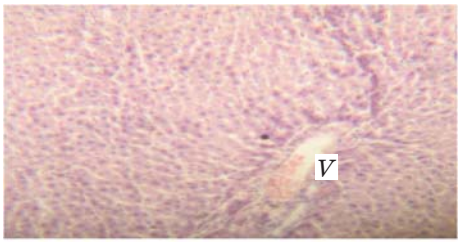

Fig. 2. **Photomicrograph of the liver section showing infiltration of perivascular space (*V*) of the hepatocytes:**
in the untreated diabetes induced group. ×200 H&E

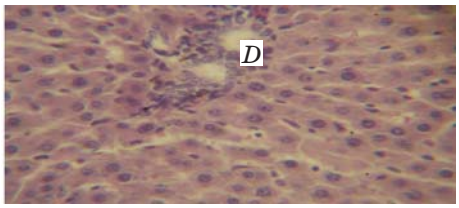

Fig. 3. **Photomicrograph of the liver section showing bile duct hyperplasia (*D*) in the hepatocytes:**
of the induced groups treated with 100mg/kg of coconut water and distilled water extraction of *P. nitida*. ×400 H&E

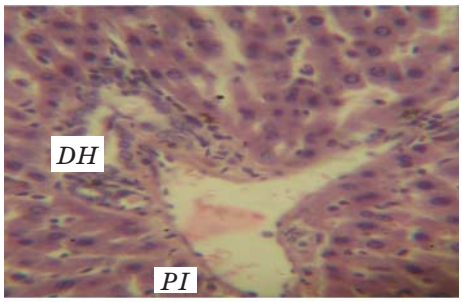

Fig. 4. **Photomicrograph of the liver section showing perivascular cellular infiltration (*PI*) and disorganized hepatic cords in the hepatocytes (*DH*):**
of the diabetic group treated with glibenclamide. ×400 H&E

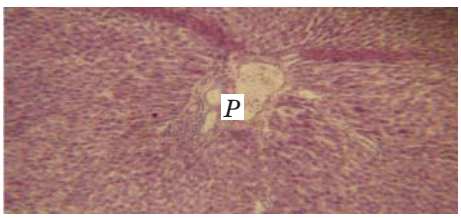

Fig. 5. **Photomicrograph of the liver section showing severe periportal cellular infiltration (*P*) and disorganized hepatic cords of the hepatocytes:**
in the diabetic group treated with coconut water and methanolic extraction of *P. nitida* seeds. ×200 H&E

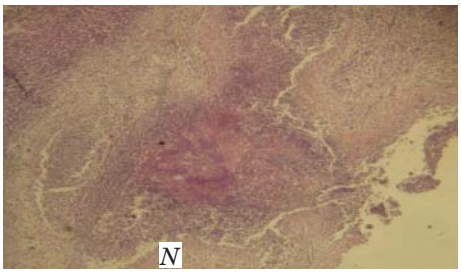

Fig. 6. **Photomicrograph of the liver section showing severe necrosis of hepatocytes (*N*):**
in the group treated with methanolic extraction of *P. nitida* seeds. ×200 H&E

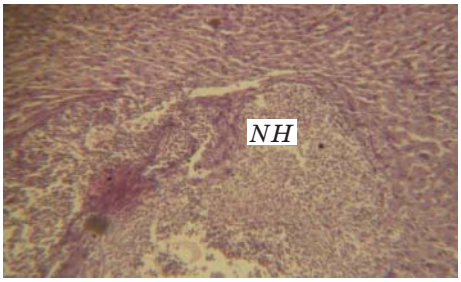

Fig. 7. **Photomicrograph of the liver section showing necrosis of the hepatocytes (*NH*).**
×200 H&E

The Kidney

Photomicrograph of the kidney of the control rats revealed normal appearance of the glomeruli, tubules and blood vessels (Fig. 8).

Histopathological examination of the untreated group showed necrosis of the Bowman's capsule, enlarged perivascular space (oedema) of the kidney and tubular necrosis of the kidney. The group treated with coconut water extraction showed diffuse massive tubular epithelial cell degeneration, glomerular necrosis, congestion of the vessels and degeneration of the renal tubules. Similar lesions or more severe than these were seen in the other treated groups.

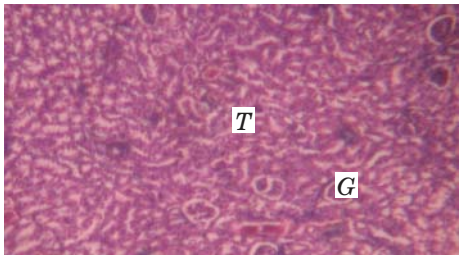

Fig. 8. **Photomicrograph of a section of the kidney of control group:**
showing the normal appearance of the glomerulus (*G*), tubules (*T*), and blood vessels of the kidney. ×200 H&E

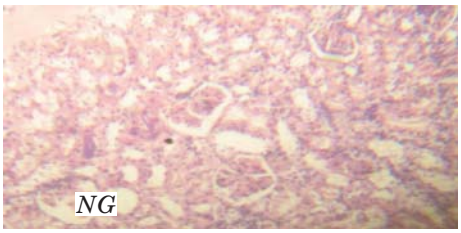

Fig. 9. **Photomicrograph of a section of the kidney:**
showing necrotic glomerulus (NG) of the diabetic groups treated with coconut water, distilled water and methanolic extraction of the seeds of *P. nitida*. ×400 H&E

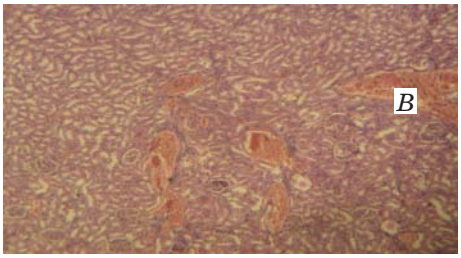

Fig. 10. **Photomicrograph of a section of kidney showing congested blood vessels (*B*):**
in the groups treated with coconut water, distilled water, methanolic extraction of *P. nitida* seeds, also in glibenclamide treated group. ×200 H&E

The Pancreas

The lesions observed in the different groups in the pancreas were as summarized in the Table 9 and Figures 11–18. These lesions include necrosis of the exocrine and fat cells, enlargement of the islet of Langerhans, hyperplastic ducts, proliferation of the blood vessels and blockage of the tubules by necrotic and inflammatory cells. Other lesions include the thickening of the tubular wall, perivascular necrosis and fibrosis as well as vascular degeneration of the cells of the islets.

Diabetes is a common and very prevalent disease affecting the people in both developed and developing countries [36]. According to the World Health organization's report in 2006, at least 171 million people worldwide suffer from diabetes [37]. The incidence of diabetes mellitus is increasing rapidly and it is estimated that by the year 2030, this number will double [37]. The primary goal of treatment of diabetes is to bring the elevated blood sugars down to a normal range in order to alleviate or prevent complications.

The use of herbal products for medicinal benefits has played an important role in nearly every culture on earth and for many years, not only because they are relatively cheap and readily available, but also due to ease with

Table 9. **Summary of the severity of lesions in the pancreas in the various treatment groups**

LESIONS	GROUPS						
	1	2	3	4	5	6	7
Necrosis of exocrine and fat cells	–	++	–	+	+	+	+
Enlarged islet of langerhans	–	++	–	+++	+++	+++	+
Hyperplastic ducts and ductular proliferation	–	–	–	+++	+++	++	+
Blockage of ductular lumen by inflammatory cells	–	++	–	+	++	++	++
Thickening of the walls of the tubules and vessels	–	–	–	++	+++	++	+
Peri-vascular necrosis and fibrosis	–	–	–	++	+++	++	++
Vascular degeneration of the cells of the islets and fat cells	–	–	–	+	+	+++	+

Notes: – not present; + mild; ++ moderate; +++ severe.

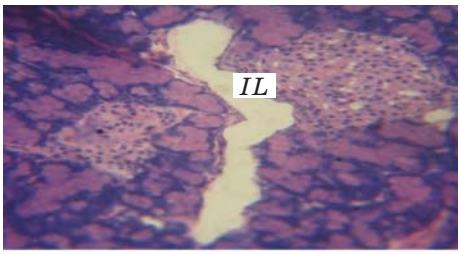

Fig. 11. **Photomicrograph of the pancreas section:** showing of normal appearance of the langerhans isletsf (*IL*) in the pancreas of normal control groups. ×400 H&E

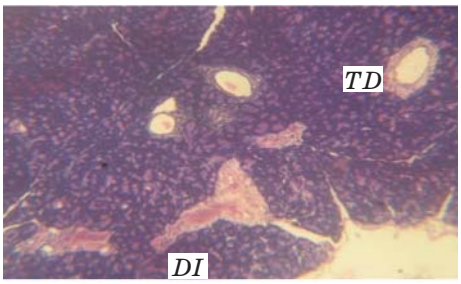

Fig. 12. **Photomicrograph of a section of the pancreas:** showing degeneration of the langerhans islets (*DI*) and thickened duct (*TD*). ×100 H&E PANCREAS: hyperplastic ductular wall

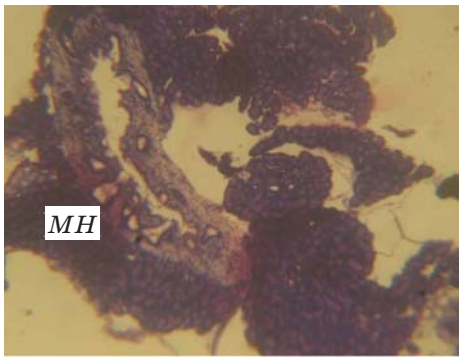

Fig. 13. **Photomicrograph of a section of the pancreas:** showing mild hyperplasia (*MH*) of the ductular wall. ×100 H&E

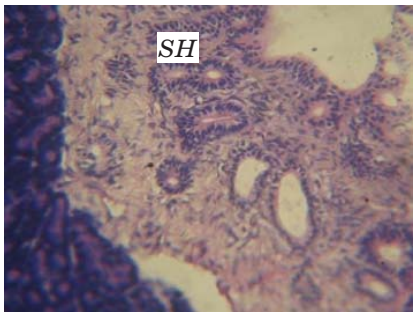

Fig. 14. **Photomicrograph of a section of the pancreas:** showing severe hyperplasia (*SH*) of the ductular wall. ×400 H&E

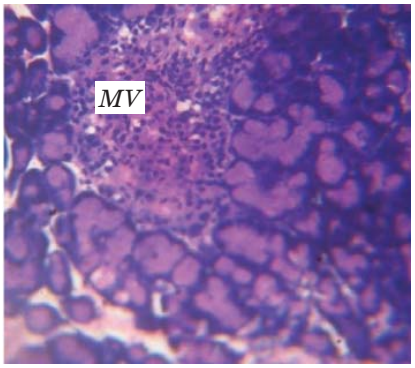

Fig. 15. **Photomicrograph of a section of the pancreas:** showing mild vacuolation (*MV*) of islet cells. ×200 H&E

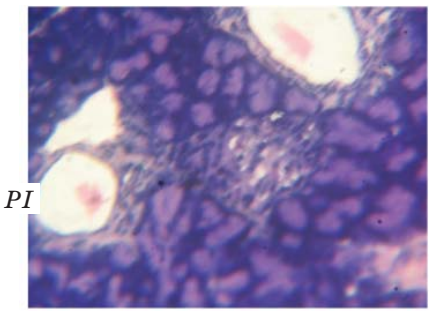

Fig. 16. **Photomicrograph of a section of the pancreas:** showing periductal cellular infiltration (*PI*). ×200H&E

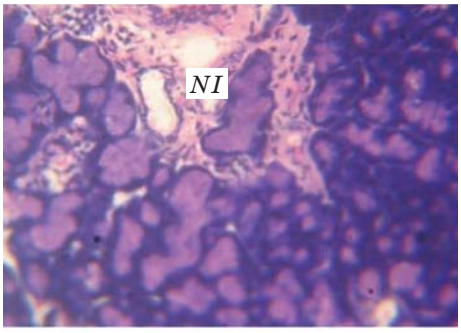

Fig. 17. **Photomicrograph of a section of the pancreas:** showing necrotic islet (*NI*). ×400H&E

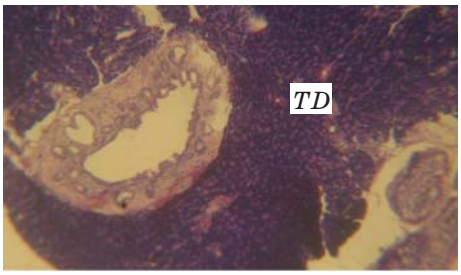

Fig. 18. **Photomicrograph of a section of the pancreas:** showing thickened pancreatic duct. ×200 H&E

which they can be administered. One of such disease condition in which herbal products is being advocated for it management is diabetes mellitus [38].

Changes in body weight are usually considered in all physiological conditions. The diabetic control group showed a remarkable decrease in body weight at the end of the study. On the other hand, increase in body weight was observed in all the seed extract treated groups and also in the control groups.

Increased oxidative reactions level shows the production of free radicals and reactive oxygen species which are formed under normal physiological conditions but may become deleterious when they are not quenched by the antioxidant molecule(s) within the systems [39]. The antiradical activity of the different extracts of *Picralima nitida* seeds showed that the methanolic extract of the seed have the highest percentage inhibition 36.73%, thus having higher free radical scavenging activity, this is followed by the aqueous extract 19.36%, while the coconut water have the least percentage inhibition of 4.09%.

There have been reports on the increase in blood glucose during diabetes in rats and humans [40–44]. The blood glucose lowering effect of *P. nitida* extract after induction by alloxan is in agreement with the reports of Salihu et al. [24] and Inya-Agha et al. [45]. This could be attributed to the presence of some active ingredient(s) that enhances its ability to increase the permeability of cell plasma membrane to glucose. There has been an increase in the prevalence of atherosclerosis and hyperlipidaemia among diabetics worldwide [46]. This is said to occur as a result of the altered lipid profile in the serum of diabetic patients [47, 48], which is as a result of increase in plasma triglycerides and total cholesterol levels. Hypercholesterolemia has been reported to occur in alloxan-diabetic rats [49, 50]. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [51]. This was observed in the lipid profile of the diabetic control group. In our study there was a significant decrease in the lipid profiles of all the three groups treated with extracts of *Picralima nitida* seeds. However, the group treated with coconut water seed extract showed a significant decrease in total cholesterol level when compared with the control, followed by the methanolic extract treated group. There was also a significant decrease in the total triglyceride and vLDL of all the seed extract treated groups. However, glibenclamide been a standard drug for diabetes showed a most pronounced decrease in the lipid profile levels. The observed hypolipidemic effect may be attributed to decreased blood glucose levels and decreased cholesterogenesis as well as fatty acid synthesis. This similar to the report of Bopanna et al. [52].

Anaemia is a medical condition caused by an abnormally low number of RBC, PCV and the Hb content. The anaemia occurring in diabetes is due to the increased non-enzymatic glycosylation of RBC membrane protein which correlates with hyperglycemia [1]. This study showed a marked reduction in the PCV and Hb content in the diabetes induced but untreated group. The treated groups showed improvement in the PCV and Hb content. This indicates that the seed extract of *Picralima nitida* might probably contain some phytochemicals that were able to increase or boast the level of packed cell volume and Hb concentration.

Aspartate- (AST) and alanine transaminase (ALT) are found predominantly in the liver, and are biochemical markers for liver injury in patients [3]. Elevated activity of both enzymes above normal is an indication of possible liver damage. Although, the diabetes induced groups all had high activities of AST and ALT when compared with the control, there was

significant reduction in the activities of these enzymes in all the extract treated groups. Only the untreated group had very high levels, thus indicating liver impairment.

Plasma GGT is a measure of the hepatobiliary system. Elevated plasma GGT activity has been reported to be found in diseases of the liver, biliary system and pancreas [53]. There was a significant reduction in the plasma GGT of the treated groups. This indicates an improvement in the hepatobilary system.

High lipid peroxidation level is a sign of oxidative damages. Oxygen free radicals species have been implicated in the pathogenesis of diabetes mellitus [54]. This was seen in all the diabetes — induced groups, but the extract treated groups showed significant decrease in the extent of lipid peroxidation. This could probably be due to the presence of the alkaloids and glycosides present in the seed extracts. However, the untreated group had a very high level lipid peroxidation.

Picralima nitida seeds have been reported to be rich in alkaloids and glycosides [8, 24]. Glycosides present in the seeds have been reported to be responsible for the blood glucose reduction through pancreatic and extra pancreatic effect. These pancreatic and extra pancreatic effects on the blood glucose could be through prevention of hepatic glucose over production, increase in glucose uptake by the muscles, inhibition of gastric emptying and/or increase in glucose permeability of plasma cell membrane.

Although, diabetes mellitus have been reported to be associated with varied histological changes in different organs, in this study, histological examinations of the pancreas of the treated and untreated groups showed varying degree of degenerations but the extent of severity in the lesions were more pronounced in the extract treated groups. The histopathological examination of the liver and kidney also revealed varying degree of degenerations in these organs. This suggests the inability of the different extracts administered to, either prevent the uptake of alloxan into the cells, or to interfere with its destructive actions in the cells. It could therefore be concluded that although, *Picralima nitida* seed extracts were seen to reduce diabetes to some extent (with the methanolic extract having highest antioxidant property than the others), however, this plant seed extract did not produce any observable ameliorative or regenerative properties to the organs damaged by alloxan. Thus, caution should be exercised while taking *P. nitida* seed extracts decoctions (traditionally) or recommending it as a possible remedy against diabetes.

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ДЕЯКІ НЕСПРИЯТЛИВІ ЕФЕКТИ, ПОВ'ЯЗАНІ З ВИКОРИСТАННЯМ БІОПРЕПАРАТУ З ЕКСТРАКТУ НАСІННЯ *Picralima nitida* ЯК АНТИДІАБЕТИЧНОГО АГЕНТА

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За допомогою біохімічних і гістологічних методів вивчено деякі несприятливі ефекти, які можуть бути пов'язані з використанням біопрепарату з насіння *P. nitida* як гіпоглікемічного засобу. Антирадикальну активність біопрепарату визначали за допомогою стабільного радикала 1,1-дифеніл-2-пікрилгідразилу. Біохімічні дослідження включали визначення активності аланін- і аспартатаміно-, гамма-глутамілтрансферази, концентрації глюкози крові, електролітів (натрій, калій і бікарбонат), рівня пероксидного окиснення ліпідів. Визначали також параметри крові (кількість

НЕКОТОРЫЕ НЕБЛАГОПРИЯТНЫЕ ЭФФЕКТЫ, СВЯЗАННЫЕ С ИСПОЛЬЗОВАНИЕМ БИОПРЕПАРАТА ИЗ ЭКСТРАКТА СЕМЯН *Picralima nitida* В КАЧЕСТВЕ АНТИДИАБЕТИЧЕСКОГО АГЕНТА

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С помощью биохимических и гистологических методов изучены некоторые неблагоприятные эффекты, которые могут быть связаны с использованием биопрепарата из семян *P. nitida* в качестве гипогликемического средства.

Антирадикальную активность биопрепарата определяли с помощью стабильного радикала 1,1-дифенил-2-пикрилгидразила. Биохимические исследования включали определение активности аланин- и аспартатамино-, гаммаглутамилтрансферазы, концентрации глюкозы крови, электролитов (натрий, калий и

сумарних клітин крові, еритроцитів і лімфоцитів), функцій печінки і нирок, а також ліпідний профіль. Гістопатологічні дослідження печінки, нирок і підшлункової залози було виконано за допомогою стандартного методу фарбування гематоксилін-еозином.

За введення щурам Вістар максимальну антиоксидантну активність порівняно з контрольними розчинами (водний і водно-кокосовий екстракти) мав метанольний екстракт насіння (36,73%), що вказує на його високий ступінь нейтралізації вільних радикалів, далі — водний (19,36%) і водно-кокосовий екстракти (4,09%). У всіх оброблених щурів наприкінці експерименту спостерігали значне зниження ($P < 0,05$) концентрації глюкози крові (в діапазоні від 55,59% до 41,66%) та істотне збільшення ($P < 0,05$) маси тіла (в межах від 9,26% до 38,89%). Для всіх груп, які отримували екстракт насіння, характерним було збільшення гематологічних показників ($P < 0,05$), а також достовірне ($P < 0,05$) зниження параметрів ліпідних профілів і активності ензимів плазми. У всіх групах, які отримували екстракт, спостерігали іонорегуляторні порушення, зокрема гіперкаліє- і гіпернатріємію, однак вони були значно меншими ($P < 0,05$) наприкінці обробки. У всіх дослідних групах були істотно нижчі концентрація сечовини і бікарбонату та рівень пероксидного окиснення ліпідів. Гістопатологічні дослідження показали, що екстракти не коригувати деякі спостережувані патологічні стани тканин, індуковані діабетом. Хоча, згідно з даними літератури, цукровий діабет супроводжується різними гістологічними змінами в різних органах, одержані в цій роботі результати морфологічного вивчення тканини підшлункової залози в групах тварин, які отримували і не отримували біопрепарат, свідчать про наявність ушкоджень різного ступеня, однак їх тяжкість в осередках ураження була більш виражена за введення біопрепарату екстракту насіння. У зв'язку з цим одержані дані, які дали змогу визначити гіпоглікемічну і антиоксидантну активність біопрепарату з екстрактів насіння *Picralima nitida* як антидіабетичного засобу, дають підстави стверджувати, що використовувати його для лікування цього захворювання слід з обережністю.

Ключові слова: цукровий діабет, екстракт насіння *Picralima nitida*, біохімічні, гістологічні дослідження.

бикарбонат), уровня пероксидного окисления липидов. Определяли также параметры крови (число суммарных клеток крови, эритроцитов и лимфоцитов), функций печени и почек, а также липидный профиль. Гистопатологические исследования печени, почек и поджелудочной железы были выполнены с помощью стандартного метода окрашивания гематоксилин-эозином.

При введении крысам Вистар максимальной антиоксидантной активностью по сравнению с контрольными растворами (водный и водно-кокосовый экстракты) обладал метанольный экстракт семян (36,73%), что указывает на его высокую степень нейтрализации свободных радикалов; затем следовал водный (19,36%) и водно-кокосовый экстракты (4,09%). У всех обработанных крыс в конце эксперимента наблюдали значительное снижение ($P < 0,05$) концентрации глюкозы крови (в диапазоне от 55,59% до 41,66%) и существенное увеличение ($P < 0,05$) массы тела (в пределах от 9,26% до 38,89%). Для всех групп, получавших экстракт семян, характерным было увеличение гематологических показателей ($P < 0,05$), а также достоверное ($P < 0,05$) снижение параметров липидных профилей и активности энзимов плазмы. Во всех группах, получавших экстракт, наблюдали ионорегуляторные нарушения, в частности гиперкалие- и гипернатриемию, но они были значительно менее выражены ($P < 0,05$) в конце обработки. Также во всех опытных группах была существенно ниже концентрация мочевины и бикарбоната, а также уровень пероксидного окисления липидов. Гистопатологические исследования показали, что экстракты не корригировали некоторые наблюдаемые патологические состояния тканей, индуцированные диабетом. Хотя, согласно данным литературы, сахарный диабет сопровождается различными гистологическими изменениями в различных органах, полученные в этой работе результаты морфологического изучения ткани поджелудочной железы в группах животных, получавших и не получавших биопрепарат, свидетельствуют о наличии повреждений различной степени, однако степень их тяжести в очагах поражения была более выражена при введении биопрепарата из экстракта семян. В связи с этим полученные данные, которые позволили определить гипогликемическую и антиоксидантную активность биопрепарата из экстрактов семян *Picralima nitida* в качестве антидиабетического средства, дают основание утверждать, что использовать его для лечения этого заболевания следует с осторожностью.

Ключевые слова: сахарный диабет, экстракт семян *Picralima nitida*, биохимические и гистологические исследования.