TROPICAL LIFE SCIENCES RESEARCH

# Antidiabetic, Antioxidant and Anti-Inflammatory Activities of Residual Aqueous Fraction of *Ethulia conyzoides* in Induced Type 2 Diabetic Rats

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## Highlights

- Sub-acute antidiabetic studies were done with varying doses (100, 200 and 400 mg/kg body weight). Treatment with the highest dose of residual aqueous fraction (RAF) of *Ethulia conyzoides* caused a 67.13% reduction in the blood glucose level of the diabetic rats.

- The in-vivo antioxidant studies revealed that serum SOD and catalase of diabetes groups treated with residual aqueous fraction of Ethulia conyzoides significantly ($p<0.05$) increased while serum MDA significantly ($p < 0.05$) decreased when compared with diabetic untreated group.

- The highest dose of 400 mg/kg b.w. was found to be the most effective dose and treatment using 400mg/kg b.w of residual aqueous fraction of *Ethulia conyzoides* caused a 30.80% and 63% reduction in TNF-α and IL-1β.

- The RAF of *Ethulia conyzoides* has ameliorative effects for Type 2 Diabetes (T2D).

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# Antidiabetic, Antioxidant and Anti-Inflammatory Activities of Residual Aqueous Fraction of *Ethulia conyzoides* in Induced Type 2 Diabetic Rats

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Running head: Antidiabetic, Antioxidant and Anti-Inflammatory Activities

## ABSTRACT

Oxidative stress and inflammation have been proved to be implicated in the pathogenesis of type 2 diabetes. Recent studies showed that *Ethulia conyzoides* had *in vitro* antioxidant activity. This study investigated the *in vivo* antidiabetic, antioxidant and anti-inflammatory potential of residual aqueous fraction of *Ethulia conyzoides* in type 2 diabetic induced male *Wistar* rats. Sub-acute antidiabetic studies were done with varying doses (100, 200 and 400 mg/kg body weight) of residual aqueous fraction for 21 days. At the end of the treatment, blood glucose level, serum insulin, *in vivo* antioxidant and pro-inflammatory cytokines- tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were analyzed. There was significant ($p < 0.05$) reduction in blood glucose, malondialdehyde (MDA), IL-1β and TNF-α levels and significant ($p < 0.05$) higher levels of (superoxide dismutase) SOD, catalase and insulin in rats administered with different concentrations of residual aqueous fraction compared to diabetic control group. Furthermore, the 400 mg/kg body weight dosage concentration was found to be the most effective. This result suggests that residual aqueous fraction of *Ethulia conyzoides* possess significant antidiabetic, antioxidant and anti-inflammatory activities.

**Keywords:** Antidiabetics, Antioxidant, Cytokines, *Ethulia conyzoides,* Type 2 Diabetes

## INTRODUCTION

Diabetes, a metabolic disorder is considered as one of the most significant global health problems afflicting both young and old in all parts of the world irrespective of gender (Animaw & Seyoum 2017; IDF 2019). The International Diabetes Federation reported an increase from 151 million in 2000 to approximately 463 million in 2019 among people living with diabetes and by 2045 it will rise to 700 million (IDF 2019). In Africa, about 19 million adults aged 20-79 years had

diabetes in 2019 and this figure is likely to rise to about 47 million by 2045; while in Nigeria about 2.7 million people (aged 20–79) were living with diabetes (IDF 2019).

Evidences have shown that chronic inflammation may play an imperative role in the insulin resistance and Type 2 Diabetes pathogenesis (Barzilay *et al.* 2001; Duncan *et al.* 2003). Studies of Badawi *et al.* (2010) and Wang *et al.* (2013) showed that several markers of inflammation are implicated in Type 2 Diabetes. Moreover, elevated plasma concentrations of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) appeared to be associated with insulin resistance and are elevated in obesity, metabolic syndrome and Type 2 Diabetes (Nilsson *et al.* 1998; Spranger *et al.* 2003).

The use of medicinal plants has been part of the history of mankind (Gidey *et al.* 2015; Odhiambo *et al.* 2011). There is high reliability, in Africa, of people on their continuous use because of the belief that they are the most effective ways of treating diverse diseases (Joshi & Joshi 2000). *Ethulia conyzoides* Linn (Asteraceae)-is an herb that grows up to 1.5 m high around wet grass land or riverside. The leaves are used as therapy for cancer in Madagascar (Burkill 1985) and South Western Nigeria (Sowemimo *et al.* 2009). The plant is a source of natural antioxidants (Aliyu *et al.* 2012). It is used as an anti-helminthic for round worms and for abdominal disorders; used to treat headaches and dysmenorrhea while crude methanol extract of the aerial parts of *E. conyzoides* has antibacterial activity (El-Bassuony 2009; Noumia *et al.* 1999). It has been reported that extracts of *E. conyzoides* contains flavonoids, triterpenoids and sterols (Mahmoud *et al.* 1983) which have been said to be responsible for their anti-diabetic effect (Gaikwad *et al.*, 2014). An interview with people living in Okpokwu Local Government Area of Benue state claimed that they have been using *E. conyzoides* to treat diabetic cases; hence the need to explore its potential. In this study, we looked at the acute effect of different doses of residual aqueous fraction on insulin, antioxidant and cytokines level.

## MATERIALS AND METHOD

### Chemicals and Reagents

Streptozotocin (STZ) procured from Sigma Aldrich (USA); Fructose (Kem light Laboratories PVT Ltd, India); Simas margarine (PT salim Ivomas pratama Tbk, Indonesia); Normal diet feed (Grand Cereals Limited, Jos, Nigeria); Rat insulin ELISA kit (Fine test kit, Wuhan, China) and all other Chemicals and reagents used were of analytical grade and procured from appropriate manufacturing companies.

### Plant Material

The whole plant of *Ethulia conyzoides* Linn was harvested from its natural habitat at the end of the raining season at Okpokwu L.G.A of Benue State. It was identified by Mr. Namadi Sanusi at the Herbarium unit of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University (A.B.U), Zaria, Nigeria with a specimen voucher number 7098 previously deposited in the herbarium.

## Experimental Animals

Forty-two (42) apparently healthy male Wistar rats weighing 120-150 g, purchased from the Animal House of the Department of Pharmacology and Theurapeutics, A.B.U Zaria, were kept in well aerated cages, given access to animal feed and water *ad libitum,* allowed to acclimatize for two weeks, then maintained under standardized environmental condition (22-28$^0$C, 60%-70% relative humidity, 12 hours dark light cycle). Ethical clearance was obtained from the A.B.U Committee on Animal Use and Care (Approval Number: ABUCAUC/2019/007). All institutional guidelines for experimental protocol adhered to as well as strict compliance to National and International Laws and Guidelines for Care and Use of Laboratory Animals in Research.

## Methods

### *Preparation and extraction of plant material*

The plant sample was rinsed in clean water to remove debris and dust particles then air dried at room temperature. The dried whole plant sample was ground into powder using a mortar and pestle. About 1700 g of the grounded sample was suspended in 70% crude methanol (1:10 *w/v*) for 48 hours at room temperature with frequent agitation (cold maceration). The mixture was filtered off using a Whatman filter paper no. 1 (1 mm mesh sieve) and the methanol solvent in the filtrate was evaporated completely using rotary evaporator at 40 ºC and the sample was concentrated by drying in a water bath maintained at a temperature of 45 ºC to obtain dried extract. The solvent-free crude methanol extract was kept in a sealed sample bottle and refrigerated at 2 - 4 °C until further required (Otsuka, 2006).

### *Partitioning of the crude methanol extract of E. conyzoides*

The solvent-free crude methanol extract (122 g) was suspended in 50 mL of distilled water and then partitioned n-hexane and ethyl-acetate consecutively to obtain n-hexane fraction, ethyl acetate fraction and residual aqueous fraction. n-hexane was added to the crude methanol extract that was dissolved in distilled water, it was then turned into a separating funnel, shaken and allowed to stand for phase separation into two fractions. The n-hexane fraction was carefully decanted after partitioning then more of the n-hexane solvent was added and the same process above was repeated several times until it was completely partitioned to obtain the n-hexane fraction. The same process above was repeated using ethyl acetate solvent to obtain the ethyl acetate fraction. The resulting residue was dissolved in water and referred to as the residual aqueous fraction. Each fraction obtained was concentrated using a rotary evaporator and the remaining solvent in the extract was allowed to evaporate at room temperature to a constant weight (Otsuka 2006). The process of fractionation (partitioning) is a purification step of the crude extract

***Induction of type 2 diabetes***

The induction of type 2 diabetes was carried out as described by S. I. Okoduwa *et al.* (2017a) with modification. Animal feed (pelleted broiler finisher - vital feed brand) was fortified with margarine in a ratio of 10 g animal feed to a gram of margarine. This was administered along with 20% fructose solution as drinking water, to the rats *ad libitum* for six (6) weeks, they were then fasted overnight and injected intraperitoneally with dissolved streptozotocin (STZ) (in a citrate buffer pH 4.5) at a single low dose of 45 mg/kg body weight. The first 24 hours after induction, the animals were given 5% glucose solution as drinking water.

***Confirmation of diabetes***

This was done ten days after STZ induction, with glucose test strip and glucometer using blood samples obtained via tail puncture of the rats. After the confirmation, animals with fasting blood glucose (FBG) ≥ 200 mg/dl, Homeostatic Model Assessment of Insulin Resistance (HOMA IR) > 5 and Homeostatic Model Assessment of β-cell (HOMA – β) < 200 were incorporated in the study as diabetic animals (S. I. R. Okoduwa *et al.* 2017b; K. Srinivasan *et al.* 2005).

***Animal grouping***

The rats were divided into seven groups of six rats each and the treatment was administered for 21 days.

Normal Control: Normal rats without induction and treatment
Diabetic Control: Diabetic rats without treatment
Diabetic rats treated with 500 mg/kg b.w Metformin
Diabetic rats treated with 100 mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*
Diabetic rats treated with 200 mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*
Diabetic rats treated with 400 mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*
Normal rats treated with 400 mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*

The fraction was administered to the animals orally.

Weekly body weight change was measured during the entire experimental period. The percentage body weight was calculated using the formula below;

$$\text{Percentage change in body weight} = \frac{\text{Final body weight-Initial body weight}}{\text{Initial body weight}} \times 100$$

Also, the percentage feed and fluid intake was extrapolated using the formula below;

Percentage change in feed/fluid intake =

Initial weight/volume of feed/fluid - leftover weight/volume of feed/fluid x 100

Initial weight/volume of feed/fluid

**Sample Collection**

After 21 days of treatment, the animals were fasted overnight, anaesthetized using chloroform and then sacrificed by decapitation. Blood was collected into plain bottles and was placed immediately on ice for 3 hr, then centrifuged at 3000 rpm for 15 mins to obtain the serum used for biochemical findings. The liver from the control and experimental groups of the rats were excised and rinsed with cold saline. The preparation of liver homogenate was done by homogenizing 1 g of liver in 4 ml of 0.1M phosphate buffer saline at pH 7.4. The homogenates were centrifuged at 3,000 rpm for 15min. The supernatant was collected as liver tissue homogenate, and was used for the *in vivo* antioxidant activity.

***Determination of in vivo antioxidant, insulin and cytokines activity***

Superoxide dismutase (SOD) was determined by a method described by (Fridovich 1989). Catalase activity was determined as described Sinha (1972). Lipid peroxidation was assessed by Thiobarbituric acid reactive substances Determination (TBARS) formation (Ohkawa *et al.* 1979). Insulin, TNF-α and IL-β was determined using ELISA assay kits according to Fine test kit manufacturers instruction.

**Insulin Sensitivity, Resistance and β-cell Function Estimation**

$$Insulin\ sensitivity = \frac{1}{Log\left\{Fasting\ serum\ insulin\left(\frac{U}{L}\right)\right\} \times Log\left\{Fasting\ blood\ glucose\left(\frac{mmol}{l}\right)\right\}}$$

$$HOMA - IR = \left\{Fasting\ serum\ insulin\left(\frac{U}{L}\right) \times Fasting\ blood\ glucose\left(\frac{mmol}{l}\right)\right\}/22.5$$

$$HOMA - \beta = \frac{20 \times Fasting\ serum\ insulin\ (\frac{U}{L})}{Fasting\ blood\ glucose\ (\frac{mmol}{l})} - 3.5$$

Conversion factor: Insulin (1U/l = 7.174 pmol/l) and blood glucose (1 mmol/l = 18 mg/dl).

**Statistical Analysis**

All statistical analyses were conducted using the computer software, statistical package for the social sciences (SPSS program version 25.0). The results are stated as mean ± standard deviation (SD). The data were analyzed by the one-way analysis of variance (ANOVA) and repeated measure ANOVA where necessary. The Duncan multiple range test was used to

determine the level of significance. P value less than 0.05 was considered as significant ($p < 0.05$).

## RESULTS

### Effect of Residual Aqueous Fraction of *Ethulia conyzoides* on Mean Fluid (mL/rat/day) and Feed intake (g/rat/day) of Induced Type 2 Diabetic Rats for the 21 days of Treatment

The mean fluid and feed intake of each experimental animal on a daily basis throughout the experimental duration is depicted in Figs. 1 and 2, respectively.

At the induction, the diabetic groups had increased fluid intake compared to normal group, although it was not significant ($p > 0.05$). At the week of diabetes confirmation, the fluid intake had significantly ($p < 0.05$) increased among the diabetic untreated group; also in week 2 and 3.

The diabetic groups at induction had significant ($p < 0.05$) increase in feed intake compared to normal group. The week during which the diabetes was confirmed, the diabetic untreated group had significant ($p < 0.05$) decrease in feed intake. While at week 2 and 3, the feed intake significantly ($p < 0.05$) increased in diabetic control compared to all the other groups.

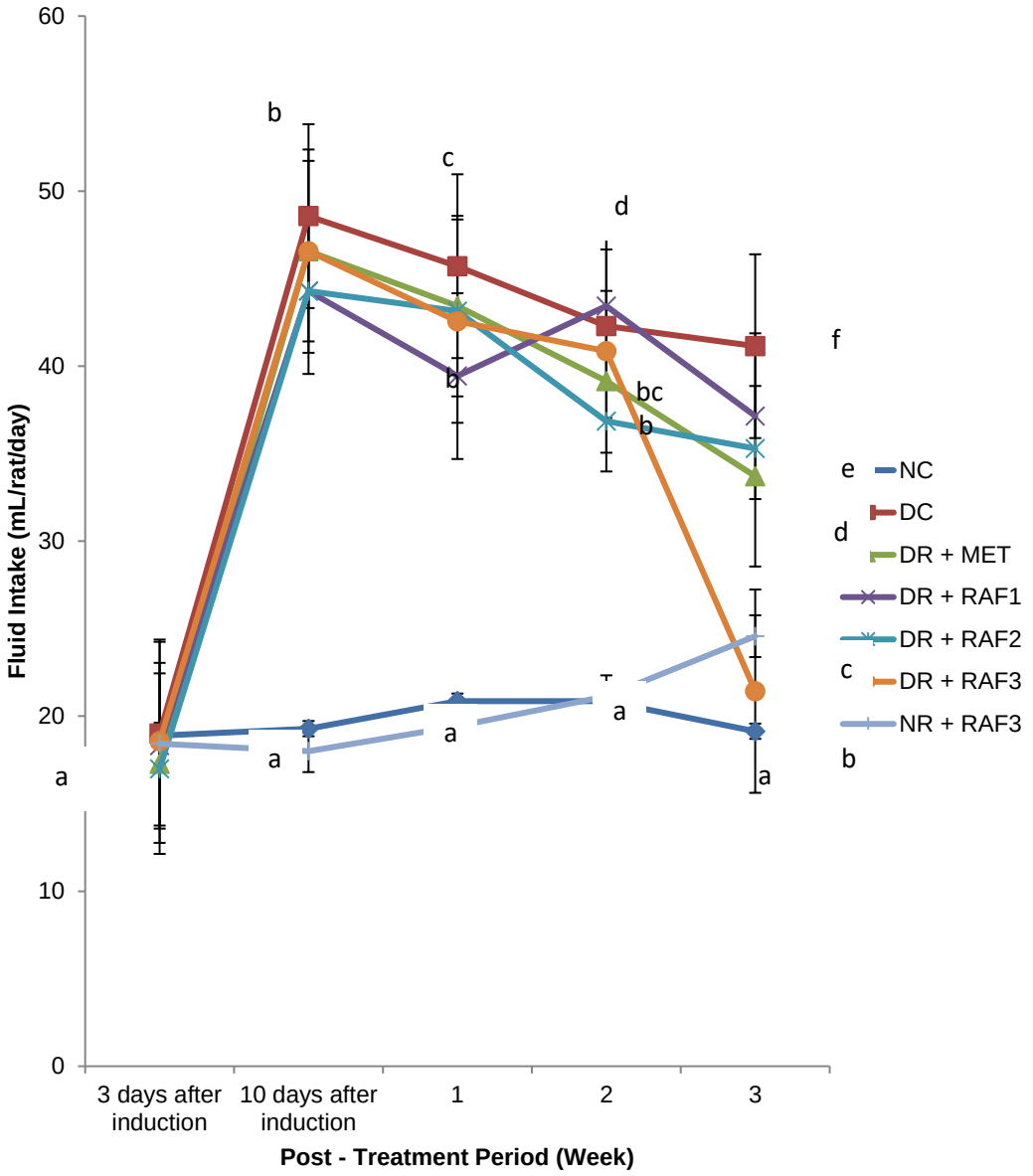


**Figure 1.** Effect of residual aqueous fraction of *Ethulia conyzoides* on mean fluid intake (mL/rat/day) of induced Type 2 diabetic rats for the 21 days of treatment.

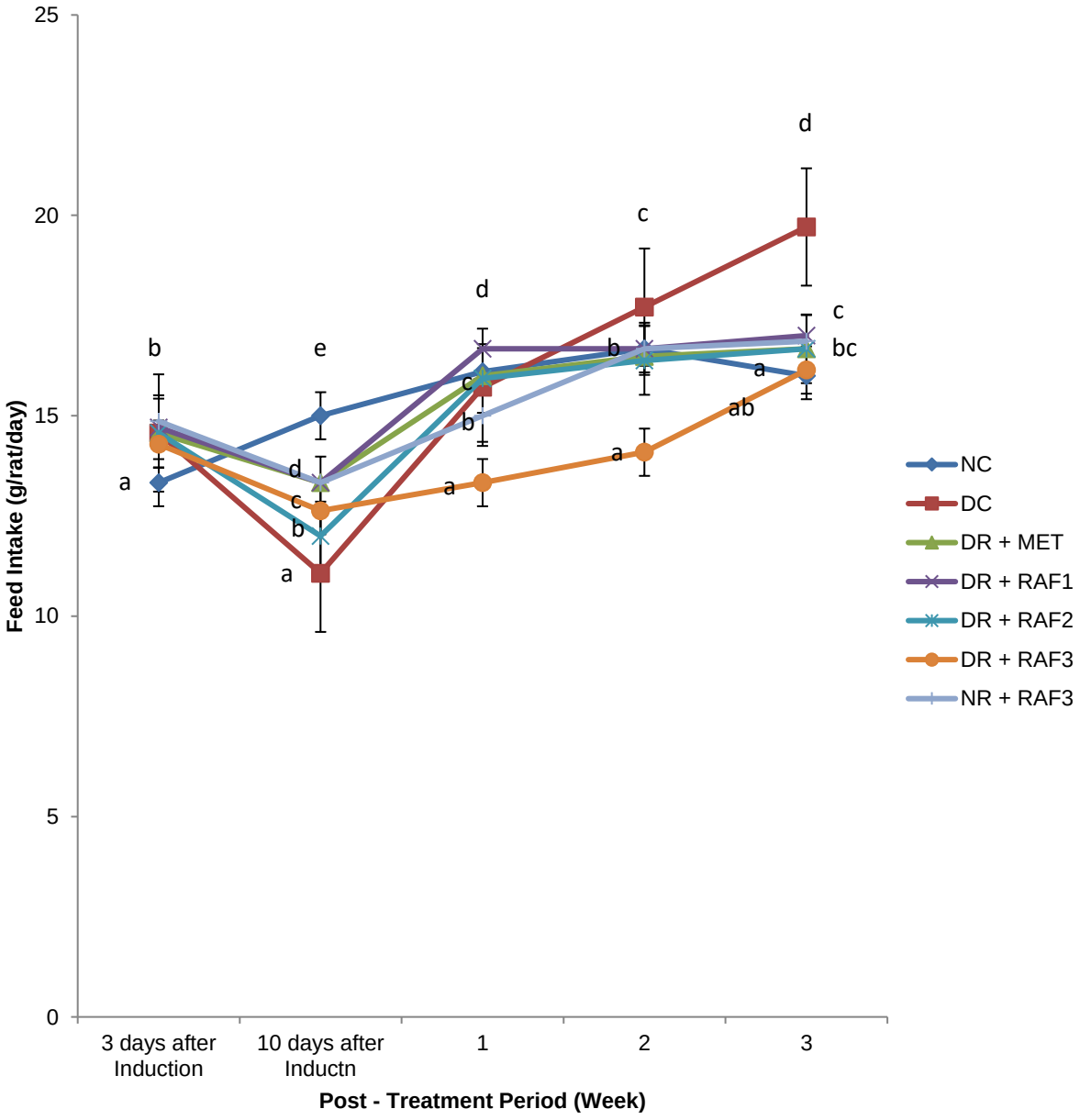


**Figure 2.** Effect of residual aqueous fraction of *Ethulia conyzoides* on mean feed intake (g/rat/day) of induced Type 2 diabetic rats for the 21 days of treatment.

**Effect of Residual Aqueous Fraction of *Ethulia conyzoides* on the Body Weight of Induced Type 2 Diabetic Rats for 21 Days of Treatment**

The result (Fig. 3) shows that diabetic untreated rats had a significant ($p < 0.05$) reduction in body weight change compared with normal control.

The treatment with standard drug (metformin) and different concentrations of residual aqueous fraction of *Ethulia conyzoides* significantly ($p < 0.05$) increased the body weight. The

rat group treated with 500 mg /kg b.w of metformin, 200 mg/kg b.w residual aqueous fraction and 400 mg/kg b.w residual aqueous fraction gave significant ($p < 0.05$) increase in body weight.

**Effect of Residual Aqueous Fraction of *Ethulia conyzoides* on the Weekly Percentage Change in Blood Glucose Level of Induced Type 2 Diabetic Rats for 21 Days of Treatment**

The initial blood glucose of all the diabetic rats was higher than the normal control rats. Treatment with different doses (100 mg/kg b.w., 200 mg/kg b.w. and 400 mg/kg b.w) of residual aqueous fraction of *Ethulia conyzoides* significantly ($p < 0.05$) lowered the blood glucose level of the diabetic rats (Table 1).

The rat group treated with 400 mg /kg body weight (b.w) residual aqueous fraction that showed the highest percentage (-73.70%) reduction in blood glucose level. The rat group treated with 100 mg/kg b.w had the least percentage (-28.42%) reduction. The result also revealed that the induced treated groups are dose dependent.

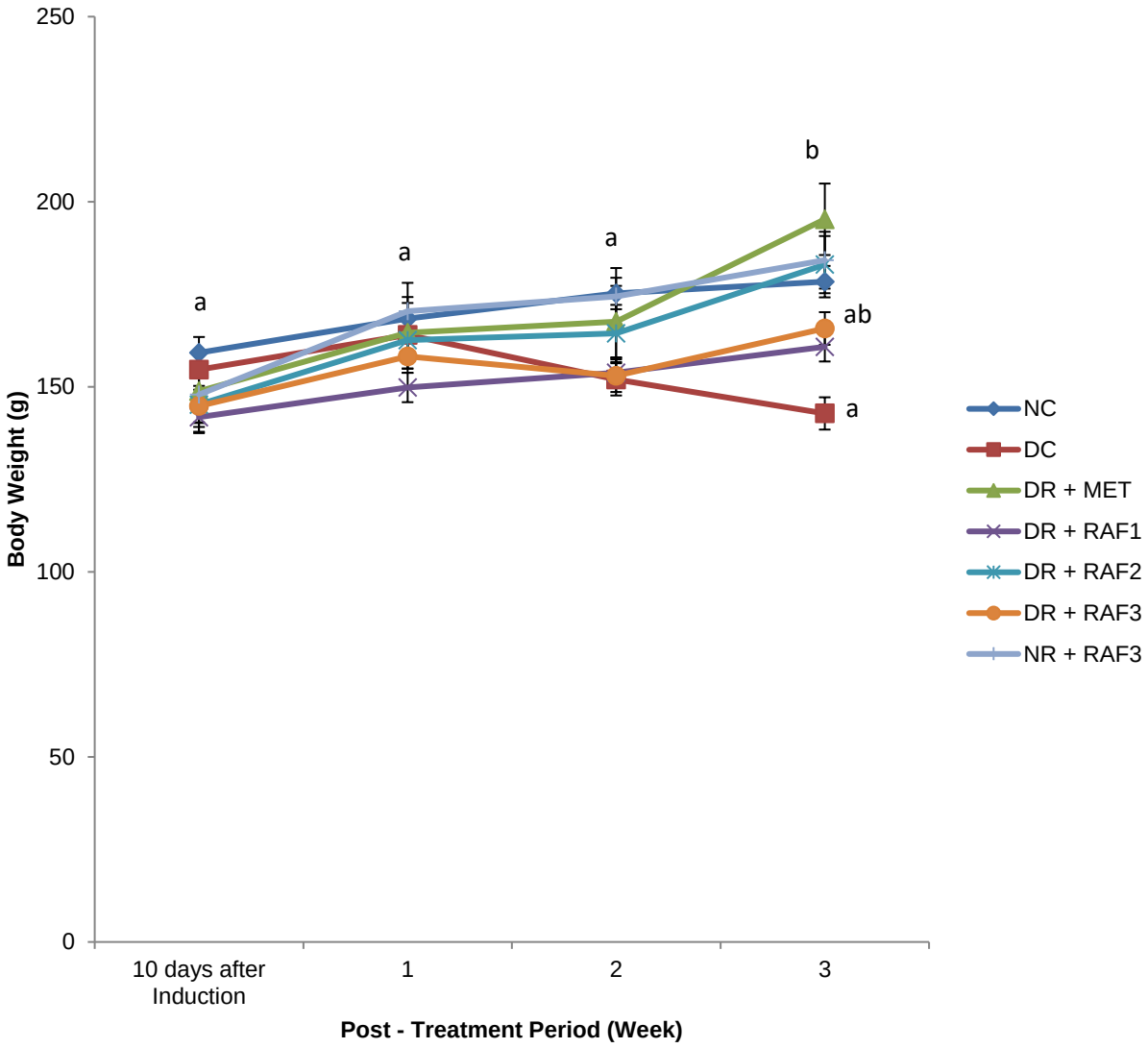


**Figure 3:** Effect of residual aqueous fraction of *Ethulia conyzoides* on the body weight of induced Type 2 diabetic rats for 21 days of treatment.

**Table 1**: Effect of residual aqueous fraction of *Ethulia conyzoides* on the weekly percentage change in the blood glucose level of induced Type 2 diabetic rats for 21 days of treatment.

| Groups | Weekly Percentage Change in the Blood Glucose level During Treatment | | |
|---|---|---|---|
| | Week 1 | Week 2 | Week 3 |
| NC | 2.67 ± 6.37$^{ab}$ | 2.75 ± 13.56$^{ab}$ | 11.01 ± 5.23$^{d}$ |
| DC | 24.26 ± 27.85$^{b}$ | 30.61 ± 62.73$^{b}$ | 6.60 ± 38.42$^{cd}$ |
| DR + MET | -21.32 ± 37.29$^{a}$ | -14.41 ± 44.11$^{ab}$ | -66.52 ± 19.31$^{a}$ |
| DR + RAF1 | 6.66 ± 30.46$^{ab}$ | -3.95 ± 19.23$^{ab}$ | -28.42 ± 13.88$^{b}$ |
| DR + RAF2 | -14.12 ± 8.10$^{a}$ | -29.20 ± 23.21$^{a}$ | -64.06 ± 34.94$^{a}$ |
| DR + RAF3 | -23.81 ± 21.22$^{a}$ | -43.59 ± 14.38$^{a}$ | -73.70 ± 16.60$^{a}$ |
| NR + RAF3 | -16.34 ± 8.60$^{a}$ | -17.82 ± 9.56$^{a}$ | -20.34 ± 10.83$^{bc}$ |

Notes: Values are expressed as mean ± Standard Deviation n = 5 (reduction from 6 to 5 as a result of mortality); Values with different superscripts down the column are significantly different ($p<0.05$).
NC: Normal Control rats; DC: Diabetic control rats; DR: Diabetic rats
DR + MET: Diabetic rats + 500mg/kg Metformin (standard drug).
DR + RAF1: Diabetic rats + 100mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*
DR + RAF2: Diabetic rats + 200mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*
DR + RAF3: Diabetic rats + 400mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*
NR + RAF3: Normal rats + 400mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*

**Effect of Residual Aqueous Fraction of *Ethulia conyzoides* on Weekly Blood Glucose Level of Induced Type 2 Diabetic Rats for 21 Days of Treatment**

There was a significant ($p < 0.05$) increase in the blood glucose level after the induction of diabetes (Fig. 4). Upon treatment, a gradual decrease in the blood glucose level was observed among the diabetic treated group when compared to the normal control and diabetic control group.

**Effect of Residual Aqueous Fraction of *Ethulia conyzoides* on the Fasting Blood Glucose, Insulin Sensitivity, Resistance and β-cell Function of Induced Type 2 Diabetic Rats after 21 Days of Treatment**

The calculated insulin sensitivity index, HOMA-IR and HOMA-β showed that HOMA-IR index was significantly ($p < 0.05$) higher in the diabetic control group when compared to the other groups while the insulin sensitivity and HOMA-β cell functioning index were significantly ($p < 0.05$) lower in the diabetic control group when compared to the other groups (Table 2).

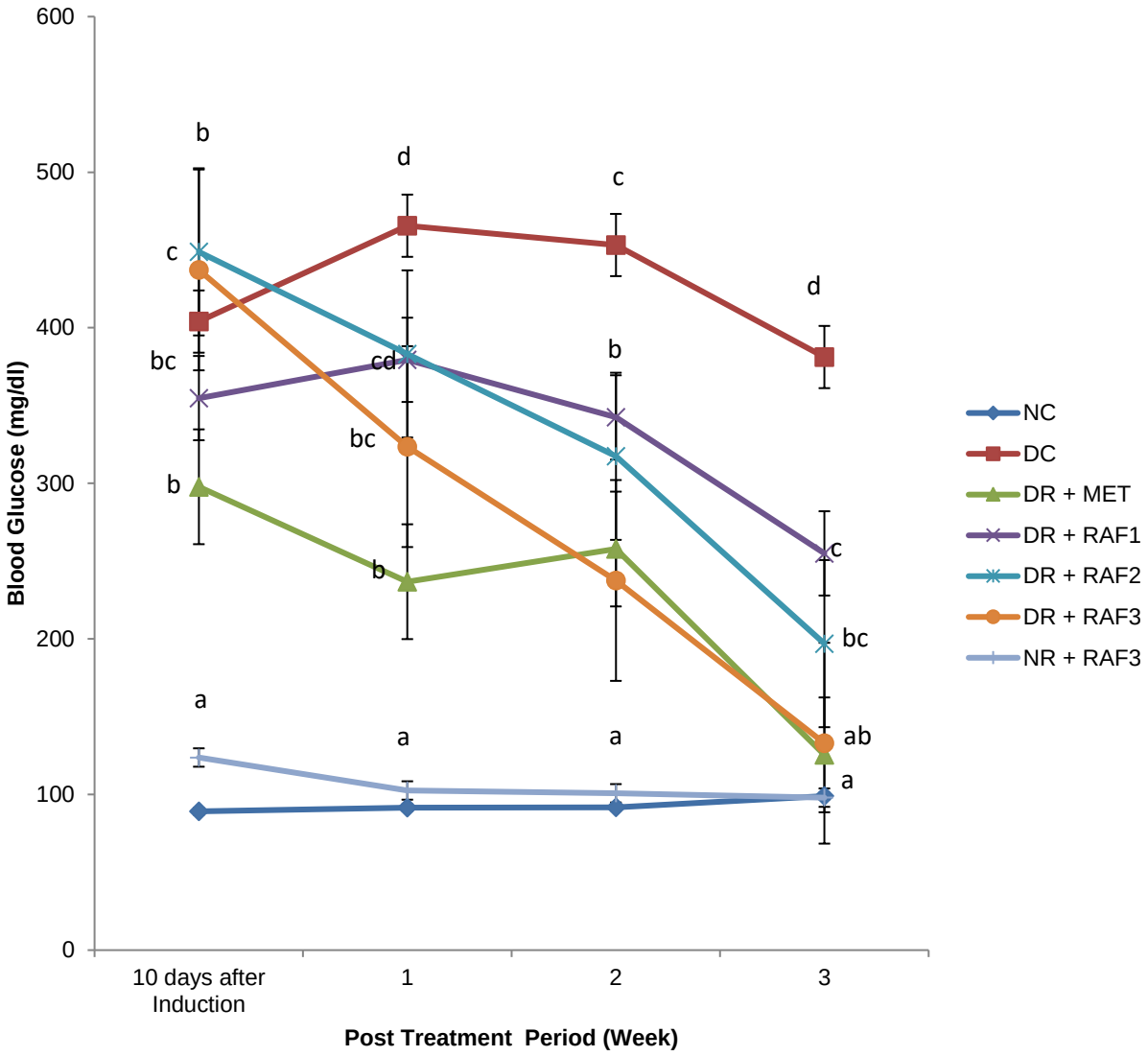


**Figure 4:** Effect of residual aqueous fraction of *Ethulia conyzoides* on weekly blood glucose level of induced Type 2 diabetic rats for 21 days of treatment.

**Table 2:** Effect of residual aqueous fraction of *Ethulia conyzoides* on the fasting blood glucose, insulin sensitivity, resistance and β-cell function of induced Type 2 diabetic rats after 21 days of treatment.

| Groups | Fasting Blood Glucose (mg/dl) | HOMA-IR | HOMA-β | Insulin Sensitivity |
|---|---|---|---|---|
| NC | 99.00 ± 4.58$^{a}$ | 0.90 ± 0.07$^{a}$ | 9.80 ± 0.74$^{bc}$ | 2.39 ± 0.11$^{d}$ |
| DC | 381.20 ± 81.34$^{c}$ | 5.46 ± 1.67$^{c}$ | 2.20 ± 1.37$^{a}$ | 1.00 ± 0.09$^{a}$ |
| DR + MET | 125.50 ± 26.35$^{a}$ | 1.80 ± 0.37$^{a}$ | 13.76 ± 3.28$^{c}$ | 1.57 ± 0.15$^{b}$ |
| DR + RAF1 | 255.00 ± 56.92$^{b}$ | 4.07 ± 0.80$^{bc}$ | 6.17 ± 2.74$^{ab}$ | 1.08 ± 0.07$^{a}$ |
| DR + RAF2 | 222.00 ± 91.98$^{b}$ | 2.53 ± 1.43$^{ab}$ | 6.36 ± 5.76$^{ab}$ | 1.50 ± 0.38$^{b}$ |
| DR + RAF3 | 133.00 ± 9.13$^{a}$ | 2.06 ± 0.17$^{a}$ | 13.42 ± 1.88$^{c}$ | 1.45 ± 0.05$^{b}$ |
| NR + RAF3 | 98.00 ± 12.57$^{a}$ | 1.14 ± 0.06$^{a}$ | 13.65 ± 0.25$^{c}$ | 2.02 ± 0.06$^{c}$ |

Notes; Values are expressed as mean ± Standard Deviation n = 5 (reduction from 6 to 5 as a result of mortality); Values with different superscripts down the column are significantly different ($p < 0.05$).
NC: Normal Control rats; DC: Diabetic control rats; DR: Diabetic rats
DR + MET: Diabetic rats + 500mg/kg Metformin (standard drug).
DR + RAF1: Diabetic rats + 100mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*
DR + RAF2: Diabetic rats + 200mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*
DR + RAF3: Diabetic rats + 400mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*
NR + RAF3: Normal rats + 400mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*

**Effects of Residual Aqueous Fraction of *Ethulia conyzoides* on Liver SOD, Catalase and MDA of Induced Type 2 Diabetic Rats for 21 Days of treatment**

SOD, catalase and MDA shows that induction of diabetes significantly ($p < 0.05$) decreased SOD and catalase and significantly ($p<0.05$) increased MDA level (Table 3).

The different concentrations of *Ethulia conyzoides* significantly ($p < 0.05$) increased SOD and catalase activities while that of MDA was significantly ($p < 0.05$) reduced compared to diabetic control. Normal rats treated with 400 mg/kg b.w residual aqueous fraction of *Ethulia conyzoides* had significantly ($p < 0.05$) higher SOD and catalase activities and significantly ($p < 0.05$) lower MDA activity compared with all treated groups This result also revealed that the diabetic treatment groups are dose dependent.

**Effect of Residual Aqueous Fraction of *Ethulia conyzoides* on the TNF-α and lL-1β Levels of Induced Type 2 Diabetic Rats after 21 Days of Treatment**

The diabetic untreated group shows significant ($p < 0.05$) increase in the levels of TNF-α and IL-1β when compared to normal group (Table 4). Upon treatment, the diabetic rats treated with the different doses of residual aqueous fraction had significant ($p < 0.05$) decrease in the levels of TNF-α and IL-1β in the rats especially the group treated with the highest dose of residual aqueous fraction.

Table 3: Effects of residual aqueous fraction of *Ethulia conyzoides* on liver SOD, catalase and MDA of induced Type 2 diabetic rats for 21 days of treatment

| Groups | SOD (mmol/min/g of tissue) | Catalase (moles of $H_2O_2$/min/g of tissue) | MDA (µmol/mg protein) |
|---|---|---|---|
| NC | $20.66 \pm 1.61^{e}$ | $16.18 \pm 0.58^{de}$ | $109.64 \pm 2.91^{b}$ |
| DC | $11.2 \pm 0.51^{a}$ | $9.38 \pm 0.91^{a}$ | $129.60 \pm 2.06^{e}$ |
| DR + MET | $16.64 \pm 1.12^{d}$ | $13.75 \pm 0.79^{c}$ | $116.80 \pm 2.37^{c}$ |
| DR + RAF1 | $12.88 \pm 0.72^{ab}$ | $10.88 \pm 0.45^{b}$ | $123.70 \pm 4.49^{d}$ |
| DR + RAF2 | $14.50 \pm 1.47^{bc}$ | $13.08 \pm 0.45^{c}$ | $121.50 \pm 3.03^{d}$ |
| DR + RAF3 | $16.20 \pm 0.84^{cd}$ | $15.50 \pm 0.63^{d}$ | $116.03 \pm 2.61^{c}$ |
| NR + RAF3 | $19.38 \pm 1.88^{e}$ | $17.05 \pm 0.83^{e}$ | $104.90 \pm 2.99^{a}$ |

Notes: Values are expressed as mean ± Standard Deviation n = 5 (reduction from 6 to 5 as a result of mortality); Values with different superscripts down the column are significantly different ($p<0.05$).
NC: Normal Control rats; DC: Diabetic control rats; DR: Diabetic rats
DR + MET: Diabetic rats + 500 mg/kg b.w Metformin (standard drug).
DR + RAF1: Diabetic rats + 100 mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*
DR + RAF2: Diabetic rats + 200 mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*
DR + RAF3: Diabetic rats + 400 mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*
NR + RAF3: Normal rats + 400 mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*

**Table 4:** Effect of residual aqueous fraction of *Ethulia conyzoides* on the TNF-α and IL-1β levels of induced Type 2 diabetic rats after 21 days of treatment.

| GROUP | TNF-α (pg/mL) | IL-1β (pg/mL) |
|---|---|---|
| NC | $6.30 \pm 0.82^{a}$ | $40.00 \pm 3.08^{a}$ |
| DC | $29.75 \pm 4.40^{e}$ | $74.33 \pm 8.14^{d}$ |
| DR + MET | $11.27 \pm 0.81^{c}$ | $41.67 \pm 2.89^{a}$ |
| DR + RAF1 | $20.55 \pm 2.01^{d}$ | $56.25 \pm 2.52^{c}$ |
| DR + RAF2 | $21.07 \pm 3.00^{d}$ | $52.67 \pm 5.13^{bc}$ |
| DR + RAF3 | $7.30 \pm 1.32^{ab}$ | $45.67 \pm 5.00^{ab}$ |
| NR + RAF3 | $10.38 \pm 1.52^{bc}$ | $45.00 \pm 11.73^{ab}$ |

Notes: Values are expressed as mean ± Standard Deviation $n = 5$ (reduction from 6 to 5 as a result of mortality); Values with different superscripts down the column are significantly different ($p < 0.05$).
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DR + RAF3: Diabetic rats + 400 mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*
NR + RAF3: Normal rats + 400 mg/kg b.w residual aqueous fraction of *Ethulia conyzoides*

## DISCUSSION

The onset of Type 2 diabetics (T2D) is strongly associated with insulin resistance and pancreatic β-cell dysfunction (WHO, 2013). The significant increase ($p < 0.05$) in food intake (polyphagia), excessive fluid intake (polydipsia) and reduction in body weight observed in the diabetic control rats in this study are characteristics features of T2D. The treatment with residual aqueous fraction was able to ameliorate these features. From literature, Yerima and Samaila

(2018) also found out that the residual aqueous fraction of their medicinal plant could be used in the treatment of diabetes as the fraction significantly ($p < 0.05$) dropped the level of blood glucose of the diabetic rats.

High fluid intake was seen in T2D rats; the reduction in fluid intake seen in the T2D treated rats could be as a result of increased intracellular water which triggers the osmoreceptor of the thirst centre of the brain leading to less water intake (S. I. R. Okoduwa *et al.* 2017c).

Weight reduction is key in the prevention and management of type 2 diabetes in the obese (Inzucchi *et al.* 2015). There was a significant ($p < 0.05$) reduction in body weight observed in the diabetic control and this could be due to decrease in appetite, feed intake or increase in catabolic effect which is evident in T2D (Russell *et al.* 2001). However, all the groups treated with residual aqueous fraction especially the group treated with the highest dose showed significant ($p < 0.05$) improvement in body weight and this indicates that residual aqueous fraction of *Ethulia conyzoides* may be able to ameliorate hyperglycaemia-induced muscle wastage; this is in line with the work of Petchi *et al.* (2014) which found out that a combination of three different plant extracts significantly improved the body weight in diabetic group treated with the combination.

Insulin is a hormone needed by the cell for the uptake of glucose (Qaid & Abdelrahman 2016). The level of insulin in the plasma conveys a signal indicating the adiposity grade to any insulin-sensitive tissue. The residual aqueous fraction of *Ethulia conyzoides* could be said to have exerted its anti-diabetic activity through its ability to decrease insulin resistance and improve the sensitivity of the cells and tissues to endogenous insulin as seen by the decreased blood glucose level. This is in line with the work of Zhang *et al.* (2016) who reported that T2D rats administered polysaccharides from *Pleurotus ostreatus* for four weeks showed significant ($p<0.05$) decrease in insulin resistance.

Hyperglycaemia results in free radical formation through various biochemical reactions Bajaj and Khan (2012) leading to lipid peroxidation, which results to tissue damage that has been observed in diabetes (Raza *et al.*, 2011). In this study, the significant ($p < 0.05$) decrease in the MDA level in the liver of diabetic induced treated groups as likened to the diabetic control group suggest that treatment with residual aqueous fractions of *Ethulia conyzoides* may exert antioxidant activities, reduce hyperglycaemic effect and protect the tissue from lipid peroxidation. This is similar to the report of Kumawat *et al.* (2013) who found out that MDA levels were significantly ($p < 0.05$) increased in T2D with or without nephropathy as compared to controls.

The therapeutic potentials of plants have been related with their antioxidant potentials (Eleazu *et al.* 2011). Endogenous antioxidant enzymes like catalase and superoxide dismutase (SOD) are body defense mechanism to prevent and neutralize free radicals-induced damage. The decreased activity of SOD and catalase in the liver tissues of T2D rats may be due to the free radicals generated by the Streptozotocin (S. Srinivasan & Pari 2012; Szkudelski 2001). The significant ($p < 0.05$) increase in superoxide dismutase and catalase were observed in the liver of diabetic treated groups compared to diabetic control rats. This indicates that residual aqueous fraction of *Ethulia conyzoides* contains free radical scavenging activity, which could exert a beneficial action against pathophysiological alterations caused by the presence of superoxide and hydroxide radicals. Daryoush *et al.* (2011) reported that a reduction in SOD

activity is a sensitive guide in hepatocellular damage and is the most sensitive enzymatic index in liver injury.

Oxidative stress mirrors the disparity between the generation of reactive oxygen species and the antioxidant defense system in the body (Sies 2015). It contributes to the progression of T2D by enhancing the secretion of pro-inflammatory cytokines like TNF-α and IL-1β. In this study, the decrease in the levels of TNF-α and IL-1β in diabetic treated group compared to the diabetic untreated group could be as a result of decrease in blood glucose level and this may have contributed to the observed decrease in the level of TNF-α and IL-1β. This is in agreement with the research done by Hämäläinen *et al.* (2007) which showed that antioxidant phytochemicals in plant extract inhibit inflammation by inhibiting nuclear factor kappa beta (NF-kB) activations. down regulation of NF-κB, which acts as a potent transcription factor in initiating inflammation, may represent a possible mechanism to inhibit T2D.

The putative mode of action can be proposed that the residual aqueous fraction of *E. conyzoides* exert anti-diabetic potential via its antioxidant and anti-inflammatory effect. The standard drug (metformin) used acts by lowering both basal and postprandial plasma glucose. It reduces hepatic glucose production, lowers intestinal glucose absorption and improves insulin sensitivity by increasing peripheral glucose uptake and utilization (Rena *et al.* 2017); so this extract too, may have worked through similar mode of action as metformin.

## CONCLUSION

T2D is an important research topic for both clinicians and research scientists. In this issue, we have discussed the residual aqueous fraction of *Ethulia conyzoides* exerting modulatory potential against some of the metabolites derangements observed in diabetes in relation to increased level of oxidative stress marker (MDA), IL-1β and TNF-α. We hope this provides useful information to basic science researcher to catalyze novel therapeutic approaches and future research directions.

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## AUTHORS' CONTRIBUTION

**HOO** proposed the idea, carried out the research, acquired data, analyzed the data, interpreted the result, contributed to writing and provided intellectual input. **MTA** performed editing, analysis of data and provided intellectual input. **OLO** assisted in the research work. **DAA** and **DBJ**

contributed to the conception and design, provided intellectual input and supervised the research.

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