Antidiabetic Potential of Tragia involucrata Linn in Alloxan-induced Diabetic Swiss Albino Mice Corroborates its Usage by Diabetic Patients in Nyamira County, Kenya

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Research Article

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Posted Date: February 19th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-6734094/v2

Additional Declarations: The authors declare no competing interests.

Antidiabetic Potential of *Tragia involucrata* Linn in Alloxan-induced Diabetic Swiss Albino Mice Corroborates its Usage by Diabetic Patients in Nyamira County, Kenya

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Abstract

Diabetes mellitus is a metabolic chronic condition that is characterized by chronic hyperglycemia and the resulting complications of the disease include nephropathy, neuropathy and cardiovascular disease. Although there are many options for using synthetic antidiabetic drugs, the use of these drugs has been limited due to side effects, the cost of the drugs and limited access to the drugs, especially in developing countries. In this research study we evaluated the chemical composition of *Tragia involucrata* Linn leaves that have been extracted using Dichloromethane (DCM), the ability of the extracts to reduce blood sugar levels in diabetic mice and the safety profile of the extracts. Blood glucose levels were monitored hourly and weekly in alloxan induced diabetic Swiss albino mice after intraperitoneal injection of extracts of *T. involucrata* at doses of 120, 170, 220 and 270 mg/kg bw. The results of the GC-MS analyses of the extracts indicated the presence of bioactive compounds such as phytol, squalene, beta-sitosterol and hydrocarbon chains of varying length which are all known to be effective in reducing elevated blood sugar levels. The extracts of *T. involucrata* were found to have a dose dependent hypoglycemic effect and the highest dose (270 mg/kg bw) reduced glucose levels in a rapid and sustained manner similar to the standard treatment. These results also show that the extracts of *T. involucrata* elicited an acute glycemic response when measured hourly but showed evidence of long-term efficacy when measured weekly over a period of 28 days. Both the acute and subacute toxicity studies of the DCM extracts of *T. involucrata* demonstrated no mortality or major toxic signs in the mice treated with the extracts. Results from biochemical markers assessment, specifically for alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, urea, creatinine and bilirubin did not reveal any significant perturbations, demonstrating that the liver and kidney were functioning normally with minor stress at higher doses. Safety evaluation was also conducted through measurement of organ weights of key organs, including the liver, kidneys, spleen, heart, lungs and brain. The absence of significant changes in these organ weights supports the favorable

safety profile of the extract. The findings validate the traditional usage of the medicinal plant by diabetic patients in Nyamira County, Kenya. Future comprehensive studies of the *T. involucrata* should focus on antidiabetic properties of pure bioactive compounds and lead molecules identification for drug discovery and development.

Keywords- Medicinal plants, antidiabetic activity, diabetes mellitus, alloxan-induced diabetes, phytochemicals, Swiss albino mice, glibenclamide.

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, action or both. It has become one of the largest public health issues globally and currently impacts approximately 463 million people and is projected to affect nearly 700 million people by the year 2045 (Saeedi *et al*., 2019). Diabetes can be divided into two primary categories: Type 1 diabetes; typically develops due to autoimmune destruction of the insulin producing beta cells and Type 2 diabetes; typically develops due to insulin resistance and subsequent impairment of pancreatic beta cell function.

Diabetes has far-reaching implications for the world's population, contributing to the increased morbidity and mortality associated with diabetes related complications (Harding *et al*., 2019). These include cardiovascular disease, diabetic neuropathy, diabetic nephropathy and diabetic retinopathy and demonstrate the need for effective strategies for managing diabetes to not only alleviate immediate symptoms, but to minimize the long-term pathologic health consequences (Yachmaneni *et al*., 2023).

The rise in diabetes is attributed to the increasing rate of urbanization, the aging of the population and changes in lifestyle that contribute to a less active life style and consumption of high calorie diets (Alhejely *et al*., 2023). The differences in the prevalence of diabetes across the globe illustrate

the interaction between lifestyle and metabolic health and therefore require more individualized approaches to diabetes management.

Diabetes causes disturbances to many biological pathways that are involved in the regulation of blood sugar, fat metabolism and the utilization of insulin. Therefore, the cells are unable to effectively absorb glucose from the blood and the liver produces excessive amounts of glucose leading to either chronically elevated or decreased levels of glucose in the blood. Additionally, problems with insulin signaling can lead to disrupted lipid metabolism thereby complicating the management of diabetes. Managing the biochemistry of diabetes requires improving how well insulin functions, allowing the body's cells to effectively utilize glucose from the blood and decreasing the amount of glucose produced in the liver (Goyal *et al*., 2020).

Lifestyle modification, oral hypoglycemic agents or insulin therapy represents current therapeutic approaches for diabetes. These methods can be successful in controlling blood glucose, but they usually fail to tackle the root biochemical problems that are beta-cell destruction and insulin resistance. The newer therapeutic options, namely Glucagon-Like Peptide 1 (GLP 1) receptor agonists and Sodium-Glucose Cotransporter 2 (SGLT2) inhibitors, focus on different aspects of diabetes pathophysiology and have better glucose control and improved cardiovascular benefit (Parfianowicz and Surma, 2025).

Recently, plant-based compounds received growing interest because they are believed to be able to control blood glucose level and impart antioxidant benefits (Rahman *et al*., 2022). In addition, these compounds are attractive candidates for the development of less invasive and more effective diabetes treatments (Ki *et al*., 2024). Research into natural products may be a means of finding novel strategies to manage oxidative stress and other diabetic related complications. One classical

example is the use of *T. involucrata* for managing diabetes by the Abugusii community in Nyamira County in Kenya although there are limited scientifically verified data to support these claims. However, while considerable progress has been made; still urgently needed are treatments addressing the basic pathophysiology of diabetes (beta-cell dysfunction and insulin resistance). This study was aimed at assessing the antidiabetic potential and safety profiles of DCM leaf extracts of *T. involucrata,* in alloxan-induced diabetic swiss albino mice in order to validate its use as a viable antidiabetic agent, contributing to the development of natural therapies that are both accessible and effective for diabetes management.

MATERIALS AND METHODS

Sample Collection and Extraction

The leaves of *T. involucrata* L. were collected from multiple sites within the Kineni area, Nyamira County, Kenya (Latitude: -0.566402, Longitude: 34.932975), based on plant availability and information from local herbalists. Approximately 5 kg of both young and mature leaves of *T. involucrata* L. were collected as described by Stéphane *et al.,* (2021). According to the local communities, both young and mature leaves are traditionally used in the management of diabetes, often without distinction, reflecting their belief in the plant's holistic therapeutic efficacy. A local taxonomist authenticated the samples and a voucher specimen was deposited at the herbarium in the Kenyatta University under the unique identifier KUH/BOA/1548/2024. The leaves were washed with tap water to wash off the surface contaminants and air dried for two weeks at Technical University of Mombasa , biochemistry laboratory.

Extraction was carried out using procedure described by Ayadi *et al*., 2023 and Kibiti & Afolayan, (2015). To begin, 2000 gms of dried leaf material was pulverized into small pieces and blended

with 4000 ml of Dichloromethane (DCM) in an appropriate container. The blend was agitated on a rotary shaker for 72 hours. A centrifuge was used to separate the liquid from the solid materials and the remaining solution was passed through number 1 Whatman filter paper to produce a clear supernatant layer. The DCM was then removed by evaporation at a low temperature and the resulting concentrated crude extract was refrigerated at 4^{o} C and kept until future experiments were conducted.

Phytochemical Screening

Phytochemical composition in the dichloromethane leaf extracts of *T. involucrata* Linn were analyzed through gas chromatography-mass spectrometry (GC-MS) techniques as outlined by Sani *et al*., (2020). The samples were introduced to the GC-MS, heated, then flowed through a capillary column and interaction between the vaporized compounds and the stationary phase affected the retention time of the compounds (Almeida *et al*., 2022). Once exited from the column, the compounds were broken down into ions and fragments in the mass spectrometer where the resulting mass spectra were compared to reference standards and an internal database to identify and quantify the compounds (Allen *et al*., 2016). In addition to identifying each of the individual compounds in the sample based on retention time, the size of the peak area indicated the quantity of each compound, while the relative abundance of each compound indicated the proportion of each compound within the extract.

Experimental Design

Twenty-eight (28) Swiss albino mice were utilized for this study; these mice were randomly split into seven (7) groups of four (4) mice each. The normal control group (Group I) was comprised of mice who did not have diabetes and which were administered intraperitoneally with normal saline (0.9%). The diabetic control group (Group II) included diabetic mice that were administered

intraperitoneally with normal saline and were left untreated. Diabetic mice that were treated intraperitoneally with glibenclamide at a dose of 5 mg/kg body weight formed the positive control group (Group III). The remaining experimental groups, including Groups IV through VII, included diabetic mice that were administered intraperitoneally with *T. involucrata* DCM leaf extract at the doses of 120 mg/kg body weight, 170 mg/kg body weight, 220 mg/kg body weight, and 270 mg/kg body weight, respectively. Inspiration for the preparation of the groups and the doses came from prior animal studies that examined the antidiabetic effects of various medicinal plant extracts (Sharmin *et al*., 2018).

Induction of Diabetes

Prior to diabetes induction, mice were placed on an empty stomach (fasted) for twelve hours and then administered once via intraperitoneal injection with a reported adequate dose of 120 mg/kg body weight of alloxan monohydrate, a chemical compound commonly used as a diabetogenic agent that is known to induce diabetes at this dosage (Tafesse *et al*., 2017). On the third day post-injection, a glucometer was utilized to determine a mouse's fasting blood glucose levels to confirm diabetes induction had occurred. Mice were determined to be diabetic if their fasting blood glucose levels exceeded 200 mg/dl; all such mice were included in the study.

Treatments and Monitoring

Mice were administered by an intraperitoneal injection (ip), with four different dosages (120, 170, 220 and 270 mg/kg bw) over a period of twenty-eight days. Blood glucose values from mice were taken and documented prior to any treatment, and also at each week interval post-blood glucose challenge induction. The lower dosage of 120 mg/kg body weight of *T. involucrata* DCM extract was selected for assessing preliminary reductions of glucose values, as well as assessing dose-related impacts on blood glucose values via the greater dosages (170, 220, 270

mg/kg body weight). In addition, to determine the immediate effects of the treatments, hourly blood glucose measurements were collected and recorded at times 0, 1, 2, 3 and 4 hr. (Sisay *et al*., 2022).

Safety Profile Assay

The acute toxicity of *T. involucrata* L. in mice was assessed following a preliminary range-finding study to identify appropriate doses, as outlined by Kifayatullah *et al*., (2015). The highest non-lethal dose (2000 mg/kg bw) was selected for further testing. A total of 24 mice were randomly divided into six groups, each consisting of four mice (n = 4). The five groups were given *T. involucrata* DCM extract orally at 5 different dosage levels by means of oral gavage with volumes of 0.25 mL for every fifty grams of body weight. In addition to these five groups, the final group was given normal saline (0.25 mL/50 g body weight) that served as a negative control.

All animals were closely observed for the first 4 hours' post-administration and monitored daily for 14 days for mortality, behavioral changes and clinical symptoms in accordance with the OECD guidelines (Organization for Economic Co-operation and Development). The negative control group was included to ensure the reliability and validity of the results. Throughout the experiment, animal welfare was strictly prioritized to minimize stress-induced confounding effects (Kifayatullah *et al.,* 2015).

Swiss albino mice (6–8 weeks old, weighing 20–25 g) were chosen for subacute toxicity studies and observed over 28 days. Three groups of animals were given 300, 1000 and 2000 mg/kg of the extract respectively while one negative control was treated with normal saline water.

Every day during the 28 days of the trial, systemic effects were examined and signs of toxicity identified. These included in specific parameters were the amounts of food and water, the condition of the fur, movements, convulsions, tremors and whether they were lethargic. Neurobehavioral

responses such as reactivity to handling, gait, and alertness were also recorded. After completing the study, each animal was euthanized by cervical dislocation. Vital organs including the liver, kidneys, spleen, heart, lungs and brain were excised and weighed to evaluate potential organ-specific toxicity, following the procedure described by Agrawal *et al*., (2016). Biochemical marker assessments were also conducted using an automated clinical chemistry analyzer under standardized conditions (37°C enzymatic assays) in order to quantify hepatic [Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Gamma-Glutamyl Transferase (GGT)] and renal (urea, creatinine, bilirubin) biomarkers.

Statistical Analysis

In order to determine if there were group differences, descriptive statistics (mean and standard deviation) and inferential statistics (ANOVA) were analyzed, and P-values ≤ 0.05 were considered significant (Holmes & Huber, 2018). Post hoc comparisons were performed using Tukey's Honestly Significant Difference (HSD) test to identify which groups differed significantly, with superscript letters (a–f) in the tables indicating statistical differences at $P \leq 0.05$. In the case of GC-MS analysis of *T. involucrata* DCM leaf extracts, compound identification was performed by using Thermo Xcalibur software. In addition, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were employed in order to uncover patterns among the compounds (Granato *et al.,* 2018).

RESULTS

Phytochemical Composition

The GC-MS analysis of *T. involucrata* Linn DCM extracts identified multiple bioactive compounds (Table 1). The compound with the highest abundance was Phytol (17.6%). Other

compounds were detected at different significant amounts; squalene (13.5%), heptacosane (9.1%), nonacosane (9.0%) and hexacosane (7.8%), Beta-sitosterol (8.2%), pentacosane (6.5%), hentriacontane (5.7%), triacontane (3.6%), 2-Methyl-1-hexadecanol (2.4%), 3-(Octadecyloxy) propyl ester (1.9%), alpha-tocopherol (1.1%) and 4-methylcholesta-8,24-dien-3-ol (1.9%).

Several other compounds in Table 1 were identified in smaller quantities; D-Germacrene (0.6%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (0.2%), 2,6,10 Trimethyltetradecane (0.5%), 3-Ethyl-5-(2-ethylbutyl) octadecane (0.8%), diisooctyl phthalate (0.9%) and ethyl iso-allocholate (1.0%).

Table 1: GC-MS Results for *T. involucrata* Linn DCM Extract showing compounds, retention times, peak area and percentage of abundance

Compound	Molecular Formula	Chemical Class	Retention Time (min)	Percentage Abundance (%)	Concentration (mg/mL)
D-Germacrene	$C_{15}H_{24}$	Sesquiterpene	12.62	0.62	0.19
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{34}O$	Diterpenoid alcohol	16.56	0.24	0.07
Phytol	$C_{20}H_{40}O$	Diterpenoid alcohol	19.21	17.60	5.28
2,6,10 Trimethyltetradecane	$C_{17}H_{36}$	Alkane	19.93	0.48	0.14
3-Ethyl-5-(2-ethylbutyl) octadecane	$C_{2o}H_{42}$	Alkane	20.79	0.79	0.24
2-Methyl-1-hexadecanol	$C_{17}H_{36}O$	Alcohol	21.61	2.41	0.72
Pentacosane	$C_{25}H_{52}$	Alkane	22.40	6.50	1.95
Diisooctyl phthalate	$C_{24}H_{38}O_4$	Ester	22.80	0.86	0.26
Hexacosane	$C_{26}H_{54}$	Alkane	23.15	7.79	2.34
Heptacosane	$C_{27}H_{56}$	Alkane	23.88	9.11	2.73
Octacosane	$C_{28}H_{58}$	Alkane	24.58	7.74	2.32
Squalene	$C_{30}H_{50}$	Terpene	24.79	13.50	4.05
Nonacosane	$C_{29}H_{60}$	Alkane	25.27	9.01	2.703
Triacontane	$C_{30}H_{62}$	Alkane	26.02	3.61	1.08
Hentriacontane	$C_{31}H_{64}$	Alkane	26.91	5.67	1.70
Alpha-tocopherol	$C_{29}H_{50}O_2$	Vitamin E	27.42	1.09	0.33
Oleic acid, 3-(Octadecyloxy)propyl ester	$C_{39}H_{76}O_3$	Ester	27.97	1.87	0.56
4-methylcholesta-8,24-dien-3-ol	$C_{28}H_{46}O$	Steroid	28.56	1.92	0.58
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	Steroid	29.22	0.96	0.29
Beta-sitosterol	$C_{29}H_{50}O$	Steroid	29.74	8.22	2.47

Table 1 lists the GC-MS data for T. involucrata Linn DCM extracts and includes the compound name; retention time and the peak area and percentage of abundance of each compound found in the sample

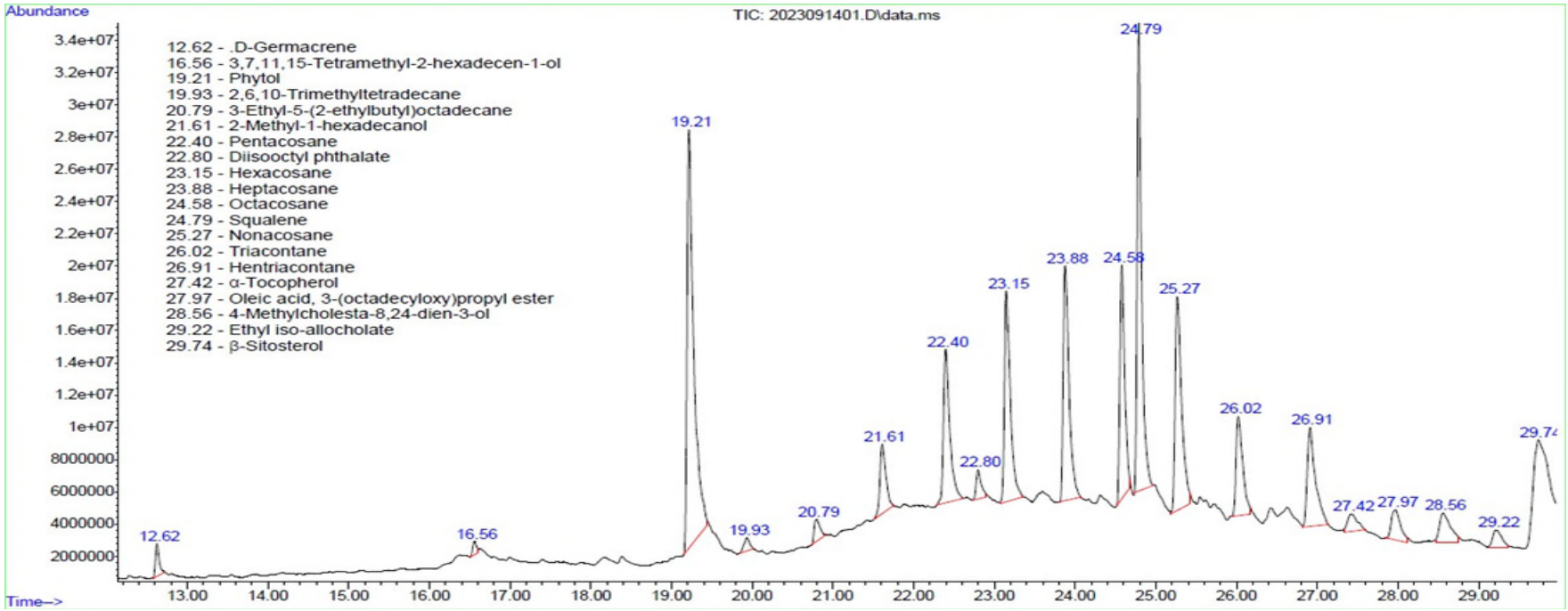

Figure 1: The chromatogram from a GC-MS analysis of T. involucrata Linn DCM extract shows retention time and relative amounts of the different compounds found in the extract.

Antidiabetic Activity of *T. involucrata* DCM Extracts in Swiss Albino Mice

Normal control mice maintained their blood glucose levels throughout the week-long study, demonstrating good glycemic stability. In comparison, untreated diabetic mice experienced a large rise in blood glucose levels, indicative of progressive diabetes due to the alloxan monohydrate used to induce diabetes (Table 2).

Treatment groups exhibited different degrees of glucose lowering efficacy over time as illustrated by each treatment group in figure 2. Glucose levels in mice receiving 120 mg/kg bw of *T. involucrata* DCM extract were reduced to some extent relative to untreated diabetic mice, but there was considerable variation among mice within this treatment group. Mice receiving 170 mg/kg bw showed a moderate reduction in glucose levels, which is likely associated with an increased dose, however considerable variability was noted between mice.

A further decrease in glucose levels was observed in mice receiving 220 mg/kg bw of *T. involucrata* DCM extract, consistent with an increasing dose producing an increasing effect as illustrated in Table 2.

Mice receiving 270 mg/kg bw of *T. involucrata* DCM extract had the lowest average glucose level on Day 28 and approached the effect of glibenclamide; although statistical power was insufficient to demonstrate a difference between these two treatments at all time points (Figure 2).

Glibenclamide treated mice also demonstrated a statistically significant reduction in blood glucose levels, supporting glibenclamide's known effectiveness as a first-line treatment for diabetes. Additionally, mice receiving 270 mg/kg bw of *T. involucrata* DCM extract exhibited glucose lowering effects similar to those of glibenclamide by the end of the study period, thus no claim of superior efficacy can be made (Table 2).

Blood glucose levels were significantly impacted by time ($F=12.85$; $p<0.001$) and there was a significant impact of treatment group ($F=105.76$; $p<0.001$) indicating the various treatments significantly impacted blood glucose levels. There was an interaction between treatment group and time, indicating that while glucose levels changed over time in each group, there was considerable variability between mice within each treatment group (Table 2).

Table 2: *In vivo* Antidiabetic Effect of *T. involucrata* L. In Swiss Albino Mice (Weekly Blood Glucose Levels)

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Normal Mice	$98.25^{d} \pm 2.94$	$95.2^{d} \pm 4.61$	$94.83^{d} \pm 1.84$	$94.75^{d} \pm 2.22$	$95.18^{d} \pm 0.62$
Diabetic Mice Untreated	$248.43^{a} \pm 1.32$	$299.10^{a} \pm 4.19$	$321.03^{a} \pm 2.20$	$335.93^{a} \pm 2.78$	$379.78^{a} \pm 1.59$
Diabetic Mice Treated with glibenclamide	$240.45^{b} \pm 1.58$	$214.25^{c} \pm 2.07$	$189.05^{c} \pm 2.67$	$169.65^{c} \pm 2.47$	$148.95^{c} \pm 3.05$
Diabetic Mice Treated with *Tragia involucrata* 120mg/kg bw	$244.78^{b} \pm 1.44$	$225.13^{bc} \pm 2.49$	$205.18^{c} \pm 3.16$	$184.78^{c} \pm 2.60$	$165.80^{c} \pm 2.36$
Diabetic Mice Treated with *Tragia involucrata* 170mg/kg bw	$239.73^{b} \pm 2.38$	$214.33^{c} \pm 1.39$	$188.10^{c} \pm 3.21$	$170.23^{c} \pm 1.30$	$150.35^{c} \pm 2.52$
Diabetic Mice Treated with *Tragia involucrata* 220mg/kg bw	$233.45^{b} \pm 3.05$	$211.65^{c} \pm 4.38$	$187.23^{c} \pm 2.79$	$168.48^{c} \pm 1.75$	$147.88^{c} \pm 3.36$
Diabetic Mice Treated with *Tragia involucrata* 270mg/kg bw	$240.93^{b} \pm 2.72$	$215.63^{c} \pm 2.94$	$185.60^{c} \pm 2.47$	$156.93^{c} \pm 1.93$	$137.35^{c} \pm 4.74$

The values in the table are presented as Mean ± Standard deviation (SD). The superscript letters (a-d) indicate statistically significant differences between groups within the same time point ($P \leq 0.05$). Groups sharing the same letter are not significantly different from each other.

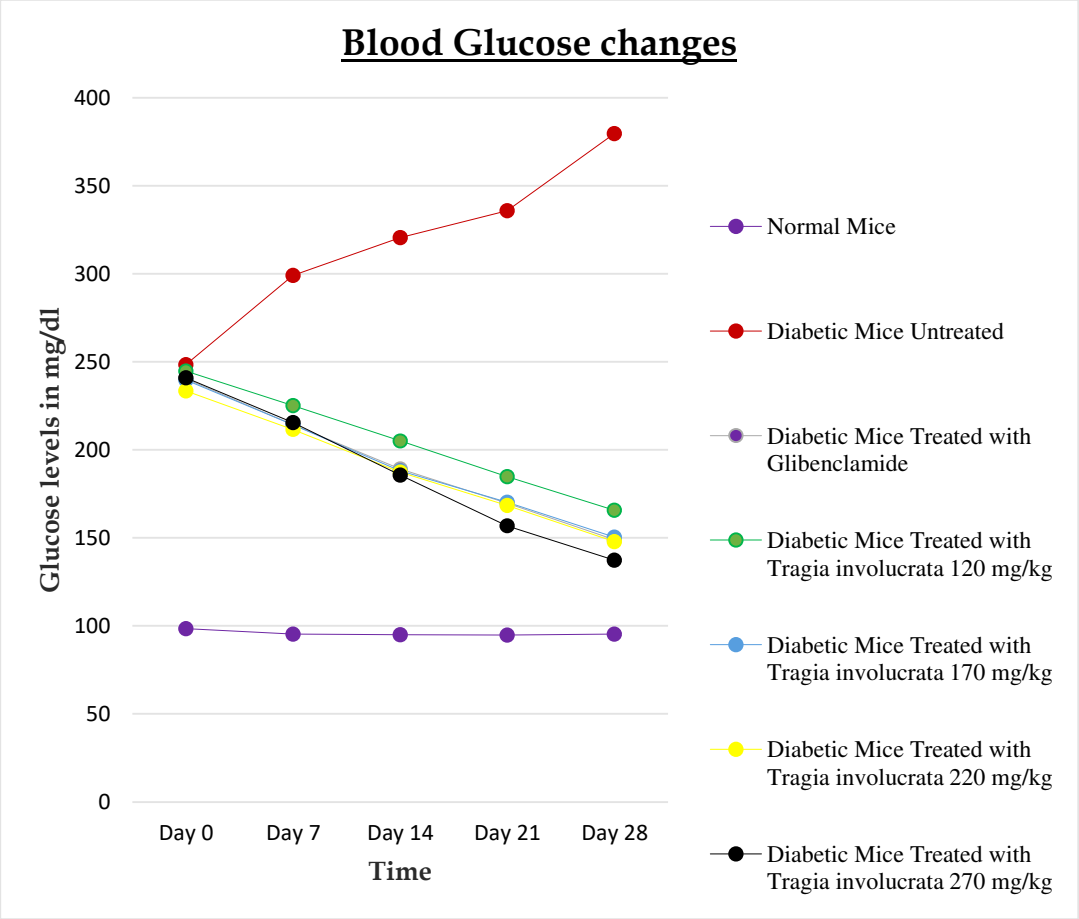

Figure 2: The line graph represents the trend in blood glucose levels over time for different experimental groups treated with glibenclamide or varying doses of T. involucrata.

Table 3 shows normal mice maintained relatively stable glucose levels, with minor fluctuations typical of biological variation (95.95–98.0 mg/dl) for the 4 hours of observation. Untreated diabetic mice showed an overall increase in glucose from 247.13 mg/dl to 255.23 mg/dl, with minor fluctuations among individual animals.

Glibenclamide-treated mice generally showed a trend toward lower glucose levels, consistent with expected antihyperglycemic effects. Mice treated with *T. involucrata* DCM extract showed

reductions in glucose, with the 270 mg/kg group reaching the lowest average levels (227.0–220.1 mg/dl), though individual variation was present. The 220 mg/kg group showed somewhat lower glucose averages compared to 120 and 170 mg/kg groups, suggesting a trend of dose-dependent effect, though not perfectly linear (table 3).

Two-way ANOVA revealed significant effects of the treatment group ($F = 4.02$, $P = 0.004$) and time ($F = 10.08$, $P < 0.001$) on blood glucose levels, indicating that both the treatments and the duration of the treatment influenced glucose levels. The interaction between treatment group and time was significant, suggesting that the trends over time differed between groups, though individual variability was observed

Table 3: *In vivo* Antidiabetic Effect of *T.involucrata* L. In Swiss Albino Mice (Hourly Blood Glucose Levels)

Time	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
Normal Mice	$98.0^{f} \pm 2.55$	$97.25^{f} \pm 1.10$	$96.65^{f} \pm 1.43$	$96.35^{f} \pm 1.43$	$95.95^{f} \pm 1.43$
Diabetic Mice Untreated	$245.13^{a} \pm 0.63$	$249.63^{a} \pm 0.63$	$252.00^{a} \pm 0.41$	$254.00^{a} \pm 0.71$	$255.23^{a} \pm 2.20$
Diabetic Mice Treated with glibenclamide	$236.88^{b} \pm 1.34$	$235.88^{bc} \pm 1.24$	$234.20^{bc} \pm 1.04$	$232.73^{c} \pm 1.29$	$229.83^{c} \pm 1.18$
Diabetic Mice Treated with *Tragia involucrata* 120 mg/kg bw	$235.00^{b} \pm 0.71$	$234.25^{bc} \pm 1.29$	$234.13^{bc} \pm 1.48$	$234.00^{bc} \pm 0.41$	$233.75^{bc} \pm 1.29$
Diabetic Mice Treated with *Tragia involucrata* 170 mg/kg bw	$243.80^{a} \pm 1.34$	$243.40^{ab} \pm 1.06$	$243.23^{ab} \pm 2.15$	$243.05^{ab} \pm 1.32$	$242.60^{ab} \pm 2.37$
Diabetic Mice Treated with *Tragia involucrata* 220 mg/kg bw	$232.33^{b} \pm 1.84$	$231.64^{bc} \pm 1.82$	$230.88^{bc} \pm 2.05$	$229.61^{c} \pm 1.96$	$228.63^{c} \pm 0.31$
Diabetic Mice Treated with *Tragia involucrata* 270 mg/kg bw	$227.00^{c} \pm 1.19$	$226.14^{c} \pm 1.70$	$224.63^{c} \pm 2.98$	$222.16^{d} \pm 2.64$	$220.10^{d} \pm 1.78$

The values in the table are presented as mean ± standard deviation (SD). The superscript letters (a-f) indicate statistically significant differences between groups within the same time point ($P \leq 0.05$). Groups sharing the same letter are not significantly different from each other.

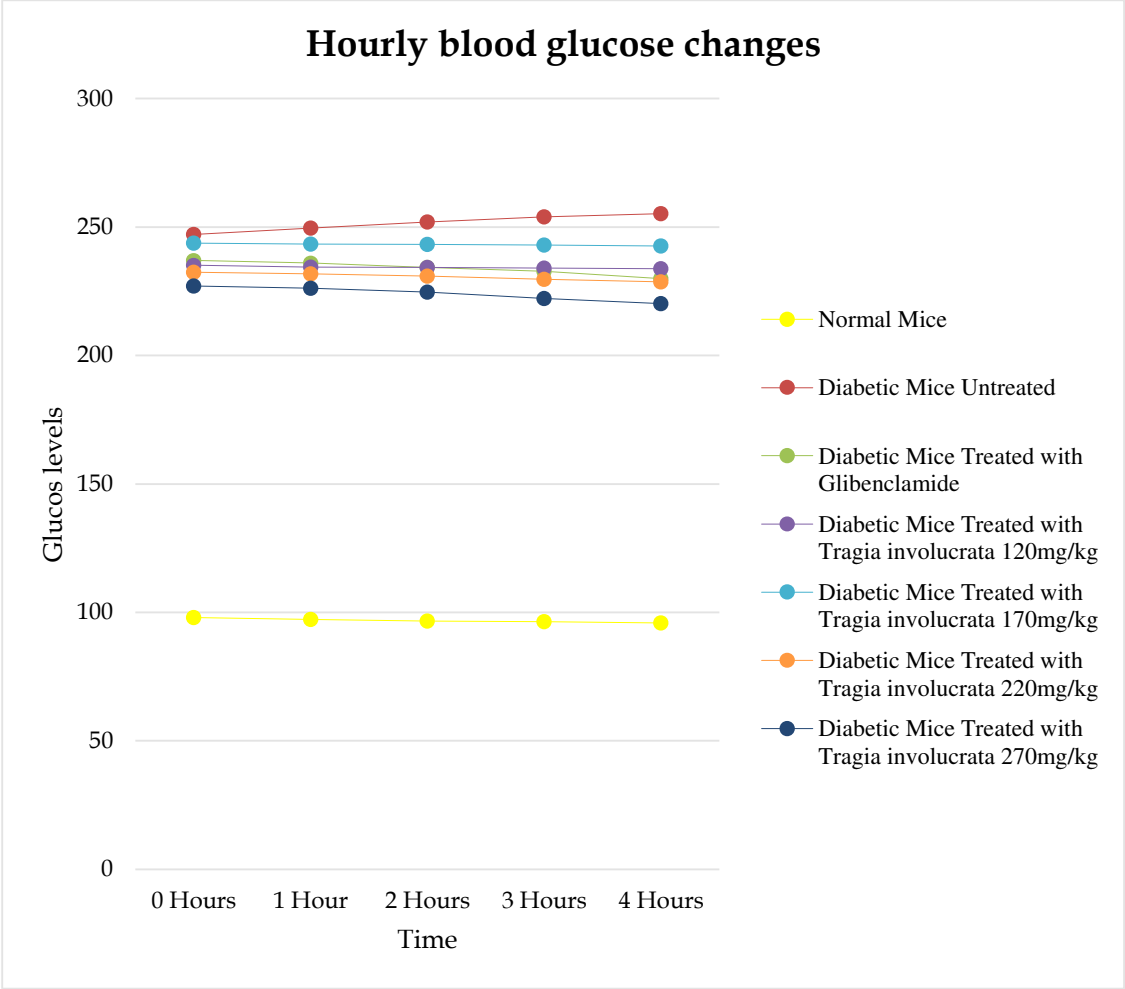

Figure 3: The line graph displays hourly blood glucose trend *over a 4-hour period for different experimental groups treated with glibenclamide or various doses of Tragia involucrata.*

Safety Profile Assay

Acute toxicity testing of the *T. involucrata* DCM extract was conducted in Swiss albino mice at doses of 50, 300, 1000, 1500, and 2000 mg/kg body weight. The animals were closely observed during the initial hours and monitored for 14 days to identify any early or delayed reactions. No mortality occurred at any of the administered doses, consistent with the observations in Table 4. Although no major behavioural abnormalities were seen, a few animals in the highest dose group

showed brief periods of reduced activity within the first 2–3 hours after dosing. These short-lived changes resolved the same day and did not progress to persistent neurobehavioral effects.

Food and water intake remained generally normal across all groups, with only a slight, non-significant reduction in feed intake noted in the high-dose animals during the first 24 hours. This mild, transient change is commonly seen following oral dosing and normalized by the following day. Skin and fur condition remained normal throughout the study, except for mild, temporary piloerection observed in two animals shortly after extract administration. This resolved spontaneously and did not indicate dermal or systemic toxicity.

Locomotor activity was largely preserved, apart from a brief decrease in exploratory behaviour observed in a few mice at the limit dose during the first hour post-treatment. Coordination and rearing remained normal thereafter. The mucous membranes of the eyes and oral cavity remained moist and pink in all groups, with only one animal showing slight, short-term dryness around the oral mucosa shortly after dosing.

The extract did not produce any marked toxic effects or mortality up to 2000 mg/kg. The mild and transient observations recorded at higher doses were consistent with typical short-term reactions seen in acute oral studies and were not indicative of systemic organ toxicity.

Table 4: Acute Toxicity Effects of *T. involucrata* DCM extract in Swiss Albino Mice.

Parameter*(All Groups)*	**Observations**	**Details**
Mortality	None observed	The extract did not cause death at the administered limit dose. However, absence of mortality alone does not rule out mild transient effects, which were monitored throughout the observation period.

Behavioral Changes	No major behavioral abnormalities were detected. A few animals in the highest dose group displayed brief periods of reduced activity within the first 2–3 hours post-administration.	Monitoring included vigilance for lethargy, tremors, unusual gait, or grooming changes. The transient reduction in activity was short-lived and resolved within the same day, suggesting mild initial physiological adjustment rather than toxicity.
Food and Water Intake	Food and water intake remained generally normal. A slight but non-significant reduction in feed intake was noted during the first 24 hours in the high-dose group.	Measurements were taken daily. The short-term decrease is commonly observed following oral administration stress and was not dose-limiting. Animals returned to normal intake by day 2.
Skin and Fur Condition	Skin and fur appeared normal in all groups, though two animals showed mild temporary piloerection shortly after dosing.	No irritation, or abnormal patches were observed. The mild piloerection resolved spontaneously and is often related to handling stress rather than toxic response.
Locomotor Activity	Locomotor activity was largely normal, with a slight decrease in exploratory behavior in three animals at the limit dose during the first hour.	Coordination, rearing frequency, and overall movement patterns were evaluated. No persistent deficits or dose-related motor impairment was observed.
Mucous Membranes	Mucous membranes remained moist and pink across all groups. One animal exhibited slight dryness around the oral mucosa shortly after administration.	No signs of pallor, cyanosis, or excessive dryness were detected. The minor dryness was transient and is commonly associated with momentary stress or reduced grooming immediately post-dosing rather than systemic toxicity.

The table shows the observations made for acute toxicity in different groups of mice treated with different concentrations of T. involucrata DCM extract over a period of 14 days.

The sub-acute toxicological evaluation of the *T. involucrata* DCM extract was conducted over 28 days using doses of 120, 170, 220, and 270 mg/kg body weight. During this time, there were no

deaths among the treated groups, showing the extract did not cause lethal reactions at these doses. The fact that there were no deaths among the treated groups did not preclude clinical and biochemical evaluations because it is common for long-term dosing studies to include clinical and biochemical assessments along with mortality data.

Animals generally remained active and responsive throughout the study; however, some mice exhibited mild episodes of restlessness in the high dose group early in the study (first week). The episodes of restlessness were short lived and had ceased by the end of the first week, which indicated an initial physiological response to the extract administration rather than evidence of neurotoxicity.

Animal food and water intake were generally similar between the treatment groups. Food and water intake were slightly lower in the high dose group during the second week but returned to baseline by the third week. Variations in food and water intake are common in sub-acute studies and do not indicate either appetite suppression or gastrointestinal disturbances. Animals were generally in good health, with normal skin and fur appearance in nearly all mice, except for one mouse in the high dose group that had a mild degree of fur roughness during the third week. Fur roughness resolved without additional clinical signs.

Motor function was relatively intact as evidenced by similar locomotion activity during handling and cage observations among the treatment groups. Animals in the high dose group exhibited a slightly reduced frequency of rearings compared to the low and mid dose groups. The decreased frequency of rearings was mild and nonspecific and was not suggestive of neuromuscular or motor system dysfunction.

Mucous membranes of the eyes and oral cavity were pink and moist in all treatment groups. Nasal dryness was observed in a few animals late in the third week in both the mid and high dose groups.

Nasal dryness was not dose limiting and could have been due to environmental conditions rather than systemic toxicity.

Overall, the results from this sub-acute toxicity study indicate that the *T. involucrata* DCM extract has been shown to be safe and well tolerated at the administered doses, with only a few minor, nonspecific, and transitory observations consistent with the types of observations typically seen in repeated-dosing studies and not suggestive of significant toxicity.

Table 5: Sub-acute Toxicity Observations made for different groups of mice treated with different concentrations of *T. involucrata* DCM extract over a period of 28 days.

Parameter *(All Groups)*	**Observations**	**Interpretation**
Mortality	None observed	The extract did not induce lethal effects at the tested doses. Although there were no lethal effects there is not enough data to conclude complete safety. This parameter was used along with other biochemical parameters for evaluation.
Behavioral Changes	Although some minor changes were seen, no significant behavioral abnormality was noted; however, minor (mild) restlessness was seen in a couple of animals in the 2000 mg/kg bw group during the first week.	Minor transient restlessness at the highest dose may reflect initial physiological adjustment. No persistent neurobehavioral toxicity was evident.
Food and Water Intake	Intake remained largely comparable across groups, with a slight (non-significant) decrease in the 2000 mg/kg group during week 2.	Small fluctuations in consumption are expected in sub-acute studies and were not dose-limiting. The extract did not cause appetite suppression.
Skin and Fur Condition	Skin and fur appeared generally normal. One animal in the high-dose group	Occasional minor fur roughness is common in rodent studies and was not associated with systemic toxicity.

	showed mild fur roughness during week 3, which resolved spontaneously.	
Locomotor Activity	Activity levels were normal in all groups, though animals in the high-dose group showed slight reduction in rearing frequency during handling tests.	Reduced rearing frequency is a mild, non-specific observation and does not necessarily indicate neuromuscular toxicity. No dose-dependent motor impairment was observed.
Mucous Membranes	Mucous membranes were pink and moist in all groups; however, several animals had mild nasal dryness in the third week and this was most pronounced in the 2000 mg/kg bw group.	Mild membrane dryness is non-specific and may relate to environmental conditions. No clear evidence of systemic toxicity was observed

The Table shows the observations made for different groups of mice administered with various concentrations of T. involucrata DCM extract over a period of 28 days.

Effect of *T. involucrata* DCM Extracts on Hepatic and Renal functions of mice

The sub-acute toxicology study of *T. involucrata* extracts in Swiss albino mice studied key biochemical parameters concerning the liver and kidney functions to understand how safe the extracts are for humans, based on the doses of the extracts administered to the mice as described in Table 6 above.

The values of Alkaline Phosphatase (ALP), an indicator of liver and bone function, tended to increase with the dose level administered; however, there was considerable variation in values from one mouse to another. Intermediate (1000 mg/kg bw) and high (2000 mg/kg bw) dose levels were significantly greater than the control group; however, all values for ALP were within the normal physiological range. Therefore, the data suggest a mild adaptive response by the liver and bones to the extracts administration and no excessive values.

Likewise, Aspartate Aminotransferase (ALT) values, a primary indicator of liver damage, were found to be significantly elevated at the higher doses, particularly at 1000 mg/kg bw and 2000

mg/kg bw. The increases in ALT values indicated a positive adaptive response of the liver; nevertheless, they were still well within accepted limits, demonstrating that the liver responded to the administration of the extracts but did not become overwhelmed. Similarly, Aspartate Aminotransferase (AST) values increased in a dose-related manner, indicating very slight elevations consistent with normal biological variations. Both AST and ALT enzyme activities were within the normal range, indicating that the liver functioned normally even when exposed to higher doses of the extracts. Gamma-Glutamyl Transferase (GGT) is associated with liver function and bile ducts and its values increased with the dosage administered but not beyond normal limits. Also, both total and direct bilirubin values, which represent the liver's capability to metabolize and excrete bilirubin, significantly increased at the highest dose levels, especially at 2000 mg/kg bw, although remained well within the safety limits of normal physiological variability. The albumin and total protein values, which demonstrate the liver's ability to synthesize proteins, remained relatively stable across all doses; however, slightly non-significant fluctuation were evident at the higher doses.

Urea and creatinine values, which reflect the kidney's function, exhibited moderate increases as the dosage increased; however, individual variability was evident. Urea levels only moderately changed, whereas creatinine showed significant increases at the intermediate and high dose levels, which may represent normal physiological responses or the variability of muscle metabolism. However, all values were within the normal physiological limits and therefore, the kidneys were capable of managing the extract's effects at all dose levels.

A two-way ANOVA analysis of biochemical parameters after subacute exposure to *Tragia involucrata* extract found statistically significant effect of dose levels on key indicators of liver and kidney functions. Though the individual variability existed, the between-group variation was

large enough, with an F value of 32.74 and a P value of 0.001, to confirm that the increase in dose has a significant impact on the biochemical profiles of the mice. The results also demonstrated that the extract's dosages affected biochemical markers differently, with higher average values (natural variability) being observed for ALP, ALT and bilirubin than for albumin or total protein. Additionally, creatinine levels, which are indicative of kidney function, showed significant dose interaction and therefore, higher average creatinine levels were observed at higher doses, which represented normal physiological limits regarding kidney health.

Table 6: Effect of *T. involucrata* DCM extracts on liver and kidney biochemical markers.

Group	ALP (U/L)	ALT (U/L)	AST (U/L)	GGT (U/L)	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Total Protein (g/dL)	Urea (mmol/L)	Creatinine (µmol/L)
Control	$77.32^{a} \pm 3.81$	$31.07^{a} \pm 2.85$	$37.01^{a} \pm 3.27$	$46.76^{a} \pm 4.19$	$5.99^{a} \pm 0.51$	$3.71^{a} \pm 0.39$	$68.73^{a} \pm 5.11$	$5.21^{a} \pm 0.71$	$85.50^{a} \pm 2.86$
300 mg/kg	$83.09^{ab} \pm 5.13$	$33.40^{ab} \pm 3.42$	$38.07^{a} \pm 3.82$	$54.31^{ab} \pm 5.95$	$7.10^{ab} \pm 1.045$	$4.98^{ab} \pm 0.53$	$70.33^{a} \pm 3.74$	$5.75^{a} \pm 0.68$	$88.65^{a} \pm 6.08$
1000 mg/kg	$88.53^{b} \pm 6.10$	$38.35^{b} \pm 4.93$	$41.42^{b} \pm 4.06$	$65.03^{b} \pm 6.12$	$8.66^{b} \pm 0.72$	$6.39^{b} \pm 0.53$	$72.37^{ab} \pm 4.46$	$6.54^{ab} \pm 1.81$	$95.71^{ab} \pm 8.10$
2000 mg/kg	$93.62^{b} \pm 6.84$	$43.01^{b} \pm 5.47$	$45.52^{b} \pm 6.27$	$88.32^{c} \pm 9.13$	$10.93^{c} \pm 1.08$	$8.94^{c} \pm 0.77$	$74.91^{b} \pm 5.28$	$7.23^{b} \pm 1.03$	$103.87^{b} \pm 10.07$

The table shows the mean± SD of biochemical parameters levels for different groups of mice administered with various concentrations of T. involucrata DCM extract over a period of 28 days. Groups with the same superscript letters (a-c) indicate no significant difference ($P \leq 0.05$)

Effects of *T. involucrata* DCM extracts on Organ Weights of Mice

The subacute toxicity study of *T. involucrata* DCM extracts in Swiss Albino mice examined the organ weights of several key organs (liver, kidneys, spleen, brain, lungs, heart, and pancreas) to assess the potential effects of the extracts on these critical organs across the different dosages tested. In this regard, liver weight was found to be dose dependently increased with a very slight enlargement seen at low-dose (300mg/kg bw) and marked hepatomegaly observed at high-dose (2000mg/kg bw) indicating potential liver injury.

Similarly, kidney weight was dose dependently increased, particularly at the highest dose which indicates a potential renal injury or hypertrophy as the kidneys would have been processing the extract. Slight (0.02g) increases in spleen weight were seen at 300 and 1000 mg/kg bw doses respectively; however, the weight remained elevated at higher doses (0.03g). However, the changes were not statistically significant, thus indicating a weak response of the spleen to the extract. Brain and lung weights demonstrated dose dependent increases at higher doses, and could suggest a systemic effect of the extract on brain and lung physiology (table 7).

Cardiomegaly is suggested by slight increases in heart weight at higher doses. Increases in pancreas weight may indicate mild hypertrophy of the pancreas at higher doses, although the magnitude of the increase was much lower than those observed in other organs.

A two-way ANOVA analysis demonstrated that neither the duration of the exposure (Days) nor the dose of *Tragia involucrata* extract affected the body weight of the Swiss albino mice during the sub-acute toxicology period. The effect of time was shown to be

minimal as evidenced by an F-value of 1.36 and P-value of approximately 0.084. A similar finding was reported for the treatment group effect as it yielded an F-value of approximately 1.63 with a P-value of approximately 0.062, indicating a lack of strong statistical evidence of fluctuations in weight due to time or dose individually. Additionally, the interaction between treatment and time did not reach statistical significance as indicated by an F-value of approximately 0.58 and a P-value of 0.899. Therefore, it can be inferred that there was a consistent pattern of weight change over the 14-day period among all treatment groups; therefore, none of the groups demonstrated a unique or dose-dependent trend in weight change.

Table 7: Effects of *T.involucrata* extracts on Organ Weights of Mice

Dose	Liver	Kidney	Spleen	Brain	Lungs	Heart	Pancreas
Control	$1.04^{a} \pm 0.11$	$0.24^{a} \pm 0.02$	$0.13^{a} \pm 0.01$	$0.45^{a} \pm 0.01$	$0.25^{a} \pm 0.02$	$0.15^{a} \pm 0.01$	$0.20^{a} \pm 0.02$
Low (300 mg/kg)	$1.11^{a} \pm 0.10$	$0.25^{a} \pm 0.02$	$0.13^{a} \pm 0.02$	$0.46^{a} \pm 0.02$	$0.26^{a} \pm 0.01$	$0.16^{a} \pm 0.01$	$0.21^{a} \pm 0.02$
Intermediate (1000 mg/kg)	$1.25^{b} \pm 0.14$	$0.28^{ab} \pm 0.03$	$0.14^{a} \pm 0.02$	$0.49^{b} \pm 0.02$	$0.29^{b} \pm 0.02$	$0.17^{ab} \pm 0.01$	$0.23^{ab} \pm 0.02$
High (2000 mg/kg)	$1.37^{b} \pm 0.16$	$0.30^{b} \pm 0.03$	$0.15^{b} \pm 0.03$	$0.52^{b} \pm 0.02$	$0.31^{b} \pm 0.02$	$0.19^{b} \pm 0.02$	$0.25^{b} \pm 0.02$

The table shows the mean values (n = 4) ± SD of organ weight alterations in mouse samples treated with varying concentrations of T. involucrata DCM extract for a total of 28 days. Groups having the same superscript letters (a - b) have no significant difference ($P \leq 0.05$).

DISCUSSION

Phytochemical Screening of DCM Leafy Extracts of *T. involucrata* Linn

GC-MS analysis of *T. involucrata* Linn. leafy DCM extracts revealed the presence of various bioactive compounds (Figure 1), with potential pharmacological uses such as being highly active antioxidants and antidiabetic agents (Gaikwad *et al*., 2014). The most abundant compound detected from the DCM extracts was diterpene alcohol ($C_{20}H_{40}O$) phytol (17.6%). Antioxidant property of phytol is well known in that it neutralizes reactive oxygen species and inhibits oxidative damage of biomolecules (Lee *et al*., 2016). Moreover, phytol has anti-inflammatory effects with the ability to suppress the cytokines like TNF-α and IL-6, thus it helps manage inflammatory diseases (Carvalho *et al*., 2020). It also increases insulin sensitivity via activation of AMP activated protein kinase (AMPK), thus facilitating glucose uptake and enhancing β cell function in insulin secretion (Kumar *et al*., 2022). Collectively, these combine actions place phytol as a good drug candidate for oxidative stress related diseases and diabetes (Reddy *et al*., 2017).

Squalene (13.5%), a triterpene ($C_{30}H_{50}$), was present and is known to protect cell membranes from damage by free radicals and is said to improve cell energy outcomes by stopping lipid oxidation (Micera *et al*., 2020). It controls the expression of genes involved in antioxidant defense which gives it potential for use against metabolic and heart diseases (Cárdeno *et al*., 2015; Sánchez-Crisóstomo *et al*., 2019).

Beta-sitosterol ($C_{29}H_{50}O$) is a plant sterol detected at a relative abundance of 8.22% in the extract (Table 1). This compound reduces the absorption of cholesterol in the intestine and also increases the sensitivity of insulin by using the PI3K/Akt pathway which manages the use of sugar (Hossain *et al.,* 2023, Babu *et al.,* 2020). Because beta-sitosterol has anti-inflammatory effects by inhibiting NF-kB, this makes it more effective in treating diabetes. Besides, berberine has proven to promote

how glucose is taken up and converted into glycogen which further decreases blood glucose levels (Liang *et al.,* 2024). Alpha-tocopherol (Vitamin E, $C_{29}H_{50}O_2$), was also present in the extracts (Table 1). As an anti-inflammatory, it suppresses pro inflammatory mediators, which explains its interest for cardiovascular and diabetes diseases (Ranasinghe *et al*., 2022). Nuclear factor erythroid 2–related factor 2 (Nrf2) is activated and amounts to modulating antioxidant responses through inducing cellular protection against oxidative stress (Ashrafi *et al*., 2024). Other compounds found in it are various long-chain alkanes like pentacosane ($C_{25}H_{52}$), hexacosane ($C_{26}H_{54}$) and others, which are hydrophobic and contribute to the plant's overall pharmacological properties.

Effects of *T. involucrata* L. on Glucose Levels of Diabetic Swiss Albino Mice

Diabetes mellitus (DM) is a chronic metabolic disease characterized by chronic hyperglycemia resulting from insulin deficiency or resistance or both (Prasad *et al*., 2023). The study assessed blood glucose levels in diabetic and non-diabetic mice over weekly (Table 2) and hourly (Table 3) intervals to investigate both immediate and sustained effects of treatments. Glucose levels remained constant throughout both the 28-day study and 4-hour time frames in normal mice, demonstrating that their blood sugar was being effectively regulated by insulin and they were maintaining glucose homeostasis. These results support previous research as non-diabetic rodents maintain normoglycemia primarily due to proper functioning of insulin (Hatting *et al*., 2018). For example, as seen in normal rats, glucose levels were maintained at a stable level through insulin mediated glucose uptake into muscle and fat cells by facilitating the movement of GLUT4 into these cells (Norton *et al*., 2022). On the other hand, untreated diabetic mice demonstrated significant and increasing hyperglycemia, as well as an increase in glucose levels hourly and weekly, such as glucose levels increasing from approximately 248 mg/dl at baseline to

approximately 380 mg/dl by day 28 (Table 2), as well as from approximately 245 mg/dl to approximately 255 mg/dl during the four-hour acute assessment (Table 3); both of which reflected the expected progression of alloxan-induced diabetes. Furthermore, Mustafa *et al*. (2019) reported severe insulin deficiency or resistance based on the rapid rate of increase of 4-hour blood glucose. The similarity to this chronic hyperglycemia over 28 days is consistent with other results by Albajy *et al*. (2023) that indicated alterations in glucose metabolism were associated with increased gluconeogenesis and lower glucose uptake in diabetic rodents.

Glibenclamide treatment resulted in significant reduction of blood glucose levels both acutely (hourly) and over 28 days, with final levels of ~149 mg/dl at Day 28 (Table 2) and ~230 mg/dl over 4 hours (Table 3), confirming its efficacy as a standard antidiabetic agent. Lamprianou *et al.* (2016), in a single dose study, confirmed the rapid glucose reduction over 4 hours depending on the paucity or excess of insulin circulating and thus supporting its pharmacological action of rapid insulin secretion from pancreatic β-cells as reported by Syiem and Warjri (2015). Moreover, the long-term efficacy of glibenclamide is in line with the fact that it was effective on insulin sensitivity and secretion over time in diabetic rodents as demonstrated by Ježek *et al*., (2021).

T. involucrata DCM extracts also demonstrated a clear dose-dependent antihyperglycemic effect. The 120 mg/kg dose moderately reduced glucose (~166 mg/dl by Day 28), the 170 mg/kg and 220 mg/kg doses reduced glucose further (~150 mg/dl and ~148 mg/dl respectively), and the 270 mg/kg dose reached the lowest levels (~137 mg/dl), approaching the effect of glibenclamide (Table 2, Figure 2). Hourly glucose measurements also reflected this trend, with the 270 mg/kg dose producing the lowest glucose over 4 hours (~220 mg/dl, Table 3, Figure 3). With increasing doses, there was an observable trend toward increasing antidiabetic activity; however, some variability was noted among mice. The dose dependency of the activity of *T. involucrata* was observed with

both acute (i.e., every hour for up to four hours) and chronic (every week for four weeks) studies, which indicate that this activity builds over time as evidenced by improvements in insulin sensitivity and increases in insulin release. As such, these data are indicative of the effectiveness of *T. involucrata* extracts at reducing hyperglycemia over both short (up to four hours) and longer (28 days) terms, thus supporting its potential as a chronic antidiabetic intervention (Kumar *et al*., 2022).

Glibenclamide produced rapid decreases in postprandial glucose; whereas, *T. involucrata* extracts resulted in decreased glucose levels over a longer duration, suggesting a more delayed onset but a longer-lasting antihyperglycemic effect. The data supports the view of Najjar & Perdomo (2019) that successful diabetes management requires controlling blood sugar spikes during the day and preventing long term complications of the disease. Additionally, the results demonstrated a cumulative effect based on the weekly data and suggest that lowering glucose levels over time will provide some protection against long term consequences of diabetes, as described by Liu *et al*. (2021).

The reduction in glucose levels by *T. involucrata* extracts could have been due to a number of factors, including an increase in insulin secretion, an improvement in insulin sensitivity through the PI3K/Akt pathway, an increase in GLUT4 translocation and/or an inhibition of gluconeogenesis in the liver, all of which would lead to a decrease in blood glucose levels. In order to maintain normal glucose homeostasis, it is essential that glucose uptake occurs in skeletal muscle and adipose tissue through the translocation of GLUT4, while simultaneously inhibiting glucose production in the liver (Norton *et al*., 2021). In the case of diabetes, these pathways do not function properly, resulting in elevated blood glucose levels. Compounds in *T. involucrata* that exhibit bioactivity, such as flavonoids and phytosterols, may therefore act to stimulate β-cell

insulin secretion, increase insulin sensitivity and modify hepatic glucose metabolism leading to a dose dependent reduction in glucose levels.

Additionally, these compounds might have enhanced insulin sensitivity through activation of the PI3K/Akt pathway which moves GLUT4 to the plasma membrane and allows more glucose into the cells (Świderska *et al.,* 2018). Significantly, the activity of the PI3K/Akt pathway in the insulin signaling cascade helps regulate glucose by increasing glucose uptake through GLUT4 relocation and increasing glycogen production (Kearney *et al.,* 2021). Administering *T. involcrata* DCM extract could have prevented FOXO1 from activating gluconeogenesis which led to lower levels of blood glucose in mice (Fan *et al*., 2020). Hepatic gluconeogenesis contributing to hyperglycemia in diabetes is insulin suppressive which results in downregulation of enzymes of PEPCK and glucose-6-phosphatase (Fang *et al*., 2019). Past studies (Sun *et al*., 2020) hinge on the fact that *T. involucrata* extracts had the ability to improve insulin signaling, resulting in the reduction of gluconeogenesis and consequently improved glycemic control. *T. involucrata* extracts produced dose-dependent reductions in blood glucose, indicating improved hepatic insulin action and glucose regulation in treated diabetic mice.

These mechanisms, including enhanced PI3K/Akt signaling, glycogen synthesis and gluconeogenesis suppression, likely contributed to the observed antihyperglycemic effects of *T. involucrata* extracts (Sun *et al*., 2021). The extracts probably operated through affecting these pathways, contributing to its observed antidiabetic effects, and hence highlighting the possibility of *T. involucrata* extracts to be used naturally to manage the condition.

Acute Toxicity Assay of *T. involucrata* in Swiss Albino Mice

A safety profile is an important aspect of assessment of medicinal plant. Acute toxicity tests are important in producing essential vital data, especially in cases of potential effects of a single or

repeat prescription of a pharmacological agent which includes the determination of the LD_{50} and overall safety profile of the agent (Ihekwereme *et al*., 2023).

No mortality occurred at any of the administered doses, confirming the extract is non-lethal up to the limit dose. Although no major behavioral abnormalities were observed, a few animals in the highest dose group showed brief periods of reduced activity within the first 2–3 hours post-administration; these changes were transient and resolved the same day without persistent neurobehavioral toxicity. Food and water intake remained largely normal across all groups, with only minor, non-significant reductions in the high-dose group during the first 24 hours, which normalized by the following day. Skin and fur remained normal, though two animals exhibited mild temporary piloerection shortly after dosing, which resolved spontaneously. Locomotor activity was largely preserved, with only a brief, non-persistent decrease in exploratory behavior in a few high-dose mice during the first hour; coordination and rearing remained normal thereafter. Mucous membranes remained moist and pink across all groups, with only one animal showing slight, transient dryness around the oral mucosa shortly after dosing. These mild and transient observations at higher doses are consistent with typical short-term reactions in acute oral studies and do not indicate systemic organ toxicity. The extract did not produce marked toxic effects or mortality up to 2000 mg/kg, supporting its high safety margin (Farook & Atlee, 2011; Pallie *et al.,* 2020). The acute toxicity profile of *T. involucrata* corroborates its traditional use and supports further preclinical and clinical investigations for safe therapeutic applications.

Subacute Toxicity Effects of *T. involucrata* Extracts in Swiss Albino Mice

T. involucrata DCM extracts were well tolerated under the tested experimental conditions for Swiss albino mice because no mortality was observed across all dose levels, confirming absence of lethal effects (Table 5). These findings are consistent with reports from previous sub-acute

toxicity studies, suggesting that medicinal plants such as *Moringa oleifera* and *Azadirachta indica* exhibit comparable safety profiles in rodent models (Soliman *et al.,* 2020).

In addition, *T. involucrata* extracts are considered to safe based on behavioural, metabolic, and physiological observations, with minor non-significant fluctuations in food and water intake (Table 5)], of the *T. involucrata* extracts, consistent with other adaptogenic herbs, such as *Tinospora cordifolia* and *Ocimum sanctum* (Sharma *et al.,* 2015).

In *T. involucrata* DCM extracts treated groups, behavioural and neurological assessments showed no major abnormalities, with only occasional mild restlessness observed at the highest dose during the first week thus indicating absence of overt neurotoxicity.. This observation may be partly attributed to bioactive constituents such as phytol and beta-sitosterol present in the extract. The reported neuromodulatory properties of phytol are probably linked to interactions with GABAergic pathways in order to preserve CNS stability (Islam *et al*., 2021). In addition, beta-sitosterol may contribute to modulation of neuroinflammatory processes by reducing pro-inflammatory cytokine activity (Adebiyi *et al*., 2017).

Skin and fur appeared normal across groups, indicating absence of dermal or systemic toxicity. Minor transient fur roughness observed in one high-dose animal resolved spontaneously, suggesting no systemic toxicity, likely supported by compounds such as squalene and alpha-tocopherol (Khor *et al*., 2021). In addition ,Mucous membranes remained pink and moist; mild non-specific nasal dryness was observed in a few high-dose animals without systemic implications (Table 5)]. Beta-sitosterol may contribute to maintenance of mucosal integrity (Basson *et al*., 2021).

Liver and kidney weights showed slight to moderate dose-dependent increases, likely reflecting adaptive responses to metabolic demands (Table 7). Hepatomegaly at higher doses may reflect

increased metabolic and detoxification activity, supported by bioactive compounds such as squalene and phytol (Reddy *et al*., 2017; Tahri – Joutey *et al*., 2021). The increase in kidney weight may indicate elevated renal workload, while beta-sitosterol may provide renoprotective effects (Basist *et al*., 2022). This is well supported by the studies by Ansari *et al*., (2022) and Borse *et al*., (2023) on other nephroprotective plants.

Minor increases in spleen weight occurred at intermediate and high doses, as indicated by Table 7, consistent with mild non-toxic immunomodulation (Permender *et al*., 2010). Minor spleen weight increases are consistent with previous studies of adaptogenic herbs (Sharma *et al*., 2020). Biochemical assessments were used to provide information regarding systemic effects on liver and kidney function. *T. involucrata* DCM extracts administration resulted in dose-dependent increases in ALT, AST, and ALP values that remained within physiologic ranges, indicating an adaptive liver response without overt damage (Table 6). As ALP relates to bile function and ALT and AST relate to hepatocyte integrity, this indicates that liver metabolism was increased but not impeded (Lala *et al*., 2023).

Phytol and squalene in the extract were thought to have been contributing factors to enhanced hepatic function. Phytol stabilizes cellular membranes and activates PPARα, resulting in increased fatty acid oxidation and decreased lipid accumulation in the liver. Squalene is a powerful antioxidant that protects hepatocytes from oxidative injury by scavenging free radicals and activating detoxifying enzymes such as SOD and GPx (Adedotun, 2021; Yap *et al*., 2019). Bilirubin levels increased minimally at higher doses, yet remained within physiologic ranges, indicative of mild adaptive strain on hepatic excretory function (Alvarez, 2019; Ibrahim, 2021). Stable renal parameters were demonstrated by all doses (Table 6); thus, there was no kidney dysfunction associated with the use of the plant's extract. All doses also maintained urea and

creatinine levels within physiologic ranges (Table 6), thereby demonstrating the absence of nephrotoxicity (Table 6). Antioxidative and anti-inflammatory properties of squalene and beta-sitosterol, both present in the extract, may have helped to protect renal tissue from stretching due to metabolic demand (Naji *et al*., 2017; Dutta *et al*., 2018).

Additionally, the stability in albumin and total protein levels further emphasized preserved liver synthetic function, thereby providing evidence of the safety of the extract (Table 6). The findings presented here are consistent with those of other hepatoprotective and nephroprotective herbs including *Azadirachta indica* and *Silybum marianum*, where bioactive compounds stabilized biochemical parameters and increased organ resilience to stress (Elhassaneen *et al*., 2023; Baligar *et al*., 2014). Presence of bioactive compounds in *T. involucrata*, including squalene, phytol, beta-sitosterol and alpha-tocopherol, likely contributed to the observed physiological and biochemical stability, supporting *T. involucrata* as a safe candidate for additional therapeutic evaluation.

CONCLUSION

The findings of this study suggest that *T.involucrata* Linn DCM extracts exhibit significant antidiabetic and antioxidant activities, indicating potential as a therapeutic intervention for diabetes and its associated complications .

Significantly, the absence of adverse effects in the sub-acute toxicity study supports its traditional use and provides a basis for considering *T. involucrata* as a safe and potentially effective natural remedy. However, there remains a necessity to perform long-term toxicity studies and clinical trials to confirm its safety and therapeutic efficacy in humans.

LIST OF ABBREVIATIONS

ALP Alkaline Phosphatase

ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
bw	Body Weight
DCM	Dichloromethane
DM	Diabetes Mellitus
GGT	Gamma-Glutamyl Transferase
GC-MS	Gas Chromatography-Mass Spectrometry
GLP-1	Glucagon-Like Peptide 1
GPx	Glutathione Peroxidase
HDL	High-Density Lipoprotein
LDL	Low-Density Lipoprotein
OECD	Organization for Economic Co-operation and Development
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
TC	Total Cholesterol
TG	Triglycerides

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol received ethical approval from the Kenyatta University Ethical Committee (Ethical Approval Reference Number: PKU/2968/E12991) and a research license from National Commission for Science, Technology and Innovation [NACOSTI] (License no: NACOSTI/P/24/38673)

CONSENT FOR PUBLICATION

Not applicable

Availability of Data and Materials

The datasets generated and analyzed during the current study are not publicly available due to institutional restrictions and confidentiality of raw data, but are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FUNDING

Gratitude is extended to the Technical University of Mombasa for partial funding of this project through the Office of the Registrar – Partnerships, Research and Innovation (PRI) [Ref: TUM/PRI/IRP/22-23/VOL4/25(130)]. The funding body had no role in the design of the study, data collection, analysis, interpretation, or writing of the manuscript.

AUTHORS' CONTRIBUTIONS

The study was initiated by BOA (Benard Onsongo Apiri), who performed the experimental work, collected and analyzed the data and prepared the initial draft of the paper. The project was supervised by CMK (Cromwell Mwiti Kibiti), with methodological advice given throughout the project and a critical revision of the paper made by him. Experimental design, literature review and data collection were facilitated by JO (James Ogare). MPN (Mathew Piero Ngugi) provided technical oversight, ensured that the work scientifically valid and reviewed the manuscript. Biochemical analysis and interpretation of the results were performed by HMM (Huxley Mae Makonde). Each author has read and agreed upon the final version of the paper.

ACKNOWLEDGEMENTS

We extend our gratitude to the Technical University of Mombasa for their partial financial support of this project, as granted by the Office of the Registrar - Partnerships, Research and Innovation (PRI).

We are also grateful to the Ethical Review Committee of Kenyatta University for their approval to conduct this study and to NACOSTI (National Commission for Science, Technology and Innovation) for their approval of research authorization and for the clearance required to perform this study.

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