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ANTI-HYPERGLYCEMIC ACTIVITY OF DOUM PALM *(HYPHAENE THEBAICA)* LEMON GRASS *(CYMBOPOGAN CITRATUS)* AND MINT LEAF *(MENTHA PIPERETA)* FORMULATION IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract
Diabetes is a serious metabolic disorder affecting millions of people globally, several medicinal plants are used to manage the disease traditionally and these natural plants have little or no side effects with high efficacy when compared to most synthetic drugs. The study was carried out to evaluate the anti-hyperglycemic activity of Doum palm *(Hyphaene thebaica)* Lemon Grass *(Cymbopogan citratus)* and Mint Leaf *(Mentha pipereta)* blended formulation (DLMF) against STZ induced albino rats. A total of 20 experimental rats were grouped into five with 4 rats each. Group I served as normal control while group II, III, IV and IV were induced with diabetic through intra-peritoneal injection of streptozotocin (150 mg/kg). Group II served as positive control while group III, IV and IV were administered with 200mg/kg of extract, 400mg/kg of extract and Metformin respectively for 14 days. Fasting blood sugar (FBS) and body weight were monitored periodically for the period of 14 days after induction of diabetes. The results show a significant ($p<0.05$) reduction in FBS in extract administered groups in a dose dependent pattern compared to diabetic control. These findings were supported by the increase in body weight diabetic rats as a result of extract administration these effects were comparable with the effects of standard anti-diabetic drug (metformin). Conclusively, this study suggests a potential efficacy for use of DLMF in management of diabetes.

Keywords: Diabetes, Streptozotocin, Doum Palm, Lemon Grass and Mint Leaf

INTRODUCTION

Diabetes mellitus has become one of the major causes of mortality at the global level after cardiovascular diseases, cancer, respiratory diseases and diabetes (Balakumar *et al.*, 2016). Diabetes is one of the most challenging diseases facing health care professionals today (WHO, 2023). These four diseases account for account for 82% of all non-communicable diseases. The problems resulting from diabetes mellitus and its complications bring high costs to the health care system (Feigin *et al.*, 2013). Current pharmacotherapy includes the use of synthetic drugs which has been associated with various side effects, reflecting the necessity of new agent in the management of diabetes (Krentz, Patel and Bailey, 2008; Tahrani, Barnett and Bailey, 2016). Diabetes mellitus is considered to have a major impact on the economic and social activity of patients, their families and society. Furthermore, uncontrolled diabetes leads to serious chronic complications such as blindness, kidney failure, heart failure and numerous other diseases (De Marsilis, 2022. *Hyphaene thebaica* is a common palm tree with edible fruit which is a rich source of macro and micronutrients (Reda, 2015) and

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natural antioxidants (Atito *et al.*, 2019). It is used in the treatment of diabetes and several diseases (Salib *et al.*, 2013). *Mentha piperita* is one of the ten most widely used plants in the world and is commonly used in the treatment of several diseases (Gulluce, *et al.*, 2007). Previous studies indicate that *Mentha piperita* has hyperglycemic activity on experimental animal (Barbalho *et al.*, 2011). *Cymbopogon citratus* is used as a taste enhancer, known to be potent antioxidants (Shah *et al.*, 2015). *Cymbopogon citratus* is reported for the potential anti-diabetic activity (Jain and Agarawal, 2020). These medicinal plants have been increasingly used in the management and treatment of chronic diseases like Diabetes Mellitus. Natural plants are an alternative to synthetic drugs and have been found to contain many phyto-constituents (Al Kury *et al.*, 2021; Zimmet *et al.*, 2001). The growing interest in the therapeutic use of natural products for diabetes, especially those derived from natural sources necessitates the demand for non-toxic, affordable drugs persists. Thus, the search for more cost effective and safer anti-diabetic agents has become an area of active research. The present study was conducted to determine the anti-hyperglycemic activity of the medicinal extracts from the formulation of lemon grass, mint leaf, and doum fruit aqueous plant extracts in streptozotocin induced hyperglycemic rats

MATERIALS AND METHODS

Collection and Preparation of Plant extract

Fresh leaves of *Mentha pipereta*, *Cymbopogan citratus*, and dried *Hyphaene thebaica* fruit was purchased from Sharada Market, Kano municipal local government area Kano. And authenticated by the Plant Biology Department, Bayero University, Kano in the month of January, 2021. The taxonomic identification and verification were obtained from the Departmental herbarium with a specimen voucher accession number given as **BUKHAN 0234, BUKHAN 0337, and BUKHAN 0380.** The leaves of these plants were shade dried over a period of 5 days under strict hygienic condition and then grinded into fine powder using wooden mortar and pestle. Aqueous extract was prepared by means of Soxhlet extraction.

The powdered samples were diluted in distilled water extraction done using Soxhlet apparatus until extraction water became colourless. The extracts were further filtered and evaporated to dryness in a vacuum dryer. The concentration of the aqueous leaf extract (filtrate) was determined as the difference in weight /final volume of the solution using the relation.

$$Conc.(g/ml) = \frac{\text{Initial weight of sample} - \text{final weight of residue}}{Final\ volume\ of\ the\ filtrate} \quad \text{(Eqn 1.0)}$$

The volume of the plant extract administered was determined based on the weight of the rats using the formula:

$$Volume\ administered\ (ml) = \frac{\text{weight of rat(kg) x dose(mg/kg)}}{\text{Concentration of the filtrate } (\frac{mg}{ml})} \quad \text{(Eqn 1.1)}$$

According to the method as described by Sani *et al.*, 2015).

Experimental Design and Procedure

Induction of Diabetes Mellitus

Diabetes was induced in wistar albino rats by a single intra-peritoneal injection of 150mg/kg of streptozotocin. The rats were kept fasted for 10hrs prior to induction of diabetes. Manifestation of diabetes, was confirmed by measuring the fasting blood sugar levels of the rats 48 hours after administration, using ACCU CHEK" glucometer and strips. Rats with Fasting Blood Sugar (FBS) greater than 130mg/dl were considered diabetic and used for the experimental study (Sani, 2015).

Grouping and Extract Treatment of Rats

The experimental animals (20) twenty albino rats were divided into five (5) groups with each group having four (4) experimental animals. Hyperglycemia was induced in group II, III, IV, and V. Group I were the non-diabetic rats (normal control), neither induced with the diabetic nor administered with the extract. Group II: Induced diabetic rats without treatment of the extract. Group III, IV were administered with 200mg/kg, 400mg/kg body weight of the extract respectively. Group V rat were administered with standard drug Metformin 500 mg/kg body weight for 2 weeks after streptozotocin.

The weight of the experimental animals was determined with the aid of a laboratory scale weighing balance. The experimental animals were weighed in grams (g) at the initial start (0) day of the research, day (7) seven and finally on day (14) days. The FBS of the rats were measured at an interval of 3 days, using the ACCUCHEK" glucometer and strips during extract administration.

RESULT AND DISCUSSION

The results of mean body weight for the test subjects for the 1st, 7th, and 14th days shown in Table 1; the mean body weight of normal rats, induced diabetic rats administered with aqueous extract formulation and metformin drug. Administrations of the extract lead to decrease in weight for the test subjects at the day 7th and a significant increase in weight of the test subjects especially at the 14th day. Similar findings were also recorded in metformin administered group. On the 7th day, there was a marked difference ($p< 0.05$) in the weight of the treatment groups compared to the values in normal and diabetic control groups. On the last day of the second week, as seen in Table 1, there was significant increase in body weight The drastic weight loss is one of the symptoms of diabetes and weight gain can be associated with signs of improvement, this can be seen in Group III (137.00 ±16.90)g initial weight, at 7days (130.00 ± 08.40)g after inducement and a significant increase in final weight at (153.000 ± 7.00)g after 14 days and in Group IV initial weight at (134.40 ± 08.80)g, then at 7 days decrease in weight at (109.80 ± 04.10)g and final weight gain at (141.20 ± 5.00)g after 14 days, to a lesser extent Group V initially at (122.20 ± 01.83)g, after inducement at 7 days (122.00 ± 2.00)g and final weight gain at (130.20 ± 03.60)g, also exhibited signs of increased body weight signifying improvement in diabetes treatment.

Table 1: Mean Body Weight(g) of Streptozotocin-Induced Diabetic Rats administered with (DLMF)For 14 Days Compared with Metformin Drug and Normal Rats

	Group	Before Inducement (g)	After 7 Days(g)	After 14 Days(g)
I.	Normal rats (NC)	106.30 ± 8.00	107.40 ± 10.10^{b}	129.70 ± 01.34^{c}
II.	Induced without treatment (PC)	123.10 ± 02.60^{c}	114.30 ±2.30^{b}	123.10 ± 02.60^{c}
III.	Treated with $DLMF_1$ (200mg/kg).	137.00 ±16.90^{a,b}	130.00 ± 08.40^{a}	153.000± 7.00^{b}
IV.	Treated with $DLMF_2$ (400mg/kg).	134.40 ± 08.80^{a,b}	109.80 ± 04.10^{a}	141.20 ±5.00^{b}
V.	Treated with Metformin drug (500mg/kg)	122.20 ± 01.83^{c}	122.00 ±2.00^{c}	130.20 ± 03.60^{d}

Key: Negative Control, PC: positive control, DLMF- Doum, Lemon and Mint leaf formulation. Values are presented as mean ± SD, n=4. Figures bearing similar superscript in the same row are significantly different ($P<0.05$).

The results of changes in blood glucose levels in Normal Rats, Diabetic Untreated Rats, and Diabetic Rats Treated with Metformin and Aqueous DLMF Extracts are presented in Table 2: The blood glucose level significantly increased at day 14 in diabetic control rats (Group II) (452 ± 7.70 mg/dL) compared to normal control rats (Group I) (120 ± 2.80 mg/dL). Rats that orally received the aqueous extract of the formulation (200 mg/kg) showed a significant reduction in blood glucose levels (133 ± 8.4 mg/dL) compared to diabetic control rats. The aqueous extract group iv (400 mg/kg) decreased blood glucose levels at day 3 from 427 ± 53.70 mg/dL to 119 ± 5.60 mg/dL after 14 days of administration. However, the hypoglycemic effect was quite evident from the 7th day onwards. Metformin also significantly reduced ($p<0.05$) blood. The hypoglycemic effect of the aqueous extracts was comparable to the metformin (standard drug) and significantly more effective the drug

Table 2: Effect of Extract Formulation and Metformin on Fasting Blood Sugar (FBS) of Streptozotocin-Induced Diabetic Rats Treated For 14 Days

Group / Dose		Day 0 (before STZ)	48 hrs after STZ	Day 3	Day 6	Day 9	Day 12
I.	Normal rats (NC)	118 ± 8.00	123±4.90[a,b,c,d]	120±3.20	122±4.50	127±2.00	120±2.80
II.	Induced without treatment (PC)	122±5.00	168±5.20[a]	232±6.30[a,b,c,]	327±21.2[a,b,c]	438.3 ±3.50[a,b,c,d]	452±7.70[a,b,c]
III.	Treated with DLMF 1 (200mg/kg)	117.5±7.70	340±6.30[b]	300.6±4.00[a]	282±13.50[a]	158.3± 5.50[a]	133±8.40[a]
IV.	Treated with DLMF 2 (400mg/kg)	115.50±9.10	427±53.70[c]	330±38.90[b]	271±29.50[b]	138 ± 7.00[b]	119±5.60[b]
V.	Treated with Metformin drug (500mg/kg)	121±7.70	428±12.00[d]	396.3±13.60[c]	306.6±79.30[c]	180 ± 18.00[c]	141.5±7.70[c]

Key: Negative Control, PC: positive control, DLMF- Doum, Lemon and Mint leaf Formulation. Values were presented as mean ± standard deviation. Values bearing similar superscript within the same column are significantly different compared to each other.

The results for the fasting blood sugar for the 1st, 7th, and 14th days as shown in Table 2. Group I maintained normal weight from Day 1 to Day 14, while untreated Streptozotocin-induced diabetic rats showed a significant decrease in body weight from 108.60 ± 2.90 g/kg before inducement to 114.3 ± 2.30 g/kg on Day 7, and subsequently decreased again to 123.10 ± 2.60 g/kg on Day 14.

Rats treated with the DLMF1 (200 mg/kg) had an initial weight of 137 ± 16.90 g/kg before inducement, 153 ± 70 g/kg after 7 days, and 130 ± 8.40 g/kg on the last day of treatment. However, rats treated with DLMF2 (400 mg/kg) had an initial body weight of 134.4 ± 8.80 g/kg before inducement, 141.2 ± 50 g/kg after 7 days, and 109.80 ± 4.10 g/kg on the last day of treatment.

Rats treated with the synthetic drug Metformin showed a steady increase in weight from Day 7 to Day 14 (122 ± 2.0 to 130.20 ± 3.60 g/kg). The medicinal formulation (DLMF1 & DLMF2) was seen from the results to have a significant effect on reducing the blood glucose level of diabetic rats, even more effectively than the synthetic anti-diabetic drug Metformin.

results of the FBS from Table 2 of normal rats (NC group) indicate a steady increase and decrease in blood glucose level (123 ± 4.90 mg/dL on Day 3 and 120 ± 2.80 mg/dL on Day 14) due to fasting, yet maintaining a normal range of fasting blood sugar (FBS). The negative control group (induced without treatment) indicates a subsequent increase in FBS on Day 3 (168 ± 52.0 mg/dL), on Day 12 (438.3 ± 3.5 mg/dL), and on the last day of treatment (Day 14, 452 ± 7.7 mg/dL), clearly showing hyperglycemia which may lead to several diseases.

In comparison, the group of rats treated with the medicinal formulation of Mint leaf, Lemon grass, and Doum palm (DMLF1, 200 mg/kg) showed a significant decrease in fasting blood sugar (FBS) from Day 3 (340 ± 6.3 mg/dL) to Day 14 (133 ± 8.4 mg/dL), indicating the high effectiveness of the plant extracts. Similarly, the group treated with formulation 2 (DMLF2, 400 mg/kg) showed a significant decrease in fasting blood sugar of the induced diabetic rats from Day 6 (330 ± 38.90 mg/dL) to Day 14 (119 ± 5.6 mg/dL), which caused extensive hypoglycemia. The Metformin (synthetic drug, 500 mg/kg) treated group showed a decrease in FBS from Day 9 (306.6 ± 79.3 mg/dL) to Day 14 (141.5 ± 7.7 mg/dL). All calculations in Table 3 and

4 are done using standard mean deviation. Administration of DLMF formulation at all doses reversed the streptozotocin-induced significant changes. The results are consistent with various previous studies on the individual extracts of Doum palm, Lemon grass, and Mint leaf.

At present, despite the availability of synthetic drugs commonly prescribed to treat diabetes, different plants are traditionally used as anti-diabetic agents. Some studies have verified that the anti-diabetic properties of these plants are related to compounds such as polysaccharides, flavonoids, terpenoids, tannins, and steroids (Hooda *et al*., 2014). The hypoglycemic activity identified in the leaves of the plants can be considered a combined action of these compounds in the leaf extract. Streptozotocin is one of the most common substances used for the experimental induction of diabetes mellitus, other than alloxan. The substance induces diabetes in animals by destroying pancreatic beta cells, which results in reduced insulin secretion and increased levels of blood glucose, cholesterol, and triglycerides, along with decreased body weight (Kang and Yang, 2020; Rask-Madsen *et al*., 2013).

The anti-diabetic properties of plants may be caused by the control of oxidative stress, which prevents free radical formation induced by streptozotocin. Some previous studies mentioned that the oral administration of the leaf extracts of these plants is likely to reduce hypoglycemic activity. These results are consistent with the findings of Barbalho *et al*., 2011. Body weight loss is one of the features of diabetes mellitus, and monitoring weight is essential for assessing the severity or response to treatment. Weight measurement is an important tool in the study of diabetes. Doum palm, Lemon grass, and Mint leaf formulation (DLMF) aqueous extract at the dose levels of 200 mg/kg and 400 mg/kg considerably improved the body weight of diabetic rats, as shown in Table 1. The ability of Mint leaf extract to reduce hyperglycemia may lead to improvement in body weight in diabetic rats.

The results of this research showed that the aqueous extract of these plants has the potential to reduce glucose levels in rats treated for 14 days. It has been proven that the active organic compounds in the DLMF are likely responsible for the anti-diabetic activity of the extract. Previous studies (Hisham *et al*., 2022; Atito, 2019; Pillai *et al*., 2012) have demonstrated that individual extracts of Doum palm, Lemon grass, and Mint leaf reduce glucose levels in induced diabetic rats. This study has shown that a combination of the three extracts is extremely effective in reducing the hyperglycemic activity in diabetes-induced rats.

CONCLUSION

The study clearly indicates that the aqueous extract formulation of doum palm fruit, mint leaf and lemon grass has high potential with effective hypoglycemia activities in diabetic rats induced with streptozotocin, which is likely to be useful for the clinical treatment of diabetes. This however does not guarantee complete treatment from diabetes. Further study is needed to identify the active compounds responsible for the anti-diabetic activity.

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