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EFFECT OF COLUMN CHROMATOGRAPHIC FRACTIONS OF METHANOL EXTRACT OF CURCUMA LONGA LINN RHIZOME ON LIVER AND KIDNEY PARAMETERS IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Medicinal plants traditionally have shown to play key role in healing and its ingredients have served as a source of encouragement for several major pharmaceutical drug. The present study was conducted to evaluate the effect of column chromatographic fractions (FI-FVI) of methanol extract of *Curcuma longa* Linn rhizome on alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total bilirubin (TB) and direct bilirubin (DB) levels as well as urea, creatinine and electrolytes (Na^+, Cl^-, K^+, HCO_3^-) levels in alloxan-induced diabetic rats. The fractions (FI-FVI) were screened at dose of 200 mg/kg body weight using a total of forty-five (45) rats which were grouped into nine (9) groups of five (5) rats each. The alloxan was administered intraperitoneal at a dose of 100 mg/kg per body weight. The administration of the fractions lasted for 21 days. There was significant ($p<0.05$) increase in weight of animals orally administered with fraction II, decrease in feed and water intake. Administration of fraction II also lowers significantly elevated activities of ALT, AST, ALP, GGT, TB and DB in diabetic rats as well as increases protection against

renal dysfunction as was shown by reduced urea, creatinine, Na^+ and K^+ levels. The fractions possesses no toxic effect as indicated by lowered AST and ALP levels and may be used for the management of diabetes mellitus.

KEYWORDS: Kidney enzymes; Liver enzymes; Column chromatography; Fractions; *Curcuma longa* Linn.

INTRODUCTION

Diabetes mellitus is a non-communicable disease which have been shown to improve with medicinal plants (Mohammed *et al*., 2021). It is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fats and protein metabolism resulting from defects in insulin secretion, insulin action or both (WHO, 2016). Diabetes is also referred to as a syndrome of disorder in metabolism usually due to the combination of hereditary and environmental causes resulting in abnormally high blood sugar levels (hyperglycemia) (Roder *et al.,* 2016). Blood glucose levels are controlled by a complex interaction of multiple chemical and hormones in the body including the hormones insulin made in beta cells of the pancreas. Diabetes mellitus develops due to diminished production of insulin (in type I) or resistances to its effects (in type II and gestational), both leads to hyperglycemia, which largely causes the acute signs of diabetes and changes in energy metabolism (Ngwen *et al*., 2018). As a result of the deficiency of insulin or inadequate insulin function there is an inadequate transfer of glucose into the cells; the utilization of glucose for energy and cellular products and its conversion to glycogen or fat and storage as such are depressed, thereby leading to accumulation of glucose in the blood, causing hyperglycemia. Fat may be mobilized from adipose tissue and broken down to provide a source of energy, which is eventually withdrawn from the body by the liver and broken down to glycerol and fatty acids leading to oxidation by the hepatic cells to ketone bodies and metabolizes by cells to produced energy, carbon dioxide and water (Mohammed *et al*., 2017). Only a limited amount of ketone acids can be utilized by cells as such if ketogenesis proceeds rapidly, exceeding the rate at which they can be metabolized, the ketone acids accumulate in the blood causing ketosis or ketone acidosis (Anssi, 2004). Tissue protein may also be broken down to amino acids which are used in gluconeogenesis contributing to the hyperglycemia. Both the uptake of amino acids by the cells and body protein synthesis are decreased. Insulin-dependent diabetes mellitus (IDDM) usually has a sudden onset in a severe, acute form. In non-insulin-dependent diabetes mellitus (NIDDM) the onset is most often insidious going undetected and

untreated for a considerable period of time. Diabetes mellitus, a chronic non-communicable disease, is ranked 7th killer disease in the world with an estimated 382 million people affected, as reported by (WHO 2015). Conventional drugs used in the treatment of diabetes are sometimes inadequate, expensive and can have serious side effects. It is therefore imperative to search for alternative drugs of higher efficacy and safety to replace and/or support the currently used drugs for the treatment and/or management. The world health organization has also recommended the evaluation of the effectiveness and safety of plants used in traditional and complementary medicines (Ibrahim *et al*., 2014)

The plant Turmeric (Curcuma longa), also known as Gangamau in Hausa language is a rhizomatous herbaceous perennial plant of the ginger family (Zingiberaceae), Zingiberaceae grows 5 - 6 feet high in the tropical regions of Southern Asia, with trumpet-shaped, dull yellow flowers. Its roots are bulbs that also produce rhizomes, which then produce stems and roots for new plants. Turmeric has a bitter and somewhat sharp taste; individual plants are about 1m tall with long oblong leaves when exposed to temperatures between 20 °C and 30 °C (68 °F and 86 °F) and a considerable amount of annual rainfall. Plants are gathered annually for their rhizomes, and are reseeded from some of those rhizomes in the following season (Aggarwal *et al*., 2007).

MATERIALS AND METHODS

Materials

Study animals

Forty-five male albino rats weighing between 65g - 85g were purchased from animal house of Biological Science Department; Bayero University, Kano. The animals were housed in well-ventilated cages in the animal house unit of Biological Science Department, Bayero University Kano. The rats were allowed to acclimatize for one week prior to the experiment and had access to food and clean water at libitum. Principles of laboratory animal care (NIH, 1996) and ethical guidelines for investigation of experimental pain in conscious animals were observed during experimentation (Zimmermann, 1983).

Plant material

The rhizomes of *Curcuma longa* Linn was collected from Toro Local Government Area of Bauchi State, Nigeria. It was identified and authenticated by a taxonomist from the Department of Plant Biology, Faculty of Sciences, Bayero University Kano and was given a

voucher number of (BUK/HAN/0188). The rhizomes was air dried under a shade at room temperature.

Methods

Preparation of alloxan

One gram of Alloxan Hydrate was dissolved in 10 mL of distilled water and to give a concentration of 100 mg/mL

Induction of diabetes mellitus with alloxan

Rats induced with Diabetes mellitus were fasted overnight for a period of 12 hours before induction of the diabetes by injecting alloxan hydrate intraperitoneally at dose of 100 mg/kg using a sterile 1 ml syringe. The volume of the solution containing 100 mg/kg given to each experimental albino rat was determined by the following relationship (Mohammed *et al*., 2017).

$$Volume\ (cm3) = \frac{Dose\ (mg/kg) \times weight\ of\ rat\ (kg)}{Concentration\ of\ \ alloxan\ (mg/cm3)}$$

Animals with fasting blood glucose ≥200 mg/dl after 48 hours of alloxan administration were considered to be diabetic and used for the study (Kumar *et al.*, 2006).

Determination of weight increase

Total body weight of diabetic and non-diabetic male wister albino rats were measured using digital chemical balance, before and after the experimental period and recorded as initial body weight (IBW) and final body weight (FBW), respectively (Kim and Ha, 2013). The weight changes were expressed as % weight increase, and were obtained as follows:

$$\frac{FBW - IBW}{IBW} \times 100$$

Packing of the column

Silica gel of mesh size of 60-120g was used as the stationary phase while varying solvents combinations were used as the eluent. Wet packing method as describe by Jerry *et al.*, (2010) was used in preparing the silica column, a slurry was formed by mixing 200 g of the silica gel in 500 ml of hexane and poured down quickly and carefully into the column, the tap was left open during packing to allow free flow of the solvent into a beaker below. At the end of the packing, the tap was closed and left to stand undisturbed for 24 hours, after which the clear

solvent on top of the silica gel was allowed to drain down to the silica gel meniscus. The flow rate of the column was noted.

Loading of the sample

Twenty gram (20g) of the dried extract was mixed thoroughly with 20g of silica gel and then gently layered on top of the column. Elution of the column was done with various solvent combination of varying polarity. The following solvents systems were used in the elution process; hexane: ethyl acetate 100:0, 80:20, 60:40, 40:60, 20:80, 0:100. For each solvent combination, the elution was done until each solvent ratio becomes clear. The eluted fractions were collected in an aliquots volume of 50ml.

Pooling of the fractions using analytical thin layer chromatography

Each fraction was spotted in a pre-coated aluminum silica gel plate and developed in a chromatographic tank in the appropriate solvent systems. With the aid of a capillary tube, a spot of the sample was applied on the plate at 1.0 cm distance from the base of the plate, the plate was allowed to dry at room temperature, and lowered in a chromatographic tank containing the solvent system saturated with the solvent vapour. The solvent was allowed to ascend the plate until the solvent front reaches about ¾ of the length of the plate. The plate was removed and allowed to dry at room temperature. The relative retention factor (Rf) was calculated

$$R_f = \frac{\text{Distance travelled by compound from origin}}{\text{Distance travelled by solvent from origin}}$$

Screening of column chromatography fractions

Forty-five (45) rats were used and grouped into nine (9) groups of five (5) rats each.

Group	Treatment
I	Normal control
II	Diabetic control
III	Diabetic, administered with Standard drug 100mg/kg body weight.
IV	Diabetic, administered with 200mg/kg body weight of fraction I
V	Diabetic, administered with 200mg/kg body weight of fraction II
VI	Diabetic, administered with 200mg/kg body weight of fraction III
VII	Diabetic, administered with 200mg/kg body weight of fraction IV
VIII	Diabetic, administered with 200mg/kg body weight of fraction V
IX	Diabetic, administered with 200mg/kg body weight of fraction VI

On 21st day after 24h of administration, blood from all animals was collected by retro-orbital puncture and after that the animals were euthanized. Blood was allowed to clot and centrifugation was performed at 3500 rpm for 10 min at 4°C to separate the serum which was used for the assay of Liver and kidney function indices.

Statistical analysis

Results were expressed as mean ± standard deviation. Statistical differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test after investigating the data for normality using Shapiro-Wilk test and for variances homogeneity to be sure that the data are normally distributed and variances would be homogenous using GraphPad Instat3 Software version 3.05 Differences of $P < 0.05$ were considered to be significant (GraphPad, 2000).

RESULTS

Table 1 showed the effect of oral administration of 200mg/kg body weight of column chromatography fractions on water intake, feed intake, and percentage weight gain in diabetic rats for 21 days. There were significant differences ($p<0.05$) in water intake, feed intake and percentage weight gain in groups administered with standard drug, fraction F_{II} when compared with diabetic control group. Also, Fraction $F_{I,}$ F_{III}, F_{IV}, F_V and F_{VI} shows no significant differences ($P>0.05$) when compared with diabetic control group.

Table 1: Effect of oral administration of 200mg/kg body weight of fractions of methanol extract of *Curcuma longa* Linn rhizomes in diabetic rats for 21 days.

Group	Initial body weight (g)	Feed intake (g/day)	Water intake (ml/day)	Final body weight(g)	Percentage Weight gain (%)
Normal control	65.20 ± 2.31	16.02 ± 1.66[a,d,e,f,g,h]	23.42 ± 2.43[a,d,e,f,g,h]	87.91 ± 1.88	34.80
Diabetic control	68.00 ± 2.77	34.12 ± 1.37[a,b,c]	98.82 ± 4.28[a,b,c]	69.40 ± 1.50	2.06
Standard drug	63.40 ± 2.10	19.67 ± 1.79[b]	27.12 ± 2.21[b]	81.35 ± 3.20	28.31
F_I	71.60 ± 3.30	26.57 ± 2.26[d]	52.33 ± 2.27[d]	79.08 ± 2.90	10.44
F_{II}	66.70 ± 2.20	18.70 ± 1.89[c]	28.22 ± 1.23[c]	80.73 ± 1.90	21.03
F_{III}	68.40 ± 3.20	30.67 ± 1.94[e]	80.24 ± 4.66[e]	70.33 ± 1.80	2.28
F_{IV}	70.40 ± 3.30	28.67 ± 1.77[f]	70.32 ± 3.32[f]	72.80 ± 2.80	3.40
F_V	71.30 ± 2.90	33.33 ± 1.37[g]	77.48 ± 3.28[g]	75.90 ± 1.80	6.45
F_{VI}	68.40 ± 2.30	33.78 ± 1.90[h]	83.42 ± 3.03[h]	69.70 ± 3.20	1.90

Table 2 showed the effect of oral administration of 200mg/kg body weight of fractions of methanol extract of *Curcuma longa* Linn rhizome on liver enzymes (AST, ALT, ALP, GGT, TB and DB) in alloxan-induced diabetic rats. There was a significant ($p<0.05$) increase in the serum levels in all the parameters analyzed in diabetic control group (Group II) compared to the normal control (Group I). Significant decreases ($p<0.05$) in all the liver enzymes analyzed were however, observed in standard drug treated group and fraction F_{II} (group IV) treated group when compared with diabetic control group (group II).

Table 2: Liver Function indices in Alloxan-induced diabetic rats orally administered with 200mg/kg body weight of fractions of the Methanol extract of *Curcuma longa* Linn rhizomes for 21 days.

Group	ALT(U/L)	AST(U/L)	ALP(U/L)	GGT(U/L)	TB (umol/l)	DB (umol/l)
Normal control	8.24 ± 0.61^{a,d,e,f,g,h}	13.82 ± 0.26^{a,d,e,f,g,h}	182.42± 4.92^{a,d,e,f,g,h}	34.61 ± 1.48^{a,d,e,f,g,h}	4.19 ± 0.33^{a,d,e,f,g,h}	8.82 ± 0.42^{a,d,e,f,g,h}
Diabetic control	91.74 ± 4.93^{a,b,c}	138.11 ± 8.62^{a,b,c}	544.95 ± 26.19^{a,b,c}	92.13 ± 3.89^{a,b,c}	18.26 ± 2.48^{a,b,c}	41.16 ± 2.94^{a,b,c}
Metformin	11.42 ± 0.62^{b}	15.25 ± 0.88^{b}	189.52 ± 2.11^{b}	39.13 ± 1.42^{b}	3.66 ± 0.67^{b}	8.26 ± 0.91^{b}
F_I	41.13 ± 0.63^{d}	59.05 ± 0.47^{d}	250.13 ± 5.31^{d}	50.27 ± 2.83^{d}	10.65 ± 1.94^{d}	19.33 ± 0.48^{d}
F_{II}	11.89 ± 1.48^{c}	18.23 ± 1.22^{c}	191.24 ± 1.64^{c}	39.97 ± 1.52^{c}	5.54 ± 0.78^{c}	9.16 ± 0.72^{c}
F_{III}	84.72 ± 3.47^{e}	147.04 ± 4.89^{e}	533.13 ± 31.14^{e}	88.38 ± 4.42^{e}	20.10 ± 2.98^{e}	36.88 ± 3.78^{e}
F_{IV}	72.64 ± 3.55^{f}	121.32 ± 5.34^{f}	486.98 ± 28.91^{f}	72.65 ± 2.88^{f}	16.95 ± 1.98^{f}	32.87 ± 2.09^{f}
F_V	82.48 ± 2.79^{g}	140.52 ± 3.89^{g}	550.89 ± 31.88^{g}	89.42 ± 3.11^{g}	17.98 ± 1.79^{g}	34.69 ± 4.77^{g}
F_{VI}	90.55 ± 5.12^{h}	120.99 ± 13.76^{h}	536.89 ± 32.70^{h}	88.11 ± 3.78^{h}	18.62 ± 1.66^{h}	38.98 ± 3.76^{h}

Values are expressed as mean ± SD, n= 5 for each group. Values in the same column bearing similar superscripts are significantly different at $p<0.05$. ALT-Alanine aminotransferase, AST- Aspartate aminotransferase, ALP- Alkaline phosphatase, GGT-gamma glutamyl transferase, TB- Total bilirubin, DB-Direct bilirubin.

Table 3 showed the effect of oral administration of 200mg/kg body weight of fractions of Methanol extract of *Curcuma longa* Linn rhizomes on serum concentrations of urea, creatinine and electrolytes (Na^+, Cl^-, K^+, HCO_3^-) in alloxan-induced diabetic rats. Significant increases ($p<0.05$) were observed in mean serum urea, creatinine, Na^+ and K^+ of diabetic control group (Group II) compared to the normal control (Group I), with a significant

decrease ($p<0.05$) in mean serum of Cl^- and HCO_3^- of diabetic control group (Group II) compared to the normal control (Group I). Significant decreases ($p<0.05$) in urea, creatinine, Na^+ and K^+ were observed in groups administered with standard drug and fraction F_{II} (group IV) when compared with diabetic control group (group II), with corresponding significant increase ($P<0.05$) in mean serum of Cl^- and HCO_3^- when compared with diabetic control group (group II).

Table 3: Kidney Function indices in Alloxan-induced diabetic rats orally administered with 200mg/kg body weight of fractions of the Methanol extract of *Curcuma longa* Linn rhizomes for 21 days.

Group	Urea (mmol/l)	Creatinine (mmol/l)	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	HCO_3^- (mmol/l)
Normal	7.18±0.98^{a,e,f,g,h}	108.17±8.41^{a,d,e,f,g,h}	145.14±10.66^{a,d,e,f,g,h}	4.92±0.32^{a,d,e,f,g,h}	115.40±4.62^{a,d,e,f,g,h}	25.76±0.55^{a,d,e,f,g,h}
Diabetic	36.82±2.19^{a,b,c,d}	494.81±10.48^{a,b,c}	120.62±9.53^{a,b,c}	6.97±0.89^{a,b,c}	90.40±5.36^{a,b,c}	11.40±0.94^{a,b,c}
Metformin	6.18± 0.81^{b}	115.86± 12.81^{b}	144.20± 11.49^{b}	5.04± 0.22^{b}	113.44± 5.49^{b}	24.41± 0.75^{b}
F_I	12.19± 0.68^{c}	222.12± 10.59^{d}	122.10± 9.77^{d}	6.17± 0.91^{d}	96.80± 3.45^{d}	13.804± 0.85^{d}
F_{II}	6.07± 0.95^{d}	120.31± 10.69^{c}	143.69± 9.55^{c}	4.98± 0.92^{c}	112.47± 6.54^{c}	24.04± 1.55^{b}
F_{III}	33.48± 3.78^{e}	404.21± 12.47^{e}	119.80± 8.84^{e}	6.22± 0.96^{e}	95.00± 1.87^{e}	11.60± 0.87^{e}
F_{IV}	30.34± 3.42^{f}	324.56± 13.95^{f}	123.04± 4.87^{f}	6.20± 1.03^{f}	92.66± 1.76^{f}	14.62± 0.64^{f}
F_V	36.98± 2.89^{g}	468.41± 13.74^{g}	120.03± 7.85^{g}	6.59± 0.98^{g}	90.98± 0.97^{g}	11.78± 0.76^{g}
F_{VI}	34.78± 3.09^{h}	498.82± 12.66^{h}	120.64± 8.52^{h}	6.99± 1.02^{h}	90.79± 1.98^{h}	11.57± 0.93^{h}

Values are expressed as mean ± SD, n= 5 for each group. Values in the same column bearing similar superscripts are significantly different at P<0.05

DISCUSSION

Alloxan, a toxic glucose analogue causes destruction of pancreatic β-cells when administered to vertebrate. This causes an insulin dependent diabetes mellitus known as alloxan-induced diabetes in the animals (Alhassan *et al.*, 2017). Alloxan is selectively toxic to insulin producing pancreatic β-cells because it preferentially accumulates in β-cells through uptake via glucose transporter-2 (GLUT2) (Luka and Mohammed, 2012). Alloxan, in the presence of intracellular thiols, generate reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The β-cell toxic action of alloxan is initiated by free radicals

formed in this redox reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells (Muhammad *et al*., 2016). Administration of alloxan elevates serum glucose, which signifies induction of diabetes mellitus. This finding is in accordance with the findings of Alhassan *et al.*, 2017 who reported induction of diabetes mellitus to experimental rats using 100 mg/kg body weight of alloxan.

The study also revealed significant loss of weight with corresponding increase in water intake of diabetic control rats compared to normal control rats and the diabetic rats administered with standard drug and Fraction F_{II} showed weights increased significantly throughout the period of study. This findings is similar to study on evaluation of the effect of aqueous leaf extract of *Corchorus Olitorius* on some biochemical parameters in alloxan-induced diabetic rats have equally reported significant weight reduction in diabetic control rats (Mohammed *et al.*, 2019). The loss in weight observed in the diabetic control may be due to the loss in muscle and adipose tissue resulting from excessive breakdown of tissue protein and fatty acids, weight loss is one of the symptoms of diabetic mellitus occurring especially when glycemic control is poor. However, the rats treated with the plant fraction showed appreciable increase in weight compared to the diabetic control group. This appreciable increased in weight as well as lowered water intake indicates that the treatment allowed the tissues to access the glucose both to supply energy and spared some to build tissue materials required for growth.

Evaluation of liver function indices shows hepatocytes damage as a result of alloxan-induced diabetes which is characterized by elevation in the level of different hepatic marker enzymes (AST, ALT and ALP) and the levels of gamma glutamyl transferase, total bilirubin and direct bilirubin. Administration of fraction II to diabetic rats significantly ameleriote the hepatic toxicity induced by decreasing the levels of hepatic marker enzymes (AST, ALT and ALP). These findings are consistent with several other studies on induced diabetic rats (Alhassan *et al*., 2017), who reported a significant decrease in the activity of liver enzymes (AST, ALT and ALP) in albino rats induced with liver damage after been administered with aqueous extract of *Khaya senegalensis* at doses of 250 mg/kg and 500 mg/kg Body weight for 5 days. Diabetes causes an increase in mean serum urea, creatinine, K^+ and Na^+ of diabetic control group compared to the normal control, while a significant decrease ($p<0.05$) was observed in mean serum of Cl^- and HCO_3^- in diabetic control group compared to the normal control.

Hence, indicating kidney damage as a result of induction of diabetes, a finding supporting similar study conducted on the effect of extract of *Citrus sinensis* peel on biochemical parameters (Luka *et al*., 2017).

CONCLUSION

The study therefore concludes that; administration of fractions of methanol extract of *Curcuma longa* Linn rhizomes may not have toxic effect on the liver and kidney at the employed dosage since it produced no significant effects on the enzymes activities as biochemical enzymes makers of liver and kidney damage. Therefore, the study shows that there is a prospective future in the use of *Curcuma longa* Linn rhizomes as a source of natural medicine for management of diabetes mellitus.

Competing interests

Authors have declared that no competing interests exist.

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