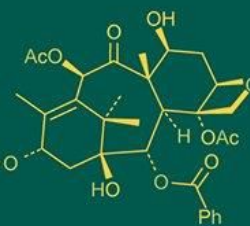


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Effect of jigsaw on serum inflammatory markers and renal indices of wistar rats with streptozotocin induced diabetes mellitus

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Abstract

This study examines the effect of jigsaw on serum renal parameters of diabetic wistar rats. Diabetes mellitus is the term used to characterize a collection of metabolic illnesses in which an individual has maintained excessive blood sugar levels as a result of either insufficient insulin production, insensitivity of body cells to insulin, or both. Individuals who have high blood sugar are more prone to have polyuria, or frequent urinating, polydipsia (Increase thirsty) and polyphagia (frequent hunger). Twenty (20) albino wistar rats weighing 80-130 g were obtained and grouped into three groups of 5 animals; group A was given only distilled water and served as the normal control, group B was induced with diabetes mellitus using Streptozotocin (STZ) but not treated and served as the negative control, group C was induced with diabetes mellitus and treated with Glucophage (standard drug) and served as positive control while group D was induced with diabetes mellitus and treated with Jigsaw. The duration of treatment was thirty-one (31) days. Animals were sacrificed 12 hours after the last treatment under chloroform vapour and whole blood was obtained via cardiac puncture, allowed to clot and then centrifuge into serum. The parameters estimated for in this study were serum creatinine, urea, electrolytes, cystatin-C and Kidney injury molecule 1 (KIM-1), Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP), serum procalcitonin (PCT). Result obtained revealed that the treatment significantly ($p < 0.05$) reduced the concentrations of biomarkers analyzed for in test group (group D) in comparison to the negative control group (group B) whose values were significantly ($p < 0.05$) increased compared with that of the normal control group (group A). Serum creatinine concentration for group D (67.10 ± 0.33) was significantly ($p < 0.05$) lowered compared with that of negative control group B (106.58 ± 0.27), Hemoglobin A1c (HbA1c) concentration for group D (3.78 ± 0.05) was significantly ($p < 0.05$) lowered compared with that of negative control group B (7.78 ± 0.08) and also Kidney injury molecule 1 (KIM-1) concentration for group D (26.60 ± 0.45) was significantly ($p < 0.05$) lowered compared with that of negative control group B (31.20 ± 1.16). It is therefore concluded that the treatment could have been responsible for the results obtained in this study and hence, Jigsaw can be said to be effective in managing diabetes mellitus and associated complication.

Keywords: Diabetes, insulin, serum, renal indices

Introduction

Elevated blood glucose levels are a common trait shared by illnesses referred to as diabetes mellitus. Diabetes mellitus is classified into two main broad categories: type 1 and type 2. Previously known as insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes mellitus, type 1 diabetes mellitus makes up 5% to 10% of all cases of diabetes mellitus and typically affects children or young adults. The autoimmune destruction of the β cells in the pancreas, which release insulin, is the cause of this condition. According to Eisenberth *et al.*, (2017) ^[1], the process entails a smoldering damaging process that can last for several years and eventually results in the failure of insulin secretion.

Ninety to ninety-five Percent of diabetes patients diagnosed with type 2 diabetic mellitus, which is becoming more common, particularly among racial and ethnic minorities. The genes that cause type 2 diabetes mellitus are varied and polygenic, and they have been found in a few subtypes of the illnesses. There are several genes associated with diabetes mellitus, and a patient's involvement in multiple genes is likely. Ageing, obesity, and a sedentary lifestyle are a few of the recognized environmental influences. Some of the hormonal, genetic, and environmental variables that predispose to obesity have been identified, and obesity is most

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likely the primary environmental factor causing the rising incidence of type 2 diabetes (Boutari and Mantzoros, 2022)^[23]. For the vast majority of individuals with type 2 diabetes mellitus, insulin resistance is a distinctive metabolic defect. This deficit can be shown several years before hyperglycemia develops in the prediabetes state (Echouffo-Tcheugi and Selvin, 2021)^[21]. Insulin resistance causes the β cell to produce more insulin, and if this is enough, compensatory hyperinsulinemia keeps glucose levels within normal limits. Relative insulin insufficiency develops when β -cell function gradually declines in those who are destined to acquire diabetes (Lackey and Olefsky, 2016)^[22]. Thus, the decompensated hyperglycemic diabetic condition is caused by β -cell loss in conjunction with insulin resistance. Obesity is the principal risk factor for type 2 diabetes mellitus, and 80–90% of those who have the condition are overweight or obese. Obese individuals may require high doses of insulin administration to sustain normal blood glucose concentrations since obesity induces insulin resistance (Wondmkun, 2020)^[11].

The pancreas, an organ located beneath the stomach which also secretes digestive enzymes, secretes the hormone insulin, which controls the levels of blood glucose. Insulin is produced by the pancreas in reaction to blood glucose levels. Insulin makes it easier for cells to absorb glucose from the bloodstream. Glucose either immediately becomes energy when it enters the cells or is stored as fat or starch glycogen until required (Kim, 2017)^[6]. Normal variations in blood glucose levels occur during the course of the day. After a meal, they get up and take around two hours to get back to their pre-meal states. Insulin production falls off as soon as blood glucose levels reach pre-meal levels. Blood glucose levels typically fluctuate within a small range of approximately 70 to 110 milligrams per deciliter (mg/dL) or 3.9 to 6.1 millimoles per liter (mmol/L) in healthy adults (Wondmkun, 2020)^[20].

Jigsimur is a traditional African medicine (TAM) officially registered with NAFDAC (A7-2720L), derived from the sap of the Aloe Ferox plant cultivated in the Western Cape of South Africa. The concentrations of calcium, magnesium, potassium, and zinc in Aloe Ferox significantly exceed those found in Aloe Vera. Folklore practitioners assert that jigsimur enhances health and bolsters the immune system, and it is utilized for the treatment and management of conditions such as arthritis, gout, fibrosis, diabetes, migraines, heartburn, colic, stomach ulcers, bladder infections, insomnia, gallstones, throat infections, hypertension, conjunctivitis, osteoporosis, and various minor ailments (LeBoff *et al.*, 2022)^[19]. It is believed to be abundant in antioxidants, vitamins, and minerals that assist in regulating blood sugar levels and promoting overall health. In contrast to conventional diabetes medications, jigsimur reportedly has no known side effects and is deemed safe for prolonged use. Research studies indicate that diabetes is among the leading causes of mortality globally. Diabetes mellitus is the primary contributor to severe conditions such as heart failure, cardiovascular diseases including stroke, retinopathy, nephropathy, vascular dysfunction, sexual dysfunction, and several types of malignancy. Many diabetic individuals have wounds that don't heal, which might lead to limb, hand, and foot amputations. Additionally, diabetes is the foremost cause of chronic kidney disease. Effective management of blood sugar levels is essential for diabetes mellitus, with a healthy

diet playing a crucial role in preventing spikes in blood sugar. Consequently, the high prevalence of diabetes mellitus necessitates research into alternative treatment options, particularly natural products that may aid in its management.

The purpose of this study was to assess Jigsimur's impact on serum renal indices and serum inflammatory markers (PCT, ESR, and C-RP) in Wistar rats that have been given diabetic mellitus.

Materials and Methods

Experimental animals

Twenty (20) Wistar strain albino rats weighing between 80 and 130 grams were acquired from the Animal House of the University of Port Harcourt's Department of Biochemistry in Rivers State, Nigeria. In accordance with the normal guidelines established by the Institute for Laboratory Animal Research (ILAR), they were kept and cared for in the animal house of the Department of Biochemistry at Federal University Otuoke in Bayelsa State.

The purchased animals were housed in plastic cages with wire mesh covers to help with ventilation, and they were given a week to acclimate. The animals were kept in an environment with a 12-hour light/dark cycle, a temperature of 28 ± 2 °C, and a relative humidity of $50 \pm 5\%$ under strict observation. The animal facility was appropriately ventilated and the animals were fed on commercial rat pellet as feed and water *ad libitum* throughout the study period.

Experimental design and treatment of animals

As indicated in Table 1, Jigsimur was administered twice daily via orogastric intubation for a duration of 30 days. Twenty albino strain wistar rats were used in the experiment, and they were split up into three groups of five animals each. The animals in Group A, which served as the standard control group, were not induced with diabetes mellitus and were given only distilled water. Group B was the negative control group, they were induced with Diabetes using Streptozotocin (STZ) and the animals in this group received distilled water. Animals in Group C which were also induced with Diabetes mellitus using Streptozotocin (STZ) intraperitoneally and were treated with 8.33mg/kgbw of Glucophage (standard diabetic drug) orally while animals in the treatment Group D which were induced with diabetes and treated with 0.25mg/kgbw of Jigsimur as treatment.

Collection of blood samples for analysis

The animals were humanely euthanized under Chloroform vapour by placing them in a chamber containing chloroform-soaked cotton or gauze allowing the rats inhales the vapour which induces anesthesia and eventually leads to euthanasia; whole blood was extracted via cardiac puncture using a sterile syringe and needle twelve hours after the last treatment. The blood samples were put into EDTA container and plain tubes. The blood sample in the plain tubes were allowed to clot by standing for 2 hours at room temperature then later centrifuged at 4000 rpm for 20 minutes to separate from the red blood cells. Sera from each centrifuged plain tube were collected into another plain tube labelled accordingly using Pasteur pipettes. The separated sera were then frozen in a refrigerator until they were required for various biochemical assays while part of the whole blood was used to assay for HbA1c.

Institutional Review Board (IRB) Approval

Ethical approval from the Institutional Review Board (IRB) Quality control was obtained for this study from the quality assurance unit of Federal University Otuoke, Bayelsa State. The IRB ensures that the research is conducted in accordance with ethical principles and guidelines for animal experimentation and human subjects research (National Institute of Health, 2020).

Compliance with Animal Welfare Regulations

All procedures involving handling of Albino Wistar rats adhered to national and international guidelines for the care and use of laboratory animals, such as the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). The welfare of animals was prioritized, and measures were implemented to minimize pain, distress, and discomfort in line with IRB approved guidelines.

Biochemical assay

All biochemical assays were carried out using kits and the required biochemical analyzers. The assay for creatinine as a biochemical parameter for renal indices is according to Jaffé's response. A reddish-orange complex is created in an alkaline solution when creatinine and picric acid combine according to the method of Levey *et al.*, 2003 [7].

The assay for Cystatin C as a biochemical parameter for renal indices is based on the principle of quantifying the concentration of Cystatin C in biological samples, primarily serum or plasma using the method of Grubb, 2020 [5].

The assay for Blood Urea Nitrogen (BUN) was carried out using fluorometric techniques as described by Meyer and Hostetter, 2007 [2].

Kidney injury molecule-1 (kim-1) was estimated using immunoassay techniques, such as enzyme-linked immunosorbent assay (ELISA) or chemiluminescent immunoassay as described by Vaidya *et al.*, 2008 [10].

The method of Tietz, (1976) [4] as modified Burtis and Ashwood, 1996 [4] was used to estimate the electrolytes concentration of sodium, potassium, chloride, magnesium, bicarbonate in the serum. These assays were performed using automated clinical chemistry analyzers.

The Erythrocyte Sedimentation Rate (ESR) was estimated using the methods of Bain and Bates, 2019 [15]. The principle of the assay for C-reactive protein (CRP) involves the quantitative measurement of CRP levels in a blood sample, typically by using immunoassays which is based on the principles of antigen-antibody interactions as described by Ridker, 2016 [14].

The assay of serum procalcitonin (PCT) concentration is based on the principle of immunoassay as described by Schuetz *et al.*, 2017 [16].

Statistical analysis

The experiment's data were subjected to one-way analysis of variance (ANOVA) using the statistical package for social sciences version 23 (SPSS 23). The results were presented as means $\pm$ standard error of mean (SEM) and values at ($p < 0.05$) were considered significant when compared to the appropriate controls.

Result

Effects of jigsimur on some biochemical parameters were assessed and the findings are shown in a table 2.

Hemoglobin A1C (HbA1c)

The laboratory results obtained showed that the Hemoglobin A1c (HbA1c) level of animal group B or the negative control group (7.78 ± 0.08) and group C (4.76 ± 0.81) were significantly ($p < 0.05$) higher compared with that of group A or normal control (3.24 ± 0.07). However, the Hemoglobin A1c (HbA1c) levels in group C (4.76 ± 0.08) was significantly ($p < 0.05$) lower than that of group B (7.78 ± 0.08). More so, group D (3.78 ± 0.05) recorded a significantly ($p < 0.05$) lower value compared with that of group B (7.78 ± 0.08) and group C (4.76 ± 0.81), but compared favorably ($p < 0.05$) well with that of group A of normal control (3.24 ± 0.07).

Kidney Injury Molecule (KIM1)

The laboratory results obtained for serum KIM1 concentration showed the serum KIM1 concentration for animals in group B (31.20 ± 1.16), C (12.40 ± 0.51) and D (26.60 ± 0.68) where all significantly ($P < 0.05$), higher compared to that of group A or the normal control group (1.54 ± 0.04). However, the serum KIM1 concentration value for animals in group D (26.60 ± 0.68) was significantly ($P < 0.05$) lower than that of group B or the positive control (31.20 ± 1.16).

Cystatin- C

The laboratory results obtained shows that serum the concentration of Cystatin- C animals in group B (34.40 ± 0.25), C (11.20 ± 0.66) and D (20.80 ± 0.20) where all significantly ($P < 0.05$), higher compared to that of group A or the normal control group (0.66 ± 0.08). More so, the serum Cystatin C value for animals in group D (13.40 ± 0.25) was significantly ($P < 0.05$) lower compared to that of group B or the positive control (34.40 ± 1.16), but compared well with that of group C (11.20 ± 0.66) which were tested with standard diabetic drugs (glucophage).

Urea (Blood Urea Nitrogen)

The blood nitrogen results obtained shows that the serum Urea nitrogen concentration of animals in group B (7.54 ± 0.07), C (4.32 ± 0.21) and D (5.42 ± 0.02) where all significantly ($P < 0.05$), higher compared to that of group A or the normal control group (2.68 ± 0.07). However, the blood urea nitrogen value for animals in group D (5.42 ± 0.02) was significantly ($P < 0.05$) lower than to that of group B or the positive control (7.54 ± 0.07), but compares favorably well with that of group C (4.32 ± 0.21) at ($P < 0.05$) which were treated with standard diabetic drugs (Glucophage).

Creatinine

The creatinine results showed that the serum creatinine concentration in group B (106.58 ± 0.27), C (51.26 ± 0.32) and D (67.10 ± 0.33) where all significantly ($P < 0.05$), higher compared with that of group A or the normal group (36.74 ± 0.79). More so, the serum creatinine value for animals in group D (67.10 ± 0.33) was significantly ($P < 0.05$) lower compared with that of group B or the positive control (106.58 ± 0.27), and that of group C (51.26 ± 0.32).

Sodium (Na)

The sodium test results showed that the serum sodium concentration of animals in group B (140.60 ± 1.21), C (141.02 ± 0.45) and D (144.08 ± 0.37) were all significantly

($P < 0.05$), higher compared with that of group A or the normal group (132.40 ± 0.68). However, the serum sodium concentration for animals in group D (144.08 ± 0.37) showed no significantly ($P > 0.05$) change compared with that of group B or the positive control (140.60 ± 1.21), but compares favorably well with that of group C (141.02 ± 0.45).

Potassium (K)

The potassium test results showed that the serum potassium concentration in group B (6.58 ± 0.08), C (4.24 ± 0.07) and D (5.50 ± 0.06) are all significantly ($P < 0.05$), higher compared with that of group A or the normal group (3.10 ± 0.03). More so, the serum potassium concentration for animals in group D (5.50 ± 0.06) was significantly ($P < 0.05$) lower compared with that of group B or the positive control (6.58 ± 0.08).

Chloride (Cl⁻)

The chloride test results showed no significant ($P > 0.05$) change in serum chloride concentration of group B (108.80 ± 0.37), C (112.00 ± 0.32) and D (116.00 ± 0.32), compared with group A or the normal group (104.40 ± 1.21).

Biocarbonate (HCO₃⁻)

The Biocarbonate test results obtained, showed that the serum biocarbonate concentration in group C (18.40 ± 0.60) and D (15.40 ± 0.25) were all significantly ($P < 0.05$), lower compared with that of group A or the normal group (20.80 ± 0.66). More so, the serum biocarbonate concentration for animals in group D (15.60 ± 0.25) was significantly ($P < 0.05$) lower compared to that of group B or the positive control (20.20 ± 0.58).

Erythrocyte Sedimentation Rate (ESR)

The result of laboratory analysis shows that the concentration of Erythrocyte Sedimentation Rate (ESR) in group B or negative control (4.00 ± 0.00), group C (3.00 ± 0.00) and group D or test group D (2.00 ± 0.00) were significantly ($P < 0.05$) higher compared with group A or Normal control (1.64 ± 0.09). However, the ESR concentration in the group C (3.00 ± 0.00) and group D (2.00 ± 0.00) was significantly ($P < 0.05$) lower than of the group B (4.00 ± 0.00).

C-Reactive Protein (CRP)

Analysis of result indicates that the C-Reactive Protein (CRP) concentration of animals in group B (64.40 ± 0.25), group C (30.80 ± 1.24) and group D (48.00 ± 0.55) were significantly ($P < 0.05$) higher compared with NC or Normal control (12.20 ± 0.37). However, the concentration of CRP in group C (30.80 ± 1.24) and group D (48.00 ± 0.55) was significantly ($P < 0.05$) lower than that of group B (64.40 ± 0.25).

Procalcitonin (PCT)

Laboratory result indicates that the Procalcitonin (PCT) concentration of animals in group B (712.20 ± 1.16), group C (217.80 ± 0.86) and group D (518.60 ± 0.51) were significantly ($P < 0.05$) higher compared with group A (45.80 ± 0.37). However, the concentration of Procalcitonin (PCT) in group C (217.80 ± 0.86) was significantly ($P < 0.05$) lower than that of group B (712.20 ± 1.16) and group D (518.60 ± 0.51).

Discussion

Chronic diabetes is a long-term medical condition that affects how the body uses its resources. It is caused by the body breaking down food (carbohydrates) into sugar (glucose), which is then discharged into the circulation. The pancreas releases insulin in response to elevated blood sugar levels. Insulin is required to enable blood sugar to enter the body's cells and be used as fuel. Diabetes mellitus is a serious worldwide health issue that affects many individuals, healthcare systems, and economy of nations.

The evaluation of serum renal indices alongside serum inflammatory markers in albino wistar rats induced with diabetes mellitus provides crucial insights into the impact of diabetes on kidney function as well as tissues inflammation. These indices includes; glomerular filtration rate (GFR), blood urea nitrogen (BUN), serum creatinine, cystatin C, and serum electrolytes as well as erythrocyte sedimentation rate (ESR), C-Reactive Protein (CRP) and procalcitonin (PCT). Diabetes mellitus can lead to alterations in GFR. Initially, there might be hyper filtration, likely due to increased renal blood flow but however, over time, GFR tends to decline as a consequence of diabetic nephropathy (Brosius *et al.*, 2009) [3]. Monitoring GFR is crucial for assessing the functional status of the kidneys in diabetes. Elevated BUN levels are often observed in diabetic rats, reflecting impaired renal function while increased BUN may result from decreased glomerular filtration and increased protein breakdown due to hyperglycemia (Yilmaz *et al.*, 2011) [12]. Elevated BUN levels signify the need for close monitoring and intervention. Serum creatinine is a commonly used marker for renal function, its sensitivity in detecting early renal dysfunction in diabetes may be limited. Creatinine levels may increase as GFR decreases, but this change may not be apparent until substantial renal damage has occurred (Weiner *et al.*, 2004) [13]. Therefore, additional markers like cystatin C and KIM-1 become imperative since it is more sensitive for early detection (Tejera *et al.*, 2015) [8]. Cystatin C and KIM-1 are novel markers and has shown promise in detecting early renal impairment in diabetic rats. It is more sensitive than creatinine and may identify renal dysfunction at an earlier stage (Tejera *et al.*, 2015) [8]. Incorporating KIM1 and cystatin C into renal assessments can aid in timely intervention and management. Diabetes mellitus can also disrupt electrolyte balance, leading to hyperkalemia, hyperphosphatemia, and other imbalances (Palmer and Clegg, 2013) [9]. These disturbances can further impact renal function and overall health. Regular monitoring of electrolytes is essential to prevent complications. The administration of jigsaw showed decreased in glycated hemoglobin level, KIM1, Cystatin-C, creatinine, BUN as well as electrolytes concentrations compared with the negative control while comparing favourably well with the group treated with Glucophage (standard drug). This suggest the hypoglycemic (occasion by the presence of saponin) as well renal protective potential of jigsaw (anti-oxidant property) which can be attributed to the various phytochemical abound in jigsaw

Apart from its primary impact on glucose metabolism, diabetes is associated with chronic low-grade inflammation. This inflammatory component plays a crucial role in the pathophysiology of diabetes and its complications. When investigating serum inflammatory markers in diabetes

mellitus, several key parameters are always considered like Cytokines, C-Reactive Protein (CRP), Adipokines, Procalcitonin (PCT), Erythrocyte Sedimentation Rate (ESR). Inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and interleukin-1 β , are known to be elevated in diabetes and play a central role in the chronic inflammation associated with the disease (Donath and Shoelson, 2011). C-Reactive Protein (CRP) an acute-phase reactant, is another important marker of inflammation. In diabetic rats, elevated CRP levels are often observed and are linked to the increased risk of cardiovascular complications (Pradhan *et al.*, 2001) [17]. Adipose tissue is now recognized as an active endocrine organ that secretes various pro-inflammatory adipokines, including adiponectin and leptin. Altered levels of these adipokines in Wistar rats with diabetes can contribute to systemic inflammation and insulin resistance (Kim *et al.*, 2010) [24]. Diabetes-induced inflammation is often associated

with increased oxidative stress, resulting in Reactive oxygen species (ROS) generation and oxidative cell injury and tissues (Juan, *et al.*, 2011) [25]. Understanding the dynamics of these serum inflammatory markers in diabetic Wistar rats is crucial for both elucidating the pathogenesis of diabetes and identifying potential therapeutic targets. Additionally, it can provide insights into the development and progression of diabetes-related complications, such as cardiovascular disease and nephropathy among others. The maintenance of the inflammatory markers within the normal control range suggesting the anti-inflammatory properties of jigsaw. The phytochemicals present in jigsaw are alkaloid, phenols, glycine, saponin, terpene, tannin, and flavonoid and is 100% alkaline. The findings of this research have indicated that the treatment is capable of preventing the progression of diabetes mellitus, reverse possible complications associated with diabetes mellitus.

Table 1: Experimental Design and Schedule of Treatments

Groups	Number of animals Administration/Treatment
A (Normal Control)	5 Distilled water
B (Positive Control)	5 Distilled water
C (Standard Control)	5 8.33 mg/kg bw of Glucophage
D (TEST)	5 0.25 ml/kg bw of Jigsaw

Results

Table 2: Effects of the various treatments on different serum biochemical parameters

	HbA1c (%)	KIM1 (ng/ml)	CYS-C (ng/ml)	Urea Mmol/L	Creat μ mol/L	Na Mmol/L	K Mmol/L	Cl ⁻ Mmol/L	Hco3 Mmol/L	ESR	CPR	PCT
A	3.24 $\pm$ 0.07	1.54 $\pm$ 0.04	0.60 $\pm$ 0.07	2.68 $\pm$ 0.07	36.74 $\pm$ 0.79	132.40 $\pm$ 0.67	3.10 $\pm$ 0.32	104.40 $\pm$ 1.21	20.80 $\pm$ 0.66	1.64 $\pm$ 0.009	12.20 $\pm$ 0.37	45.80 $\pm$ 0.037
B	*7.78 $\pm$ 0.08	*31.20 $\pm$ 1.16	*34.40 $\pm$ 0.24	*7.54 $\pm$ 0.068	*106.58 $\pm$ 0.27	*40.60 $\pm$ 1.21	*6.58 $\pm$ 0.08	*108.80 $\pm$ 0.37	*20.20 $\pm$ 0.58	4.00 $\pm$ 0.00	64.40 $\pm$ 0.25	712.80 $\pm$ 1.16
C	*a4.76 $\pm$ 0.81	*a12.40 $\pm$ 0.51	*a11.20 $\pm$ 0.66	*a4.32 $\pm$ 0.21	*a51.26 $\pm$ 0.32	*a141.00 $\pm$ 0.45	*a4.24 $\pm$ 0.07	*a112.00 $\pm$ 0.32	*a18.40 $\pm$ 0.60	3.00 $\pm$ 0.00	30.80 $\pm$ 1.24	217.80 $\pm$ 0.86
D	*ab3.78 $\pm$ 0.05	*ab26.60 $\pm$ 0.45	*ab20.80 $\pm$ 0.20	*a5.42 $\pm$ 0.02	*ab67.10 $\pm$ 0.33	*a144.08 $\pm$ 0.37	*ab5.50 $\pm$ 0.06	*ab116.00 $\pm$ 0.32	*ab15.40 $\pm$ 0.25	2.00 $\pm$ 0.00	48.00 $\pm$ 0.55	518.60 $\pm$ 0.51

Values are expressed as mean $\pm$ standard error of mean (SEM), n = 5.

* = significant @ p < 0.05 compared with group 1 (A)

* a = significant @ p < 0.05 compared with group 2 (B)

* ab = significant @ p < 0.05 compared with group 3 (C)

Keys: KIM1= Kidney Injury Molecule 1, cystc = Cystatin-C, Creat = Creatinine, Na = Sodium, K= Potassium, Cl⁻ = chlorine, HCO₃ = Bicarbonate, HbA1c=Hemoglobin A1C, ESR=Erythrocytes Sedimentation Rate, CPR= C-Reaction Protein, PCT=Procalcitonin.

Conclusion

The results obtained from this study indicates that jigsaw has the potential lowered Hemoglobin A1c (HbA1c) as well as highlights the correlation between diabetes-induced renal damage, and inflammation in Wistar rats. Jigsaw treatment demonstrated effectiveness in restoring renal function as well as inflammatory markers, suggesting its potential anti-hyperglycemic and anti-inflammatory properties.

However, it's important to note that while jigsaw treatment led to improvements in the above mentioned parameters, it also essential to carry out regular monitoring of blood sugar level in order to avoid potential hypoglycemia as well routine micronutrient and lipid profile estimations.

Compliance with ethical standards

This study adheres to all conventional ethical practices as applied to this research.

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Disclosure of conflict of interest

The authors declare that none of the work reported in this study could have been influenced by any known competing financial interests or personal relationships.

Statement of ethical approval

The Research and Quality Control Unit of Federal University Otuoke granted ethical permission for this study in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes ETS-123.

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