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Comparative Antioxidant Potential of Fractionalised Extracts of *Detarium senegalense* on Streptozocin Induced Diabetic Rat Models

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Abstract

Oxidative stress is acknowledged as a significant contributor to the initiation and advancement of Diabetes Mellitus and its associated consequences. Consequently, antioxidant-based treatment approaches have garnered heightened interest in diabetes research. *Detarium Senegalense* (DS), a plant extensively used in traditional West African medicine, has antidiabetic and antioxidant characteristics. The present study therefore assessed and compared the antioxidant potential of fractionalized extracts of *Detarium Senegalense* in diabetic Wistar rat models. Male Wistar rats, with weights ranging from 150-200 grams, were deployed and randomly separated, with each of the 6 different groups having 7 rats per group. The first group took just water and ordinary feed (control), while groups 2-6 were subjected to streptozotocin (ST-Z) induction (60 mg/kg intraperitoneally). Group 2 was the control and received 60mg/kg streptozocin only intraperitonially, group 3 received ST-Z and 50mg/kg metformin (MET-F). Group 4, 5 and 6 received 250 mg/kg each of *D. Senegalense* (DS) extracts of ethyl acetate (DS_EA), N-hexane (DS_HE), and chloroform (DS_CE) respectively. Fasting blood glucose levels, Superoxide Dismutase (SOD), Malondialdehyde (MD-A), Catalase (CATL), were assessed and compared weekly over a 10-week period, before and after therapy. The experimental animals exhibited superior antioxidant bioactivity compared to control animals. Diabetic rats demonstrated a substantial elevation in MD-A, a depletion in CATL along with SOD. Administration of 250mg/kg each of hexane (DS_HE), ethyl acetate (DS_EA) as well as chloroform fractions (DS_CE) derived from the ethanol extract from the bark of the stem in conjunction with 50mg/kg of the anti-diabetic drug metformin (MET), substantially decreased MD-A levels while enhancing CATL and SOD, with the ethyl acetate extract demonstrating the greatest efficiency. The DS extract exhibited considerable antioxidant capabilities, substantiating its use in traditional medicine.

Keywords: Diabetes Mellitus, *D. Senegalense*, Anti-Oxidants, Phytochemical Components.

INTRODUCTION

Diabetes mellitus commonly abbreviated as DM, is a longterm metabolic condition marked by consistently elevated levels of blood sugar. This arises from insufficient insulin production, impaired insulin use, or a combination of both, resulting in the disturbance of glucose homeostasis. Diabetes mellitus often results in persistent polyuria, visual impairment, blindness, renal failure, neuropathy, cardiovascular disease, and several systemic illnesses [1]. Diabetes is a prevalent metabolic disorder globally, affecting both affluent and impoverished populations, with over 347 million individuals being impacted by the illness.

Despite several research examining the anti-diabetic and antioxidant properties of medicinal plants, there is a lack of knowledge on the blood glucose-lowering effects and systemic metabolic influences of *Detarium senegalense* (*D. senegalense*). According to

research by David *et al*., [2], the extract of the root of *D. senegalense* ameliorated blood glucose levels by up to 67.3% in diabetic rats, exceeding the typical medicine glibenclamide, which demonstrated a 57.5% reduction at the 32nd hour of treatment [2].

Detarium Senegalense tree is notable for its nourishing fruits, its use in traditional medicine, and its worth as a timber resource. Its many uses suggest potential advantages for food security and sustainable land management.

Phytochemical examination of *D. Senegalense* has a significant abundance of compounds with anti-oxidant and anti-inflammatory properties [3]. Historically, its aqueous formulations have been used in the treatment of respiratory tract infections, gastrointestinal disorders, and dermatological conditions throughout tropical Africa. Furthermore, it has exhibited promising antibacterial effect against *S. aureus, S. epidermidis, S. faecalis* as well as antifungal properties such as the Aspergillus species [4].

Although the exact ways in which *D. Senegalense* (DS) lowers blood sugar are not fully understood, a more pressing concern is the widespread non-adherence to standard oral anti-diabetic drugs among the patients. This underscores the demand for novel treatments with fewer adverse effects for Type 2 DM. Interest in affordable, locally sourced remedies with antioxidant effects has been increasing, particularly among rural communities in Africa [5].

The present study therefore assessed and compared the antioxidant potential of various fractionalized stem bark extracts of *D. senegalense* (DS) in diabetic Wistar rat models.

MATERIALS AND METHODS

Study Area

The research was done in the Pharmacology and Therapeutics department at the Rivers State University (RiSU) located in the city of Port-Harcourt, Nigeria.

Experimental Study Protocol

The animal's fasting blood glucose (FBS) levels and their weight were assessed on a weekly basis for ten weeks. Male Wistar rats, with weights ranging from 150-200 grams, were deployed and randomly separated, with each of the 6 different groups having 7 rats per group. The first group took just water and ordinary feed (control), while groups 2-6 were subjected to streptozotocin (ST-Z) induction (60 mg/kg intraperitoneally). Group 2 was the control and received 60mg/kg streptozocin only intraperitonially, group 3 received ST-Z and 50mg/kg metformin (MET-F). Group 4, 5 and 6 received 250 mg/kg each of *D. Senegalense* (DS) extracts of ethyl acetate (DS_EA), N-hexane (DS_HE), and chloroform (DS_CE) respectively. Fasting blood glucose levels, Superoxide Dismutase (SOD), Malondialdehyde (MD-A), Catalase (CATL), were assessed and compared weekly over a 10-week period, before and after therapy.

Collection of the Plant Sample

The collection of *Detarium Senegalense* (DS) stem bark samples was done in its native habitat in Chaza village situated situated in Niger State, a northern state in Nigeria. The plant material was confirmed by a Taxonomist at the Medicinal Plants and Traditional Medicine Department located at the National Institute of Pharmaceutical Research and Development (NIPRD), Federal Capital Territory (FCT), Nigeria. The voucher number (VN), was designated # NIPRD/H/7082, and deposited at the NIPRD herbarium. The certified plant material was sent to the Pharmacology Department Laboratory at Rivers State University for extraction.

Method of Plant Extraction

The extraction was done by cleansing the stem bark followed by air drying, then grinding using a grinding machine, converting it into powder. A total of 1500 grams of powdered bark was submerged in 6 liters of ethanol. The process lasted for forty eight hours. A filtration process of the solution was done through the Whatman filtration paper [1]. This was followed by air-drying to form a paste which was kept dry in universal bottles at a tempeture of 4°C for future experimental use.

Phytochemistry (GC-MS Analysis)

An analysis of the ethanol extract of D. Senegalense was done using Gas Chromatography Mass Spectrometry (GC_MS).

Ethanolic Extract Separation

The separation of the ethanolic extract was done by utilising the serial liquid separation technique as described by Ekam *et al*., [6].

Fractionation Protocol:

Crude ethanol extract (200 ml) placed in a 500 ml separation funnel, was stabilized with a retort stand, followed by the addition of 200 ml of n-hexane. The mixture was thereafter exposed to intense stirring and let to stand for 30 minutes. The hexane-soluble top layer was extracted, and the residual layer was used for further fractionation. The residual extract was then combined with 200 mLlof chloroform and isolated using the same procedure. The chloroform-soluble layer was collected, and the remaining substance underwent a further extraction with 200 ml of ethyl acetate. Each fraction was air-dried and the final fractions (n-hexane, chloroform, and ethyl acetate) were preserved at 4°C in sealed containers to prevent microbial contamination or deterioration.

Identification of Phytochemicals in Flora

Qualitative phytochemical screening was conducted on the extracts using established chemical methods to determine the presence of bioactive substances. These include the tests for tannins, saponins, alkaloids and glycosides.

Animal Handling

Male Wistar rats were procured from the the Department of Pharmacology and Therapeutics, Rivers State University (RiSU) in Port Harcourt, Nigeria. Acclimatization of the study animals, which lasted for one week, was carried out in properly ventilated cages under natural 12-hour light/dark cycle at about 25°C.

Ethical Considerations

The Research Ethics Committee of Rivers State University (RiSU) issued ethical clearance with reference number RSU/FBCSEC/A/20.

Evaluation of Acute Toxicity

Acute oral toxicity was assessed using a modified Lorke's approach [7], recognized for its reduced animal consumption, cost-effectiveness, and ease of implementation. Fifteen (15) rats were randomly allocated into five groups (three rats per group) and administered ethyl acetate stem bark extract of *Daniellia senegalense* at dosages of 100, 1000, 1600, 2900, and 5000 mg/kg orally. Monitoring of the animals for behavioral alterations or mortality was done for 72 hours.

Induction of Diabetes

Diabetes was developed using approaches similar to those outlined by Naiho [8]. Rats were subjected to fasting for 18 to 24 hours while having full access to water. They were then assigned randomly to control and experimental groups. 60 mg/kg of newly synthesized streptozotocin (ST-Z) was administered intraperitoneally to induce diabetes.

Diagnosis of Diabetes Mellitus

Three days post-STZ injection, fasting blood sugar (FBS) levels were evaluated and Diabetes mellitus (DM) was diagnosed when FBS level exceeded 200 mg/dL.

Specimen Acquisition

The animals were executed humanely, using the cervical dislocation technique. A conclusive fasting blood glucose assessment was conducted prior to euthanasia. A laparotomy was performed to examine internal organs for further assessment. Blood samples were acquired by cardiac puncture using 5 mL syringes and 23G needles, and collected in standard sample containers.

Statistical Analysis

To present the data, standard error of the mean (SEM) plus or minus the mean was used. We used an analysis of variance (ANOVA), which is one-way, to compare items statistically. Posthoc analysis using Fisher's Least Significant Difference (LSD) was deployed when statistically significant differences were found. Statistical analysis was carried out using the Statistical Product and Services Service solutions – SPSS 21.0V software. A significance of $p < 0.05$ was established.

RESULTS

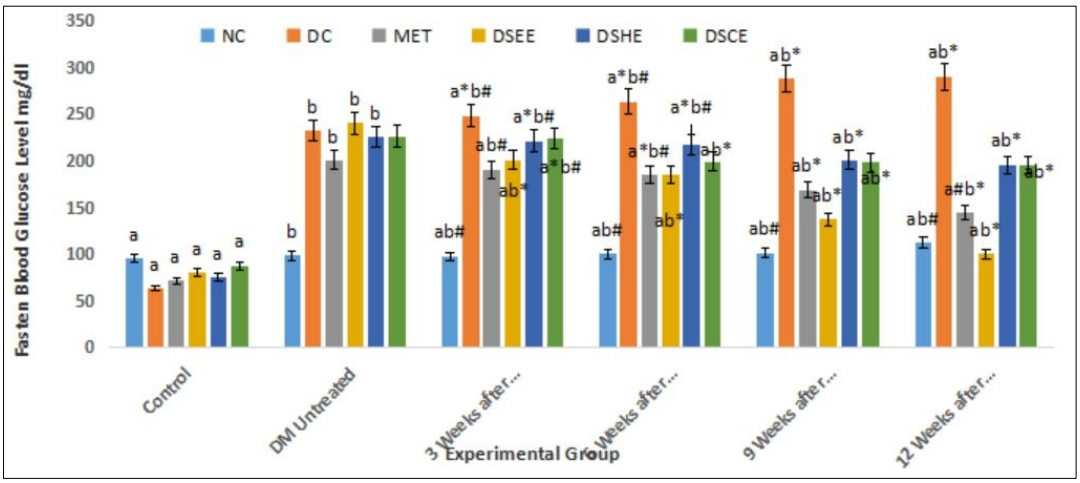

Figure 1: **The Effects of fractionalized D. Senegalense compared with different fractionalized extracts on fasting blood sugar levels (FBS) in ST-Z induced diabetic rat models**

Values represent mean ± SEM. Several range tests with LSD follow the analysis of variance. The bar chart of identical colour codes and without a similar asterick (*) or pound symbols (#) and/or letters (a or b), implies that the control and Negative Control groups led to a significant change at a $p < 0.05$ level of significance.

Colour Codes:

Blue: Normal Control (NC), Orange: Diabetic Control (DC), Gray: Metformin (50 mg/kg) (MET), Yellow: D. Senegalense Ethyl Acetate Extract (DSEE), Blue: D. Senegalense n-Hexene Extract (DSHE), Green: D. Senegalense Chloroform Extract (DSCE), *: statistically significant; #: statistically not significant.

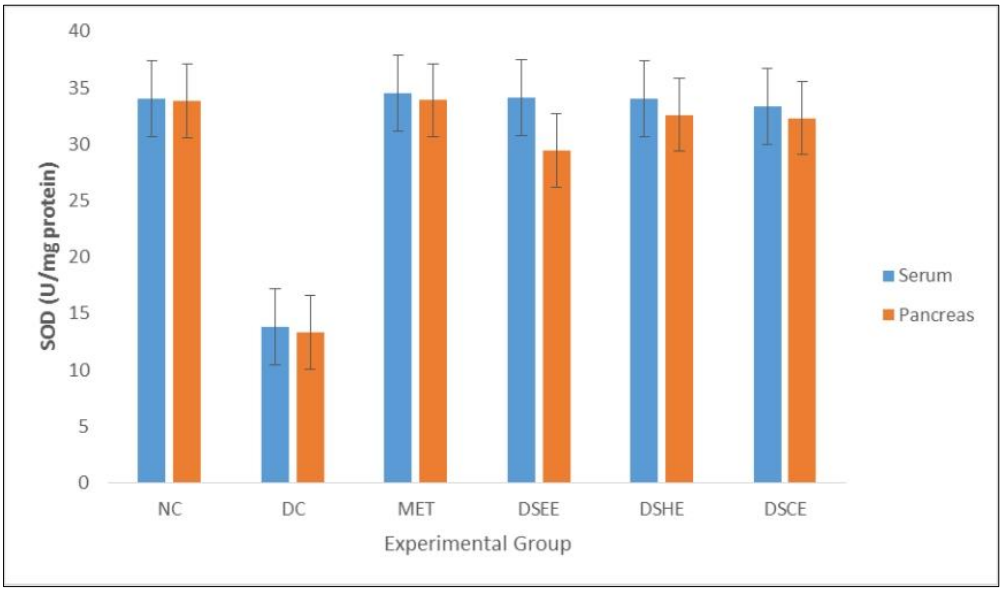

Figure 2: The Protective effects of extracts of *D. Senegalese* bark on Superoxide Dismutase (SOD)

The values represent the mean ± SEM. Several range tests with LSD follow the analysis of variance. The data are shown using the mean± SEM. Several range tests with LSD follow the analysis of variance. Values with different superscripts show a significant amount of variance at a significance level of P<0.05.

Key: Normal Control (NC); Diabetic Control (DC); Metformin (MET); *D. Senegalense* bark ethyl acetate extract (DSEE); *D.Senegalese* bark N-hexane extract (DSHE); Chloroform extract of *D. Senegalense* bark (DSCE).

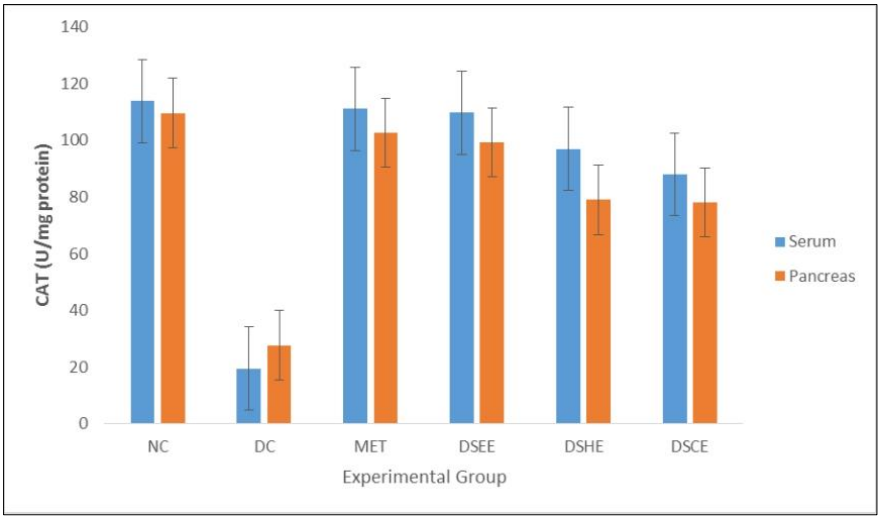

Figure 3: The protective effectiveness of D. Senegalese bark extracts related to oxidative stress and Catalase (CAT) levels in diabetic rats

The impact of different *D. Senegalense* doses on Catalase (CAT)L levels in ST-Z induced diabetic rat models.

The data are shown using the mean± SEM. Several range tests with LSD follow the analysis of variance. Values with different superscripts show a significant amount of variance at a significance level of P<0.05.

Key: Normal Control (NC); Diabetic Control (DC); Metformin (MET); *D. Senegalense* bark ethyl

acetate extract (DSEE); *D.Senegalese* bark N-hexane extract (DSHE); Chloroform extract of *D. Senegalense* bark (DSCE).

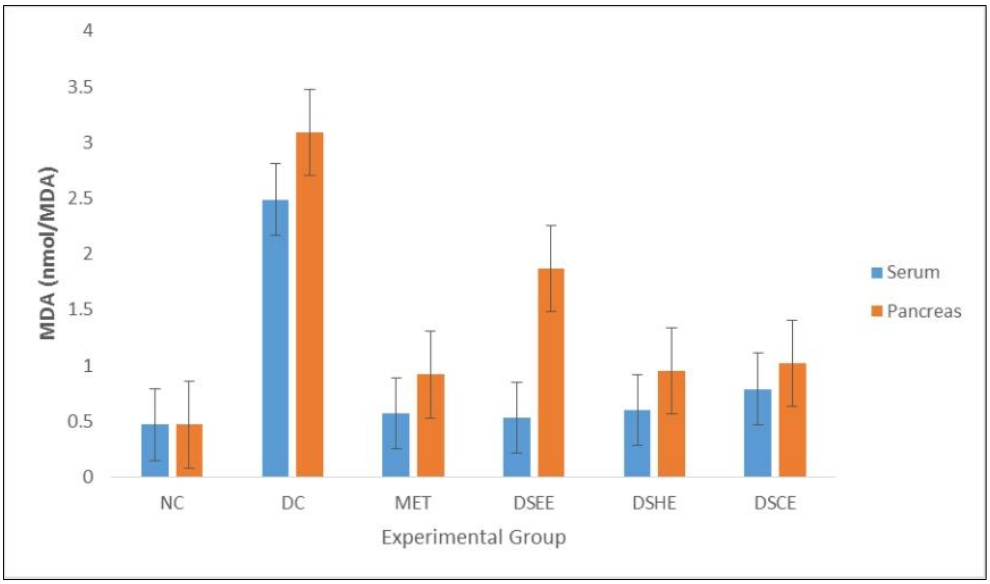

Figure 4: Different doses of *D. Senegalense* extract and their effects on Malondialdehyde (MDA) levels in ST-Z-induced diabetic rat models.

The data are shown using the mean± SEM. Several range tests with LSD follow the analysis of variance. Values with different superscripts show a significant amount of variance at a significance level of $P<0.05$.

Key: Normal Control (NC); Diabetic Control (DC); Metformin (MET); *D. Senegalense* bark ethyl acetate extract (DSEE); *D.Senegalese* bark N-hexane extract (DSHE); Chloroform extract of *D. Senegalense* bark (DSCE).

DISCUSSION

This research assessed and compared the antioxidant potential of fractionalized extracts of *Detarium senegalense* in diabetic Wistar rat models. Blood glucose levels in experimental animals were evaluated weekly during the study period to determine the efficacy of *D. Senegalense* for the elimination of glucose from their bloodstream.

In Group 2 (control group), FBS levels initially significantly rose but it was followed by a dose dependent amelioration of elevated glucose levels due to treatment with the various extracts of *D. Senegalense*. Metformin, a routine antidiabetic, led to a marked reduction in blood glucose levels within 7 days. However, there was a significant rise in glucose levels after administration of ethyl acetate extract *D. Senegalense* (250mg/kg) which began to decline after 4 days after treatment. Overall, the DS-treatment groups showed a more prominent amelioration in FBS levels compared to both the metformin-treated and control groups.

Ethanol as an extraction solvent choice of extraction solve reflects traditional practices in the study region, where such combinations are commonly used to treat illnesses. According to Abuzeid *et al*., [9], extracts from this plant are tradionally used for managing DM, suggesting the presense of bioactive compounds [10]. This traditional knowledge provided the basis for investigation the effects of constituents of the extract, leading to the extraction of *D. Senegalense* stems for phytochemical analysis.

Phytochemical analysis of the plant presents an array of tannins, flavonoids, alkaloids, terpenoids, saponins, carbohydrates, and steroids. These phytochemical compounds are recognized for their pharmacological effects, particularly on the body's vital organs [9]. Recent study indicates that the ethanolic extract comprises many phytochemical constituents, including proteins, amino acids, sterols, terpenoids, and acetogenins [11]. Moreover, the leaf extract contains anthraquinones [12], recognized for their laxative properties. Flavonoids, present in *D. Senegalense* confer antioxidant properties, and the alkaloid may be used in the treatment of several diseases.

Most cells depend on protective mechanisms to prevent or repair oxidative damage for survival [13-16]. Antioxidants like SOD, Catalase, and MDA serve as biomarkers for assessing oxidative stress levels in biological tissues [15]. The results of this investigation

indicated that the extract of *D. Senegalense* at a dosage of 250 mg/kg significantly elevates the levels of Catalase and SOD enzymes in pancreatic tissue and blood (Figure 2 and Figure 3). Previous research has corroborated these findings [16-21], highlighting the antioxidant properties of phytochemicals in a review entitled "The Significance of Antioxidants in the Management of Diabetes and Its Consequences." The data presented in Figures 2 and 3 indicate that hyperglycemia is associated with a reduction in superoxide dismutase (SOD) and catalase activities in pancreatic tissues, as previously documented by Ojieh [17].

Figure 4 illustrates a significant rise in the oxidant activity of Malondialdehyde (MD_A) in DM rat models. However, treatment with ethyl acetate, hexane and chloroform fractions of the of *D. Senegalense* (250 mg/kg) as well as antidiabetic drug, metformin (50 mg/kg) substantially boosted pancreatic antioxidant activity. Among these, Group 4 (ethyl acetate extract group) showed the most prominent effects. A similar outcome was reported by seven *et al*., [22]. Oxidative stress in DM is intensified by factors such as non-enzymatic and auto-oxidative glycosylation as well metabolic-driven stress [23]. Elevated levels of lipid peroxidation products have been detected in ST-Z-induced diabetic rat models and it is well established that hyperglycemia aggravates lipid peroxidation, potentially leading to long-term tissue damage [23].

CONCLUSION

Proportions of the extract from *D. Senegalense* has antioxidant characteristics, demonstrating in vivo antioxidant activity. The experimental animals exhibited superior antioxidant bioactivity compared to control animals. Diabetic rats demonstrated a substantial elevation in MD-A, a depletion in CATL along with SOD. Administration of 250mg/kg each of hexane (DS_HE), ethyl acetate (DS_EA) as well as chloroform fractions (DS_CE) derived from the ethanol extract from the bark of the stem in conjunction with 50mg/kg of the anti-diabetic drug metformin (MET), substantially decreased MD-A levels while enhancing CATL and SOD, with the ethyl acetate extract demonstrating the greatest efficiency. The DS extract exhibited considerable antioxidant capabilities, substantiating its use in traditional medicine.

More studies are needed to validate, isolate, and quantify the exact components found in extract from *D. Senegalense* that may elucidate the recorded antioxidant effects.

Conflicts of Interest: The authors declare no conflict of interest.

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