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Research Article

Combination Therapy of *Pistia stratiotes* and *Helixanthera parasitica* in Alloxan-induced Diabetic Rats: Reversal of Abnormal Lipid Metabolism and Hepatomegaly

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ABSTRACT

Diabetes Mellitus (DM) remains a major global health threat with prevalence increasing rapidly across the world. Although several therapeutic agents are available, the drugs are expensive, hence not affordable to patients, particularly in developing countries. Moreover, most of the drugs are faced with certain drawbacks necessitating the search for newer drug candidates. In this study, combined extracts of *Pistia stratiotes* and *Helixanthera parasitica* at 100, 200, and 400 mg/kg Body Weight (BW) were administered to alloxan induced-diabetic rats for 21 days followed by assessment of lipid profile, hepatic and renal damages as well as organs enlargement. All diabetic rats had initial Fasting Blood Glucose (FBG) > 300 mg/dL following induction. The combined extracts at tested doses significantly ($P < 0.05$) decreased the FBG of the diabetic animals to their normal glycemic level. The diabetes-induced alteration of the lipid metabolism was significantly ($P< 0.05$) ameliorated following treatment with the respective doses of combined *P. stratiotes* and *H. parasitica* extracts. Similarly, liver damage and hepatomegaly were significantly ($P < 0.05$) reversed. Findings from this study showed that the combined extracts of *Pistia stratiotes* and *Helixanthera parasitica* could prevent hyperglycemia, reverse the compromised lipid metabolism and hepatomegaly caused by alloxan-induced DM.

Keywords: *Alloxan, Diabetes, Hepatomegaly, Helixanthera parasitica, Lipid metabolism, Pistia stratiotes*

INTRODUCTION

Diabetes mellitus (DM) is an endocrinological disorder caused as a result of a group of metabolic or heterogeneous afflictions resulting from an irregularity in insulin secretion, action, or both (Lyons and Benvenuti, 2016). The disease leads to several complications such as neuropathy, retinopathy, and nephropathy. These complications are a result of derangements in the regulatory systems involved in the mobilization and storage of metabolic fuels (Srivastaven, 2016). In addition to the persistently elevated blood glucose level, individuals with DM experienced lipid abnormalities termed "diabetic dyslipidemia" (Bhowmik *et al.*, 2018; Stöhr *et al.*, 2021). It is generally noted that DM patients have a 2-3 fold increased risk of coronary artery disease which is considered as the leading cause of death among diabetic patients (Aronson and Edelman, 2014).

The prevalence of DM is increasing rapidly, with an estimate of about 463 million adults suffering from the disease worldwide in 2019. This shows a clear-cut increase

in the previously estimated 422 million people found to be diabetics (WHO, 2016). However, the current number is projected to increase by almost 50% with an estimate of about 700 million living with the disorder by 2045 (IDF, 2019). Lifestyle changes and the use of synthetic hypoglycemic agents have remained by far, the available options for the treatment and management of DM. Although the lifestyle changes are highly promising, the drugs used in disease management are faced with certain drawbacks and are expensive (Sunil *et al*., 2020). Because of the expensive nature of the drugs, most of the patients in developing countries rely on traditional herbal preparations (Zaruwa *et al.,* 2018).

One of the important strategies used in tackling diseases is combination therapy (Corrao *et al.,* 2015). Combination therapy has gained more attention in diabetes control (Marín-Peñalver *et al.,* 2016). The strategy can be employed to increase efficacy and reduce drug resistance faced in diabetes. In a way to increase efficacy, many extracts have been combined and investigated for possible synergistic effects (Dawoud *et al.,* 2013). In this study, combined extracts of *Pistia stratiotes* and *Helixanthera parasitica* was investigated for anti-diabetic potentials.

This is because, *P. stratiotes*, commonly known as water lettuce are classified as perennial monocotyledonous plants occurring either naturally or introduced due to human activities. They are found in almost every tropical and subtropical waterway. It is usually floating on a water surface having its roots hanging below floating leaves. Phytochemicals including alkaloids, glycosides, flavonoids, and steroids have been reported in the plant hence, giving it diverse pharmacological activity (Tripathi *et al.,* 2010). The extracts from *P. stratiotes* are used in the treatment of inflammation, worm infections, tuberculosis, among others in addition to its anti-diabetic potential that was recently reported (Lawal *et al.,* 2019). While in the case of *H. parasitic*, it is a parasitic plant species belonging to *Loranthaceae* and they have a self-supporting growing form. Extracts from this plant have antioxidants and anti-metastatic effects (Rajachan *et al.,* 2020). Most importantly, a combination of the plant with other parasitic plants has been utilized in the treatment of liver diseases (Kwanda *et al.,* 2012).

Considering the potentiality of the extracts towards a wide spectrum of pharmacological activities coupled with the reported phytochemicals, herein, we reported the effect of the combined extracts on alloxan-induced diabetic experimental animals. The study could give more insights on finding alternative ways to tackle the menace of diabetes mellitus.

MATERIALS AND METHODS

Chemicals and reagents

Alloxan hydrate was purchased from Kem Light laboratory, Mumbai- India while Assay kits for Lipid profiling were obtained from Randox Laboratory limited, United Kingdom. Similarly, kits for serum aspartate aminotransferase, alanine aminotransferase, urea, and creatinine determinations were also procured from Randox Laboratory limited, United Kingdom. Glibenclamide used as the standard drug was produced from Hovid bhd, Malaysia, and was procured from a local pharmaceutical company.

Collection and preparations of plants materials

The whole plants of *P. stratiotes* and *H. parasitica* were collected in July 2021 from Katsina, Nigeria. The plants were identified and authenticated at the herbarium section of the Department of Biological Sciences, Umaru Musa Yar'adua University, Katsina, Nigeria where voucher numbers V176 and V4046 were assigned to the *Pistia stratiotes* and *Helixanthera parasitic*, respectively. Thereafter, the leaves of the plants were separately sorted, washed, and air-dried at room temperature for three (3) weeks before they were pulverized to the coarse powder prior to storage in an air-tight container. Thereafter, exactly 50 g of each of the *P. stratiotes* and *H. parasitica* powdered samples were mixed, and exactly 1 L of distilled water was added. The mixture was thoroughly mixed and was allowed to stay for 48 hrs before decanting. The solution collected was filtered using Whatman No. 1 filter paper and the filtrate was concentrated by passing it through an oven set at 45ºC. The collected dry extract was stored in a refrigerator before subsequent use.

Experimental animal and experimental design

A total of thirty-five (35) apparently healthy Wistar albino rats of both sexes ranging from 150- 250 g were used in the study. The animals were purchased from the Department of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria, and were housed in the animal house of the Department of Biochemistry, Umaru Musa Yar'adua University. The animals were randomly grouped into seven (7) groups containing five (5) rats each as follows; Normal control (NC), Extract control, Diabetic control (DC), Diabetes-induced treated with 100 mg/kg BW (DI+100E), 200 mg/kg BW (DI+200E), 400 mg/kg BW (DI+400) of the extracts and diabetes-induced treated with glibenclamide. Strict compliance with the guidelines of Good Laboratory Practice regulations of the World Health Organization (WHO) was ensured while handling the animals and the study was conducted and reported according to the ARRIVE guidelines (https://arriveguidlines.org/). Before the

commencement of the study, the animals were acclimatized for two (2) weeks.

Induction of diabetes and determination of fasting blood glucose (FBG)

For the induction of diabetes, after overnight fasting, animals in the diabetic groups were injected with 120 mg/kg BW of alloxan monohydrate intra-peritoneally. Thereafter, the animals were fed with 10 % D-glucose solution for 4 hours and then replaced with water. Successful induction was ascertained by the appearance of the diabetic symptoms (excessive urination and drinking water) followed by the subsequent increase in FBG after 72 hours of alloxan injection. At the same time, the initial FBG of the non-diabetic groups was also determined. As diabetes was established, treatment immediately commenced as earlier described and lasted for 3 weeks (21 days).

Animals' sacrifice, blood, and organ sample collections

At the end of the experiment, the FBG of the animals along with their final weights were recorded. The animals were euthanized under mild anesthesia and blood was collected in plain containers. Serum was generated by centrifuging the blood at 1000 ×g for 15minutes. On the other hand, the liver, kidney, and spleen were removed, thoroughly rinsed in normal saline, blotted with cotton wool, and relative organs weight were determined using the formula;

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Live weight of rat on the day of sacrifice}} \times 100$$

Assessment of biochemical parameters and lipid profile

Serum aspartate aminotransferase (AST), alanine aminotransferases (ALT), alkaline phosphatase (ALP), total bilirubin, and direct bilirubin in addition to serum urea and creatinine were determined using Randox Laboratory reagent kits while following the instructors' manual. Lipid profile parameters (total serum cholesterol, serum triglycerides, and high (HDL-C) density lipoprotein cholesterol) were determined using Randox Laboratory kits according to the instructors' manual. The LDL-Cholesterol (LDL-C) was determined by the Friedewald Formula (Friedewald *et al*., 1972);

$$(LDL - C) = Cholesterol - (HDL - C - \frac{Triacylglycerol}{5})$$

Statistical analysis

Data obtained were expressed as mean ± standard deviation and data analysis was conducted using a statistical software package (SPSS) version 21. For the FBG, paired sample t-test was conducted while the remaining data were analyzed using One Way Analysis of Variance (ANOVA) followed by Tukey's-HSD multiple posthoc test, and P-values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Results

The FBG of the diabetes-induced groups was > 200 mg/dL after 42 hrs induction (Figure 1). In the DC group, although the final FBG of the animals was reduced, the value was non-significant (P > 0.05) when compared with the initial (Figure 1). The increased FBG was reversed following treatments with the respective doses of the combined extracts of *P. stratiotes* and *H. parasitica.* This was evident as there was no significant (P > 0.05) difference in final FBG values of the animals with their initial FBG status (Figure 1). Comparatively, both the extracts and glibenclamide appeared to significantly (P < 0.05) decrease the final FBG with the same efficacy (Figure 1).

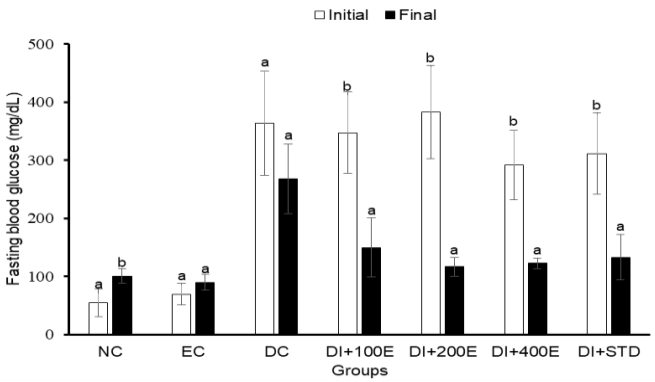

Figure 1. Effect of Combined Treatment with *Pistia stratiotes* and *Helixanthera parasitica* Aqueous Extracts on Fasting Blood Glucose in Diabetes-induced Experimental Rats.

Data are presented as Mean ± standard deviation of 5 determinations and values with different letters within the groups are considered significantly different (P< 0.05). NC– Normal Control, EC – Extract Control, DC – Diabetic Control, DI+100E – Diabetes-induced treated with 100 mg/kg BW combined extract, DI+200E – Diabetes-induced treated with 200 mg/kg BW combined extract, and DI+400E – Diabetes-induced treated with 400 mg/kg BW combined extract (400mg/kg), DI+STD- Diabetes-induced treated with glibenclamide.

Investigation of lipid profile showed a significant (P< 0.05) increase in the levels of triglycerides, total cholesterol, and LDL-C with a concomitant decrease in HDL-C in the DC group when compared with the normal control group (Table 1). Treatment with the respective doses of the combined extracts of *P. stratiotes* and *H. parasitica* significantly (P<0.05) normalized the lipid profile in the diabetic control animals (Table 1). The levels of triglycerides, total cholesterol, and LDL-C, as well as the HDL, were not significantly affected in the EC I group compared with the NC group (Table 1).

Induction of diabetes caused a significant ($P < 0.05$) increase in serum activities of AST, ALT, and ALP in the DC group when compared with the NC group (Table 2). Similarly, dir. Bilirubin and total bilirubin of the animals in the DC group significantly ($P < 0.05$) increased when compared with the animal in the normal control group (Tables 2). Treatment with the respective doses of the combined extracts of *P. stratiotes* and *H. parasitica* significantly reduced the serum activities of AST while only 400 mg/kg BW of the combined extracts of *P. stratiotes* and *H. parasitica* significantly ($P < 0.05$) reduced dir. Bilirubin and total bilirubin levels in the diabetic-induced animals (Table 2). However, the low dose (100 mg/kg B.W) of the combined extract showed a non-significant ($P > 0.05$) effect on direct bilirubin when compared with the diabetic group (Table 2). In the same vein, serum urea and creatinine non-significantly increased in the DC group when compared with the NC group (Table 2). Although treatment with the respective doses of the combined extracts of *P. stratiotes* and *H. parasitica* reduced the renal biomarkers, the effect was non-significant ($P > 0.05$) when compared with the DC group (Table 2).

Table 1. Effect of Combined Treatment with *Pistia stratiotes* and *Helixanthera parasitica* Aqueous Extracts on Lipid Profile in Diabetes-Induced Experimental Rats.

Groups	Triglycerides (mg/dL)	Total Cholesterol (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)
NC	64.79 ± 4.56^{b}	52.45 ± 2.20^{b}	19.38 ± 9.50^{a}	113.50 ± 9.94^{b}
EC	44.87 ± 4.00^{a}	39.20 ± 9.00^{a}	12.73 ± 4.00^{a}	106.44 ± 7.64^{b}
DC	201.47 ± 10.22^{d}	202.54 ± 1.89^{f}	113.95 ± 10.00^{d}	55.91 ± 13.31^{a}
DI+100E	179.73 ± 29.21^{c}	182.18 ± 2.18^{e}	74.19 ± 4.00^{c}	69.16 ± 8.93^{a}
DI+200E	157.45 ± 7.03^{c}	118.18 ± 10.05^{d}	38.35 ± 8.00^{b}	95.46 ± 5.20^{b}
DI+400E	153.82 ± 20.13^{c}	129.33 ± 26.54^{d}	16.84 ± 10.00^{a}	106.20 ± 6.22^{b}
DI+STD	139.58 ± 19.27^{c}	87.34 ± 7.00^{c}	16.96 ± 6.05^{a}	110.15 ± 10.07^{b}

Data are presented as Mean ± standard deviation of 5 determinations and values with different letters between the groups are considered significantly different ($P< 0.05$). LDL-C – Low-density lipoprotein cholesterol, HDL-C – High-density lipoprotein cholesterol, NC – Normal Control, EC – Extract Control, DC – Diabetic Control, DI+100E – Diabetes-induced treated with 100 mg/kg BW combined extract, DI+200E – Diabetes-induced treated with 200 mg/kg BW combined extract, and DI+400E – Diabetes-induced treated with 400 mg/kg BW combined extract (400mg/kg), DI+STD- Diabetes-induced treated with glibenclamide.

Table 2. Effect of Combined Treatment with *Pistia stratiotes* and *Helixanthera parasitica* Aqueous Extracts on Diabetes-Induced Hepatic and Renal Damages in Experimental Rats.

Groups	AST (i.u./mL)	ALT (i.u./mL)	ALP (i.u./mL)	Total Bilirubin (mg/dL)	Dir. Bilirubin (mg/dL)	Serum Urea (mg/dL)	Serum Creatinine (mg/dL)
NC	39.03 ± 2.38^{b}	18.81 ± 1.82^{b}	14.21 ± 1.71^{ab}	0.91 ± 0.07^{b}	0.34 ± 0.05^{a}	36.76 ± 7.52^{a}	2.10 ± 0.50^{a}
EC	32.54 ± 1.46^{a}	14.66 ± 2.04^{a}	10.43 ± 2.53^{a}	0.72 ± 0.06^{a}	0.32 ± 0.03^{a}	37.45 ± 6.87^{a}	1.16 ± 0.06^{a}
DC	79.84 ± 5.40^{d}	29.16 ± 2.97^{d}	17.67 ± 0.58^{c}	1.93 ± 0.07^{e}	1.39 ± 0.12^{c}	52.25 ± 14.48^{a}	2.70 ± 0.68^{a}
DI+100E	52.22 ± 11.96^{c}	21.70 ± 4.40^{bcd}	17.33 ± 0.85^{c}	1.64 ± 0.04^{d}	1.32 ± 0.13^{c}	44.90 ± 19.89^{a}	2.67 ± 0.16^{a}
DI+200E	46.40 ± 5.05^{c}	21.45 ± 1.29^{c}	16.53 ± 1.14^{c}	1.53 ± 0.06^{c}	1.15 ± 0.11^{b}	40.06 ± 5.65^{a}	1.84 ± 0.83^{a}
DI+400E	45.58 ± 4.26^{c}	23.82 ± 1.94^{cd}	16.88 ± 1.38^{c}	1.50 ± 0.03^{c}	1.22 ± 0.11^{c}	48.13 ± 10.73^{a}	2.20 ± 0.60^{a}
DI+STD	45.69 ± 2.16^{c}	24.92 ± 1.76^{d}	14.00 ± 0.35^{b}	1.49 ± 0.03^{c}	0.93 ± 0.11^{b}	47.84 ± 4.23^{a}	2.40 ± 0.33^{a}

Key: Data are presented as Mean ± standard deviation of 5 determinations and values with different letters between the groups are considered significantly different ($P< 0.05$). AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; ALP = Alkaline phosphatase. NC – Normal Control, EC – Extract Control, DC – Diabetic Control, DI+100E – Diabetes-induced treated with 100 mg/kg BW combined extract, DI+200E – Diabetes-induced treated with 200 mg/kg BW combined extract, and DI+400E – Diabetes-induced treated with 400 mg/kg BW combined extract (400mg/kg), DI+STD- Diabetes-induced treated with glibenclamide.

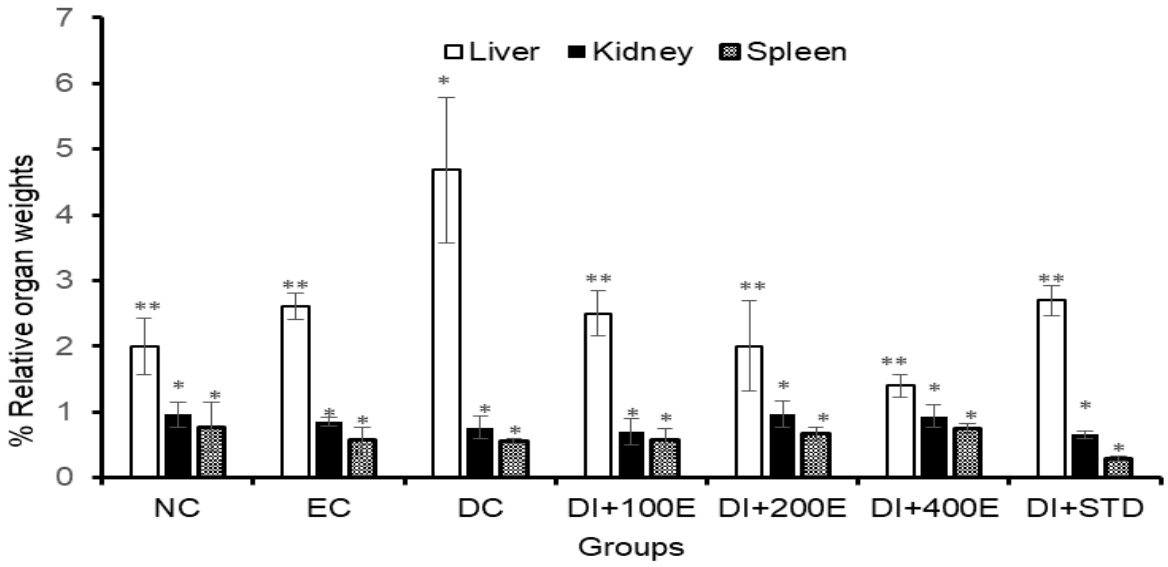

Figure 2: Effect of Combined Treatment with *Pistia stratiotes* and *Helixanthera parasitica* Aqueous Extracts on Relative Organ Weights of Diabetes-induced Experimental Rats.

Data are presented as Mean ± standard deviation of 5 determinations and values with the different numbers of asterisks along the organs are considered significantly different (P< 0.05). NC – Normal Control, EC – Extract Control, DC – Diabetic Control, DI+100E – Diabetes-induced treated with 100 mg/kg BW combined extract, DI+200E – Diabetes-induced treated with 200 mg/kg BW combined extract, and DI+400E – Diabetes-induced treated with 400 mg/kg BW combined extract (400mg/kg), DI+STD- Diabetes-induced treated with glibenclamide.

Investigation of the relative liver weight of animals in the DC group revealed a significant (P< 0.05) increase in the organ weight when compared with the normal control group with a non-significant (P > 0.05) increase in the relative weights of kidney and spleen (Figure 2). As the relative kidney and spleen weights were not significantly (P> 0.05) affected by the respective doses of the combined extracts of *P. stratiotes* and *H. parasitica*, treatment with the extracts significantly (P< 0.05) decreased the relative liver weight of the diabetic-induced animals (Figure 2). The effect was found to be dose-dependent with 400 mg/kg BW of the combined extracts showing more effects (Figure 2).

Discussion

As DM remains a major global health threat, the increased drawbacks associated with the therapeutic agents have necessitated the search for new drug candidates against the disease (Ighodaro *et al.*, 2021). As an important strategy, we investigated the potential of the combined extracts of *P. stratiotes* and *H. parasitica* against disease-induced hyperglycemia, lipid metabolisms, and organs damage.

The choice of alloxan as a diabetogenic agent in the current study was due to its informed ability to cause diabetes via partial degradation of β-cell of the pancreatic islets in experimental animals (Lenzen, 2008). The high FBG levels > 200 mg/dL after 42 hrs induction in the DC group coupled with the observed diabetic symptoms was indicative of a successful diabetic induction in the experimental animals. The sustained FBG in the DC group revealed the absence of reversion to normoglycemic conditions in the group. The progressive decline in FBG observed in the groups treated with respective doses of the combined extracts of *P. stratiotes* and *H. parasitica* could indicate the presence of some anti-hyperglycemic principle in the extracts that had the same degree of potency as the standard hypoglycemic drug (Glibenclamide). Several plant extracts have been reported to have anti-diabetic action in laboratory models (Kibiti and Afolayan, 2015) and this activity has been attributed to diverse groups of secondary metabolites found in these extracts (Gaikwad *et al.*, 2014; Mishra *et al.*, 2015). Many of these bioactive compounds act by either potentiating the secretion of insulin or prolonging the half-life of circulating insulin (Domínguez *et al.*, 2017); some others improve the sensitivity of peripheral tissues to insulin (Miyazaki *et al*., 2001) or reduce the contribution of hepatic gluconeogenesis to diabetic hyperglycemia. Studies have confirmed the presence of some of these phytochemicals in the individual extracts of *P. stratiotes* and *H. parasitica* (Tripathi *et al.*, 2010; Kwanda *et al.*, 2012), and most importantly, we have previously reported the anti-

diabetic effect of *P. stratiotes* (Lawal *et al.*, 2019) which could support the observed FBG lowering effects of the individual extracts investigated.

The generalized increases in serum lipids (TC, TG, LDL-C) with decreased HDL-C in the DC group observed in this study have been a well-known phenomenon in diabetic animals (Rizzo and Berneis, 2005; Bitzur *et al.*, 2009). High levels of these lipids in the blood have been strongly associated with the cardiovascular complications found in diabetic patients (Sadeghi *et al.*, 2020) so any treatment regimen that also significantly decreases the levels of these lipids in the serum would be of immense benefit as observed with respective doses of the combined extracts of *P. stratiotes* and *H. parasitica*. Similarly, the respective doses of the combined extracts of *P. stratiotes* and *H. parasitica* elevated the HDL-C level which is usually considered beneficial to health (Ali *et al.*, 2012).

Organ degeneration occasioned by diabetes is consistently seen in later stages of the disease (Soumya and Srilatha, 2011). Signs of degenerative changes in the liver and kidneys of the DC group were manifested by the increased serum activities of investigated aminotransferases, total and direct bilirubin as well as serum urea and creatinine levels. As organ degeneration is usually occasioned in the later stage of the disease (Soumya and Srilatha, 2011), the non-significant increase in some of the parameters could occur as a result of the short duration of the study. Administration of the combined extracts of *P. stratiotes* and *H. parasitica* ameliorated the liver damage but not the kidney in addition to hepatomegaly induced in the diabetic rats. The effect of the combined extracts on the liver could be due to *H. parasitica* since it is reported to have efficacy in the treatment of liver diseases (Kwanda *et al.*, 2012). It is pertinent to note that the extracts when given to non-diabetic rats caused no change in these parameters; suggesting that the extract may not be toxic to the two organs in question.

CONCLUSION

From the present study, we concluded that Administration of the combined extracts of *P. stratiotes* and *H. parasitica* could prevent hyperglycemia, dyslipidemia and hepatomegaly caused by DM. Hence, the combination of the extracts may play a role in fighting diabetes mellitus. Our future work will focus on finding the active ingredients responsible for the above observations.

AUTHORS' CONTRIBUTIONS

Conceptualization: IAU, NUM, FAD, SA; Methodology: SA, MMS, AY, UAW, AM, US, NUM, IAU; Formal analysis and investigation: IAU, NUM, SA; Writing - original draft preparation: SA; Writing -review and editing: IAU, NUM, SA, and FAD. All authors have read and approved the Manuscript.

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CONFLICT OF INTEREST

We declared that there is no potential conflict of interest.

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