ABSTRACT: Diabetes is one of the commonest global diseases affecting both sexes and phytomedicine is currently among the foremost replacements for orthodox drug. Polyalthia longifolia is among the locally used herbal remedies for various ailments. The onus of this research was to investigate the phytochemical constituents and potential of P. longifolia aqueous leaf extract against alterations in liver and kidney functions in rats injected with alloxan. Phytochemical evaluation of the aqueous plant leaf extract indicated that terpenes, non-reducing sugar, flavonoid, resin, phenol, gums and mucilage were present. The contents of total flavonoids and phenol in the plant leaf are 55.56 µg catechin equivalent/g and 1.62 g/ 100g DW, respectively. The plant extract administered reduced the glucose concentration of the diabetic-induced animals in a dose dependent manner. This reducing potential of glucose by the plant is as a consequence of the availability of these phytochemicals in the extract of the plant. Alterations in liver function biomarkers (serum ALP, ALT, AST, GG, TB and DB) caused by the hyperglycemic state of the test animals were reversed as the extract was given to the diabetic rats. Kidney function makers such as creatinine, urea and uric acid were also reduced upon administration of hypoglycemic drug and aqueous plant extract and improved as time progressed. Thus P. longifolia (mast tree) aqueous leaf extract has ameliorative effects on liver and kidney functions of rats induced with diabetes and could be used in management of type 2 diabetes even at a concentration of 100 mg/kg bwt.

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Key words: Polyalthia longifolia, hypoglycemic agent, hepato-renal toxicity, amelioration

MATERIALS AND METHODS

Experimental Animals: Adult male rats that were used in the work were of the weight range of 100-200g. They were bought from the animal house, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria and then fed for a period of one week with grower mash purchased from Dutch Farm Limited, Abraka and water ad libitum. Diabetes was induced in these animals by the injection of alloxan monohydrate which caused hyperglycemia via the destruction of the pancreas.

Chemicals: All reagents and the alloxan monohydrate used for this research were bought from Alpha Chimika, Mumbia, China.

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Collection and Identification of Plant Material: Leaves of mast tree (*Polyalthia longifolia*) were collected from Ovu-Inland, Delta State and identified in the Department of Botany, Delta State University, Abraka, Delta State, Nigeria.

Preparation of Extract: The obtained plant leaves were washed with water and then air-dried for three weeks. These were grinded to power form with the help of Waren blender. 800g of powdered solute (*P. longifolia*) was immersed in 3200ml (3.2L) of distilled water (1:4 ratio). It was marinated for 48hrs to obtain crude. This was followed by filtration using Whatman No110 filter paper. The supernatant (filtered crude) was then concentrated using a vacuum rotary evaporator at 50°C and water bathe at 40°C. This extract concentrate, which was dark in colour and weighed 141.88g (17.7%/w/w), was packaged in an airtight plastic container and stored at 4°C until when required for use.

Induction of Diabetes: The rats were starved for 12 hours and 150mg/kg alloxan monohydrate solution was administered intraperitoneally. Main while, the fasting blood glucose level (FBGL) was recorded using glucometer before the induction to know the glucose levels. The alloxan monohydrate solution was prepared by dissolving 4g of alloxan in 100ml of normal saline (40mg/ml.). After 72 hours of alloxan monohydrate injection, blood glucose level of over 200mg/dl was recorded which is an indication of hyperglycemic state (Iweala et al., 2013; Islam et al., 2015).

Experimental design: The design of the study is as shown in Table 1.

| Group | Treatment |
|-------|-----------|
| NC    | Negative control (Healthy rats + no treatment) |
| PC    | Positive control (Diabetic rats + no treatment) |
| F1    | Diabetic rats + 100 mg/kg of extract of *P. longifolia* |
| F2    | Diabetic rats + 200 mg/kg of extract of *P. longifolia* |
| F3    | Diabetic rats + 300 mg/kg of extract of *P. longifolia* |
| STD   | Diabetic rats + 100 mg/kg of hypoglycemic drug (Metformin) |

Phytochemical screening: Preliminary phytochemical screening of aqueous leaf extract of *P. longifolia* was carried out using standard methods as described by Njoku and Obi (2009), Borokini and Omotayo (2012), Anigboro et al. (2014) and Tonukari et al., (2015) to screen for the presence of various chemical constituents.

Determination of Biochemical Parameters: Determination/Estimation of liver enzymes, lipid profile and kidney function were carried out using the Prietest easylab Biochemistry analyzer. It measures theoretical densities of samples and it uses algorithm to calculate results, which are used for biochemical investigations. Serum Glucose, Urea, Creatinine, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Total Bilirubin (TB), Direct Bilirubin (DB), Alkaline Phosphatase (ALP), Uric Acid, Urea, and Gamma glutamyltransferase (GGT), were assayed using Prietest easylab Biochemical analyzer.

Statistical Analysis: The data was analyzed using a computer software (SPSS version 21) and compared using Bonferroni post hoc test. The results were represented as Mean ± SD. Values in tables carrying different superscript letters (a,b,c,d,e) within a column or row are considered statistically significantly different (P<0.05) while those with the same letter are not (P>0.05).

RESULTS AND DISCUSSION

Based on the phytochemical evaluation of *Polyalthia longifolia*, the presence of terpenes and non-reducing sugar was observed with mild detection of flavonoid, resin, phenol, gums and mucilage (Tables 2a and 2b). In the management of diabetes mellitus, one approach is the slowing down of glucose absorption through reduction in carbohydrate catabolism in the intestine by inhibiting hydrolyzing enzymes of carbohydrate such as α-amylases, α-glucosidases and maltase (Hamden et al., 2013). Terpenes such as triterpenes are effective in inhibiting α-glucosidases and maltase (Nazaruk and Borzym-Kluczyk, 2015). Aldose reductase inhibition is another means through which terpenes are used in the upkeep of diabetes; aldose reductase is involved in polyol pathway also called sorbitol-aldose reductase pathway responsible for the catabolism of un-phosphorylated glucose. The metabolism of glucose through the sorbitol-aldose reductase is greatly increased in hyperglycemic state and it results to microvascular complications in diabetes (Petrash, 2004). In a diabetic state, the quantity of free radical generated increases leading to a reduction of the antioxidant capacity of the body (Rahimi et al., 2005). Terpenes function to boost the antioxidant potential of the body (Tonukari et al., 2013; Anigboro et al., 2014; Tonukari et al., 2015; Aganbi et al., 2017). Liver and renal dysfunction is linked with metabolic disorder. Previous findings have proven that *Polyalthia longifolia* leaves and stem extract elicit its action through anti-hyperglycemic activity and inhibition of α-glucosidase and α-amylase (Gosh et al., 2010; Sivashanmugam and Chatterjee, 2017). Liver and renal dysfunction is linked with metabolic disorder. Previous findings have proven that *Polyalthia longifolia* leaves and stem extract elicit its action through anti-hyperglycemic activity and inhibition of α-glucosidase and α-amylase.
Phenolic compounds including flavonoids and phenol from plants serve as natural antioxidants and hence are also important in the treatment of diabetes as phenols and flavonoids have the ability to inhibit digestion enzymes like α-amylase and α-glucosidase required in the catabolism of complex carbohydrate to glucose (Lin et al., 2016). Mucilage derived from plant has long been employed in Persian world for treatment of various ailments including type 1 diabetes through its inhibition of α-glucosidase and α-amylase. Mucilage also carries out antimicrobial activity etc (Ameri et al., 2014); mucilage is important in drug delivery because of its binding and gelling potential alongside its sustaining capacity (Wadhwa et al., 2013).

Table 2a: Qualitative analysis of phytochemical constituents of *Polyalthia longifolia* leaf.

| Phytochemical          | *Polyalthia longifolia* |
|------------------------|-------------------------|
| Saponin                | -                       |
| Tannin                 | -                       |
| Terpenes               | ++                      |
| Flavonoid              | +                       |
| Phlobatannins          | -                       |
| Alkaloid               | -                       |
| Glycosides             | -                       |
| Resin                  | +                       |
| Phenol                 | +                       |
| Micronutrients         | -                       |
| Steroids               | -                       |
| Proteins               | -                       |
| Carbohydrates          | -                       |
| Amino acids            | -                       |
| Gums & mucilage        | +                       |
| Non reducing polysaccharides | -               |
| Non reducing ++ simple sugar | -                 |

+ = Mildly present; ++ = highly present; +++ = more highly present; - = Absent. The phytochemical evaluation of the mast tree leaf extract indicated that terpenes, non-reducing sugar, flavonoid, resin, phenol, gums and mucilage were present.

Table 2b: Total phenol and flavonoid contents of *Polyalthia longifolia* leaf.

| Phytoconstituent | *Polyalthia longifolia* |
|------------------|-------------------------|
| Total phenol (g/100g DW) | 1.62                    |
| Total flavonoid (µg CE/g) | 55.65                   |

The quantitative analysis showed that mast tree leaf is rich in total phenol (1.62 g/100g dry weight) and total flavonoids (55.65 µg catechin equivalent/g).

During the period of study, the elevated glucose level of the untreated diabetic rats remained within the same range while the diabetic treated rats’ glucose concentration decreased as the time of study progressed (Table 3); at each five-day interval the glucose concentration was lower than the previous five-day value. *P. longifolia* leaf extract reduced the glucose concentration in a dose dependent manner, higher dosage had better effect. The reduction in glucose level by plant extract and hypoglycemic drug in treated rats was markedly lower than in diabetic rats that were not treated at day 10 and day 15. This noticed reduction in the concentration of glucose indicates that the extract could be used in treatment of hyperglycemic condition and supports previous claims of the plant’s use in the upkeep of diabetes (Khan et al., 2013). Phytochemical composition of this plant has a lot to play in regards to the reduction in glucose concentration. As stated earlier, phenolic compounds and terpenes possesses the potential to inhibit α-glucosidase and α-amylase which are required in the breakdown of carbohydrate to glucose, these phytochemicals present in *P. longifolia* also have antioxidant ability to protect the cells from increased free radicals caused by oxidative stress due to the diabetic state. The activity of ALT (alanine aminotransferase) in the blood of test rats at the end of experiment showed decrease in activity when compared against the untreated diabetic rats; the higher dosage had a better impact (Table 4). Levels of liver function enzymes (ALT, AST, ALP and GGT) were significantly increased (P<0.05) in the untreated diabetic rats (PC) compared to the healthy control group (NC). Administration of *P. longifolia* aqueous leaf extract to the diabetic rats, however, led to significant (P<0.05) decrease in levels of these enzymes in groups F1, F2 and F3. Bilirubin levels in the treated diabetic group rats (F1, F2 and F3) were also reduced compared to the untreated group (PC) though not significantly (P>0.05). The diabetic experimental animals treated with hypoglycemic drug had a lower level of ALT, even lower than that of the normal rats (negative control) and significantly lower than all groups. AST (aspartate aminotransferase) activity of test diabetic rats given aqueous leaf extract of *P. longifolia* was noticeably lower than the diabetic rats without treatment and normal rats while diabetic rats treated with hypoglycemic drug had the least activity of AST and significantly different from all groups (Table 4). ALP (alkaline phosphatase) activity of diabetic rats treated with plant extract and hypoglycemic drug reduced when compared against untreated diabetic rats and the reduction was significantly different (Table 4).
The significant increased levels of serum creatinine and serum urea in the untreated diabetic animal group (PC) compared to the healthy control group (NC) were significantly (P<0.05) decreased in levels of these enzymes in groups F1, F2 and F3. Bilirubin levels in both untreated (PC) and treated diabetic group rats (F1, F2,) increased (P<0.05) compared to the negative control group (NC). The significantly increased levels of serum creatinine and serum urea in the untreated diabetic animal group (PC) compared to the healthy control group (NC) were significantly (P<0.05) reduced in groups F1, F2, and F3 treated with the extract of P. longifiola. Serum uric acid levels in diabetic rats were also reduced by the plant extract intervention, though not significantly (P>0.05). Inducement of diabetes to the experimental animals resulted in an increase of GGT (gamma glutamyltransferase) activity, the GGT activity only reduced in diabetic animals treated with hypoglycemic drug which was markedly lower than all groups (Table 4). Total bilirubin (TB) level and direct bilirubin (DB) were elevated in untreated diabetic rats and those treated with plant extract compared to the negative control, whereas the hypoglycemic drug treated group reduced significantly in TB and DB levels when correlated with those treated with plant extract and untreated diabetic rats (Table 4). The rise in serum level of liver function enzymes (serum ALT, AST, ALP and GGT) has been attributed to the damaged structural integrity of the liver (Asayama et al., 1994; Lee et al., 2016) as a result of a metabolic alteration that occurs in diabetic animals. Bilirubin acts as an antioxidant to mop off free radicals generated; in diabetic condition bilirubin guides against lipid peroxidation. Thus the elevated level of bilirubin in hyperglycemia state is a protective action of the body and bilirubin concentration is reduced as the concentration of glucose in the blood reduces or tends towards normal (Zhu et al., 2017).

Table 3: Hypoglycemic effect of P. longifiola aqueous leaf extract on alloxan-induced diabetic rats

| GROUP  | ALT (U/L) | AST (U/L) | ALP (U/L) | GGT (U/L) | TB (mg/dl) | DB (mg/dl) |
|--------|----------|----------|----------|-----------|------------|------------|
| NC     | 23.00 ± 2.00d | 56.70 ± 2.00d | 31.00 ± 1.00d | 72.00 ± 1.00d | 53.00 ± 1.00d | 23.00 ± 2.00d |
| PC     | 25.00 ± 2.00c | 58.00 ± 2.00c | 32.00 ± 1.00c | 74.00 ± 1.00c | 55.00 ± 1.00c | 25.00 ± 2.00c |
| F1     | 27.00 ± 2.00b | 60.00 ± 2.00b | 33.00 ± 1.00b | 76.00 ± 1.00b | 57.00 ± 1.00b | 27.00 ± 2.00b |
| F2     | 29.00 ± 2.00a | 62.00 ± 2.00a | 34.00 ± 1.00a | 78.00 ± 1.00a | 59.00 ± 1.00a | 29.00 ± 2.00a |
| F3     | 31.00 ± 2.00 | 64.00 ± 2.00 | 35.00 ± 1.00 | 80.00 ± 1.00 | 61.00 ± 1.00 | 31.00 ± 2.00 |
| STD    | 33.00 ± 2.00 | 66.00 ± 2.00 | 37.00 ± 1.00 | 82.00 ± 1.00 | 63.00 ± 1.00 | 33.00 ± 2.00 |

Values are expressed in Mean ± Standard error of mean. F1 = P. longifiola aqueous extract concentration at 100mg/kgbwt, F2 = 200mg/kgbwt, F3 = 300mg/kgbwt, STD = Standard antidiabetic drug (Metformin) given at 100 mg/kgbwt. The plant extract administered reduced elevated glucose level of the diabetic animals in groups F1, F2 and F3 in a dose dependent manner and also with respect to duration of administration from Day 1 to 15.

Table 4: Effect of P. longifiola aqueous leaf extract treatment on liver function biomarkers in alloxan-induced diabetic rats.

| GROUP  | ALT (U/L) | AST (U/L) | ALP (U/L) | GGT (U/L) | TB (mg/dl) | DB (mg/dl) |
|--------|----------|----------|----------|-----------|------------|------------|
| NC     | 23.00 ± 2.00d | 56.70 ± 2.00d | 31.00 ± 1.00d | 72.00 ± 1.00d | 53.00 ± 1.00d | 23.00 ± 2.00d |
| PC     | 25.00 ± 2.00c | 58.00 ± 2.00c | 32.00 ± 1.00c | 74.00 ± 1.00c | 55.00 ± 1.00c | 25.00 ± 2.00c |
| F1     | 27.00 ± 2.00b | 60.00 ± 2.00b | 33.00 ± 1.00b | 76.00 ± 1.00b | 57.00 ± 1.00b | 27.00 ± 2.00b |
| F2     | 29.00 ± 2.00a | 62.00 ± 2.00a | 34.00 ± 1.00a | 78.00 ± 1.00a | 59.00 ± 1.00a | 29.00 ± 2.00a |
| F3     | 31.00 ± 2.00 | 64.00 ± 2.00 | 35.00 ± 1.00 | 80.00 ± 1.00 | 61.00 ± 1.00 | 31.00 ± 2.00 |
| STD    | 33.00 ± 2.00 | 66.00 ± 2.00 | 37.00 ± 1.00 | 82.00 ± 1.00 | 63.00 ± 1.00 | 33.00 ± 2.00 |

Table 5: Effect of P. longifiola aqueous leaf extract treatment on serum creatinine, urea and uric acid levels in alloxan-induced diabetic rats.

| GROUP  | Creatinine (mg/dl) | Urea (mg/dl) | Uric acid (mg/dl) |
|--------|--------------------|--------------|-------------------|
| NC     | 2.23 ± 0.06 | 18.73 ± 0.06 | 3.23 ± 0.06 |
| PC     | 2.57 ± 0.06 | 20.23 ± 0.06 | 3.57 ± 0.06 |
| F1     | 2.83 ± 0.06 | 21.47 ± 0.06 | 3.83 ± 0.06 |
| F2     | 3.03 ± 0.06 | 22.73 ± 0.06 | 4.03 ± 0.06 |
| F3     | 3.23 ± 0.06 | 23.93 ± 0.06 | 4.23 ± 0.06 |
| STD    | 3.43 ± 0.06 | 25.23 ± 0.06 | 4.43 ± 0.06 |

The significantly increased levels of serum creatinine and serum urea in the untreated diabetic animal group (PC) compared to the healthy control group (NC) were significantly (P<0.05) reduced in groups F1, F2, and F3 treated with the extract of P. longifiola. Serum uric acid levels in diabetic rats were also reduced by the plant extract intervention, though not significantly (P>0.05).
P. longifiola aqueous leaf extract. Inducement of diabetes caused a great increase of urea concentration that was significantly different (P<0.05) from all groups. Administration of the extract and hypoglycemic drug reduced, non-significantly, the elevated urea concentration. The reduced urea concentration by the drug and aqueous leaf extract was not as low as that of the negative control.

Hyperglycemia is one of the main reasons of gradual renal damage. This improper functioning of the kidney leads to an elevation of urea in the blood (Ishraq, 2014). Elevated serum concentration of creatinine and urea in diabetic state indicates destruction to the kidney. Serum uric acid has been proven to be associated with kidney disease; the reason behind high uric acid concentration in a diabetic patient might be as a result of the inhibition of reabsorption of uric acid in the proximal tubule (Pavani and Anoop, 2011). Based on the observed results of the study, P. longifiola (mast tree) aqueous leaf extract carries out ameliorative actions on the liver and kidney; this is evident through the ability of the plant aqueous extract to reduce and bring to normal range the concentration of creatinine, urea, uric acid and liver enzymes (ALP, AST, ALT and GGT) in the blood. The reduction in direct bilirubin and total bilirubin upon administration of plant extract to the diabetic animals also proves further that the plant extract carries out ameliorative action against alterations in liver and kidney functions in diabetic rats injected with alloxan; the plant aqueous leaf extract also exhibited ability to reduce glucose level in diabetic rats.

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