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## Research Article

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# Ethanollic Extract of Watermelon (*Citrullus lanatus*) Seeds on Diabetic Wistar Rats: A Histological, Histochemical, and Neurobehavioural Study

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## Abstract

**Background:** Diabetes mellitus (DM) affects 11.11% of adults globally in 2024, with prevalence projected to reach 13% by 2050. Chronic hyperglycaemia drives oxidative stress, neuroinflammation and structural brain injury which is termed diabetic encephalopathy, manifesting as anxiety-like behaviour, locomotor deficits and autonomic dysregulation. Conventional therapies have limitations, prompting interest in nutraceuticals like *Citrullus lanatus* (watermelon) seed, rich in citrulline, phenolics and flavonoids with documented antioxidant and antidiabetic properties.

**Methods:** Diabetes was induced with STZ, and rats received EWS or metformin for six weeks alongside untreated diabetic and normal controls; an extract-only non-diabetic group assessed intrinsic extract effects. Parameters assessed included body and brain weight, blood glucose, open-field neurobehavioural indices, oxidative stress markers (MDA, SOD, GSH, CAT), glucose-metabolising enzymes (ALP, ACP, G6PDH, LDH), inflammatory/apoptotic markers (caspase-3, IL-6, TNF- $\alpha$ ), and frontal cortical histoarchitecture.

**Results:** Diabetic controls exhibited significant weight loss, hyperglycaemia, anxiety-like and hypoactive behaviour, elevated oxidative stress and inflammatory markers, depleted antioxidants

and metabolic enzymes, cortical neuronal necrosis, reactive astrogliosis and collagen deposition. Treatment with EWS or metformin significantly attenuated nearly all derangements, normalising blood glucose and restoring behavioural, biochemical and histological parameters close to normal, with only partial mitigation of collagen deposition. The extract-only group showed no differences from normal controls.

**Keywords:** Watermelon seed, Diabetes mellitus, Frontal cortex, Oxidative stress, Enzyme of glucose metabolism, Neurobehavioural study

## **Introduction**

### **Background**

Diabetes mellitus (DM) remains one of the fastest-growing non-communicable diseases of the modern era. According to the 11th edition of the International Diabetes Federation (IDF) Diabetes Atlas, 11.11% of adults worldwide were living with diabetes in 2024, corresponding to approximately 589 million people, with prevalence projected to rise to nearly 13% (853 million people) by 2050 (Genitsaridi et al., 2026). A parallel Global Burden of Disease analysis similarly documents a rising trajectory of type 2 diabetes prevalence across all socio-demographic strata between 1990 and 2021, with continued increases projected through 2044 (He et al., 2024). This escalating burden is driven by urbanisation, sedentary lifestyles, dietary transition and population ageing, and is compounded by a substantial proportion of undiagnosed cases, particularly in low- and middle-income settings (Genitsaridi et al., 2026).

Beyond its classical metabolic and vascular complications, chronic hyperglycaemia exerts profound effects on the central nervous system. Persistently elevated glucose promotes mitochondrial dysfunction, excessive generation of reactive oxygen species, and impairment of endogenous antioxidant defences, culminating in neuronal injury, reactive astrogliosis and disrupted glial–neuronal signalling (He et al., 2024). This constellation of central changes, termed diabetic encephalopathy, is now recognised to affect a substantial proportion of people living with diabetes and manifests clinically and pre-clinically as cognitive decline, anxiety-like behaviour and altered psychomotor activity (Alrefaei & Elbeeh, 2025). In rodent models of streptozotocin (STZ)-induced diabetes, these central derangements are paralleled by increased astrocytic glial fibrillary acidic protein (GFAP) expression, neuronal necrosis and disturbances in exploratory,

anxiety-related and autonomic behaviours assessed using paradigms such as the open-field test (Alrefaei & Elbeeh, 2025).

Metformin remains the first-line pharmacological agent for type 2 diabetes and has additionally been reported to exert central benefits through activation of AMP-activated protein kinase (AMPK), with downstream effects on neuroinflammation, oxidative stress and neurotrophic signalling (Ameen et al., 2022; Khaleghi-Mehr et al., 2023). However, its central efficacy is not uniform across studies, and gastrointestinal intolerance limits use in some patients, sustaining interest in complementary or alternative approaches, including nutraceuticals with combined antioxidant and antidiabetic activity (Alrefaei & Elbeeh, 2025).

## **Rationale**

*Citrullus lanatus* (watermelon), a widely cultivated member of the Cucurbitaceae family, has long been used in traditional medicine for the management of hypertension, renal stones, urinary tract infection and diabetes. Its seeds are particularly rich in protein, unsaturated fatty acids, minerals and phytochemicals, including phenolics, flavonoids and the non-protein amino acid citrulline, which collectively confer antioxidant, anti-inflammatory and metabolic benefits (Benmeziene & Derradji, 2023). An ethanolic extract of *C. lanatus* seed has been shown to be well tolerated in Wistar rats, with no adverse behavioural, biochemical or histopathological effects at doses up to 1000–2000 mg/kg, and with evidence of a protective effect on hepatic and renal histoarchitecture and on serum glucose and lipid profiles (Belemkar & Shendge, 2021). Related preparations of the plant, such as a leaf extract, have similarly been shown to attenuate hyperglycaemia, dyslipidaemia, oxidative stress and structural organ damage in high-fat diet/streptozotocin-induced diabetic rats (Jibril et al., 2022), lending biological plausibility to a central protective effect of seed-derived extracts as well.

Despite this accumulating evidence for the peripheral antidiabetic and antioxidant activity of watermelon-derived extracts, the majority of existing studies have focused on metabolic, hepatic, renal or cardiovascular endpoints. Comparatively little is known about the effects of watermelon seed extract on diabetes-associated neurobehavioural change and frontal cortical histoarchitecture, an important gap given the growing clinical recognition of diabetic encephalopathy as a major, under-treated complication of diabetes (Alrefaei & Elbeeh, 2025).

## Objectives

The present study was therefore designed to characterize, in a single integrated model, the histological, histochemical, biochemical and neurobehavioural effects of an ethanolic extract of watermelon seed in STZ-induced diabetic male Wistar rats, using metformin as an active comparator. Specifically, the study evaluated:

1. The qualitative and quantitative phytochemical composition and *in vitro* antioxidant activity of the extract;
2. The effects of the extract on body weight, relative brain weight, and blood glucose levels;
3. The Neurobehavioural outcomes including locomotor activity, anxiety-related behaviours, and autonomic functions;
4. The oxidative stress markers (MDA, SOD, GSH, CAT), glucose-metabolising enzymes (ALP, ACP, G6PDH, LDH), and inflammatory/apoptotic markers (caspase-3, IL-6, TNF- $\alpha$ );
5. The frontal cortical histoarchitecture using H&E, PTAH, Cresyl Fast Violet, GFAP immunohistochemistry, and Masson's trichrome staining.

The overarching goal was to establish whether this readily available agricultural by-product holds promise as a nutraceutical adjunct for the neurological complications of diabetes.

## Materials and Methods

### Acute oral toxicity (LD<sub>50</sub>) study

The acute oral toxicity of the ethanolic extract was evaluated in healthy adult Wistar rats using Lorke's (1983) up-and-down two-phase method, as adapted in recent toxicological studies (Olubodun et al., 2024). In Phase One, animals were randomly allocated into three groups (n = 3 per group) and administered the extract orally as a single dose of 100, 400, or 1000 mg/kg body weight. Animals were observed continuously for the first 4 hours and then intermittently over 24 hours, and daily thereafter for up to 14 days, for signs of toxicity including changes in behaviour, motor activity, salivation, tremors, convulsions, and mortality. As no mortality or overt toxicity was observed in Phase One at any dose, Phase Two was conducted using three new groups of rats (n = 3 per group) administered higher single oral doses of 2000, 3000, or 4000 mg/kg body weight, with the same observation protocol applied (Dumon et al., 2019). All animal procedures were

carried out in accordance with institutional and national guidelines for the care and use of laboratory animals, following approval by the relevant institutional animal ethics committee.

### **Qualitative phytochemical screening**

The crude ethanolic extract was screened for the presence of tannins, phenols, phlobatannins, alkaloids, saponins, flavonoids, steroids, anthraquinones, terpenoids, cardiac glycosides, and reducing sugars using standard qualitative colour and precipitation reactions (Shah et al., 2023). Tannins and phenols were detected by the ferric chloride test, in which a few drops of 5% ferric chloride solution were added to the extract and observed for a blue-black or greenish-black colouration. Phlobatannins were identified by boiling the extract with 1% aqueous hydrochloric acid and observing for a red precipitate (Noor & Terefe, 2025). Alkaloids were detected using Mayer's and Wagner's reagents, with the appearance of a cream or reddish-brown precipitate, respectively, indicating a positive result. Saponins were assessed by the froth test, in which the extract was vigorously agitated with distilled water and observed for persistent, honeycomb-like froth. Flavonoids were tested using the sodium hydroxide test, where a yellow colouration that disappeared on addition of dilute acid confirmed their presence. Steroids and terpenoids were detected using the Salkowski and Liebermann–Burchard tests, indicated by a red-brown or blue-green colouration at the interface of chloroform and concentrated sulphuric acid. Anthraquinones were identified by Borntrager's test, involving the appearance of a pink to red colouration in the ammoniacal layer. Cardiac glycosides were detected by the Keller–Kiliani test, indicated by a brown ring at the interface. Reducing sugars were confirmed using Fehling's test, with the formation of a brick-red precipitate on heating. Each test was recorded as positive (+) or negative (–) based on the characteristic colour change or precipitate formed.

### **Quantitative phytochemical analysis**

Following confirmation of their presence, the concentrations of the seven quantifiable constituents (tannin, phenol, reducing sugar, alkaloid, flavonoid, saponin, and cardiac glycoside) were determined and expressed as mg per 100 g of extract, using standard spectrophotometric and gravimetric procedures (Nugrahani et al., 2025). Total tannin and total phenolic contents were determined by the Folin–Ciocalteu colorimetric method, with absorbance read at 700–760 nm against a tannic acid or gallic acid standard curve, respectively. Total flavonoid content was determined by the aluminium chloride colorimetric method, with absorbance measured at 415 nm

against a quercetin standard curve (Nyadanu et al., 2020). Alkaloid content was determined gravimetrically following precipitation with concentrated ammonium hydroxide and re-extraction into chloroform. Saponin content was determined by the vanillin–sulphuric acid spectrophotometric method, with absorbance read at 544 nm. Cardiac glycoside content was determined spectrophotometrically using the Baljet reagent, with absorbance read at 495 nm. Reducing sugar content was determined by the 3,5-dinitrosalicylic acid (DNS) method, with absorbance read at 540 nm against a glucose standard curve. All determinations were performed in triplicate, and results were expressed as the mean concentration in mg/100 g of dried extract.

### **In vitro antioxidant assays**

The antioxidant capacity of the ethanolic extract was evaluated across five complementary assays at concentrations of 50, 100, 200, 400, and 500 µg/mL, with ascorbic acid used as the reference standard in each assay (Knez et al., 2025). The ferric reducing antioxidant power (FRAP) assay was based on the reduction of the  $\text{Fe}^{3+}$ –TPTZ (2,4,6-tripyridyl-s-triazine) complex to its blue  $\text{Fe}^{2+}$  form in the presence of antioxidants, with absorbance measured at 593 nm (Fialho et al., 2025). The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed by mixing the extract or standard with a methanolic DPPH solution, incubating in the dark for 30 minutes, and measuring the decrease in absorbance at 517 nm, with percentage inhibition calculated relative to the control. Nitric oxide (NO) scavenging activity was assessed using sodium nitroprusside as a nitric oxide donor in phosphate-buffered saline; the nitrite generated was quantified using the Griess reagent, with absorbance read at 546 nm. Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) scavenging activity was determined by incubating the extract with a hydrogen peroxide solution prepared in phosphate buffer (pH 7.4) and measuring the residual  $\text{H}_2\text{O}_2$  concentration spectrophotometrically at 230 nm. Lipid peroxidation inhibitory activity was evaluated using the thiobarbituric acid reactive substances (TBARS) method, in which lipid peroxidation was induced in an egg-yolk or tissue homogenate substrate using ferrous sulphate, and the malondialdehyde-thiobarbituric acid chromogen formed was measured at 532 nm. In each assay, percentage activity was calculated relative to the untreated control, and all determinations were performed in triplicate.

### **Gas chromatography–mass spectrometry (GC-MS) analysis**

The chemical composition of the ethanolic extract was profiled using a gas chromatograph coupled to a mass spectrometer (GC-MS-QP series) fitted with a non-polar capillary column (Rtx-5MS or

equivalent, 30 m × 0.25 mm internal diameter, 0.25 µm film thickness), following the general approach of Shah et al., (2023). Helium was used as the carrier gas at a constant flow rate of approximately 1 mL/min. The injector temperature was maintained at approximately 250°C, with a sample injection volume of 1 µL in split mode. The column oven temperature was programmed from an initial 60°C, held for 2 minutes, and ramped at 3–10°C/min to a final temperature of 280°C. Mass spectra were acquired in electron impact ionisation mode at 70 eV, scanning a mass range of approximately 40–600 m/z. Individual constituents were tentatively identified by comparing their mass spectral fragmentation patterns and retention times against reference spectra held in the National Institute of Standards and Technology (NIST) mass spectral library, and the relative percentage of each constituent was calculated from its corresponding peak area relative to the total ion chromatogram.

## **Plant materials**

Ripe Water melon fruits (*Citrullus lanatus*) were harvested from Chipakama area Kapiri mposhi, central province, Zambia. The fruits were washed and cut open; the seeds were harvested, washed and dried under room temperature and were subjected to identification at the University of Zambia School of Natural Sciences under the Department of Biological Sciences before the study began. The seeds were manually extracted from the fruits, cleaned, and airdried before being processed into powder in an electric blender. Prior to analysis, they were stored in an airtight plastic container at 4°C. The dried seed powder was macerated in 2 liters of distilled water for 24 hours at room temperature. The extractive solvent was then filtered, and the aqueous soluble extract was dried in a hot air oven that was set at 45°C. To acquire the extract's flavonoid-rich parts, the oven-dried extract was first dissolved in distilled water at room temperature. This was done in accordance with the procedure outlined by Bazabang et al.,(2023), the procedure described entailed defatting the extract first by adding N-hexane to the aqueous component of the extract and allowing it to stand overnight. The two distinct layers generated were separated, and the water residue was treated with a hydroalcoholic solution (70:30 methanol to water). Polar compounds were removed further by soaking the hydroalcoholic extract in a diethyl ether solution overnight and collecting the two different layers that formed. Butanol was added to the water residual, which was violently mixed and left to stand overnight. The butanolic fraction contains saponins and will be split into two parts after the two different layers were separated. After the two unique layers had developed,

they were separated; the butanolic fraction, which includes saponins, was then split into two halves. One portion received 1% KOH in addition to being acidified with strong hydrochloric acid to produce the flavonoid fraction. The crude flavonoid fraction was the final product employed in this investigation.

## **Animals**

Thirty adult presumably healthy male Wistar rats (*Rattus norvegicus*) were used for this study. The animals were between 8 to 10 weeks old; body weight (180-220 g). Animals were kept in five cages (6 rats per cage) and housed in the Animal house of, Mulungushi University School of Medicine and Health Sciences, Department of Human Anatomy, Livingstone campus. They were maintained on standard animal feeds (Wealth-gate pelletized feeds) and were given free access to clean water and feeds.

## **Induction of diabetes**

Streptozotocin (STZ) was used for induction. The feeds were withdrawn overnight from the rats, the rats were weighed and a baseline glucose level was obtained. An injection of STZ was administered at a dose of 70 mg/kg body weight intraperitoneally, the rats were returned to their regular feeding schedule (Luambia, Siabwacha, & Ngosa, 2024). After 72 hours of induction, a tail vein puncture was used to obtain fasting blood glucose level in order to ascertain the onset of diabetes. The rats with fasting blood glucose level of more than 8 mmol/l or more than 220 mg/dl, were classified as diabetic.

## **Experimental design**

**Table 1 Experimental design**

| <b>Group</b> | <b>Code</b> | <b>Description</b>                            |
|--------------|-------------|-----------------------------------------------|
| 1            | NC          | Normal control                                |
| 2            | WSO         | Watermelon seed extract only                  |
| 3            | DW          | Diabetic treated with watermelon seed extract |
| 4            | DM          | Diabetic treated with metformin               |
| 5            | DC          | Diabetic control                              |

## **Watermelon seed extract mode of administration**

Watermelon seed extracts were dissolved in physiological saline daily and administered orally with the use of an oro-gastric cannula to Group WSO and DW rats (n=6 each group) at 400 mg/kg bw, (at 9.00 – 10.00 a.m. each day), for a maximum period of six weeks. Group DM (n=6) received 10mg/kg bw of Metformin. Group NC rats (n=6) received neither STZ nor Guava leaves extract. Group DC rats (n=6) received STZ but no treatment was given (Luambia, Siabwacha, & Ngosa, 2024).

### **Measurement of the body weight (g)**

Body weight (g) of the rats was recorded for two weeks (acclimatisation period) prior to induction of diabetes and on a weekly basis during the experimental treatment for a duration of six weeks. Body Weight was taken with a weighing scale (Venus VT 30 SL);(Luambia, Siabwacha, & Ngosa, 2024)

### **The relative organ weight (%)**

Using a sensitive weighing balance (SonyF3G brand), the relative organ weight of the rat was calculated as the ratio of the weight of its brain to its terminal body weight; the unit of measurement was recorded as a percentage (%)(Luambia, Siabwacha, Ngosa, et al., 2024).

### **Measurement of blood glucose**

Between 9:00 and 10:00 in the morning, blood glucose levels in Wistar rats that have fasted overnight was assessed using the glucose oxidase method of the One Touch Ultra 2 glucometer (Accu-Chek Compact Plus). By puncturing the tip of the tail, blood was drawn from the tail's median caudal vein. Weekly blood glucose monitoring began two weeks before to the induction of diabetes and continued for six weeks during the course of treatment (Luambia, Siabwacha, Ngosa, et al., 2024).

### **Neurobehavioural studies**

Anxiety, exploration and locomotor activities: The Anxiety, Exploration and Locomotor activities were studied using open field maze method (Kehinde et al., 2018). The open field maze that was used was the large size (72 × 72 cm). The large open field was used for measuring anxiety and exploration as well as locomotion as it had a large center arena. The open field apparatus was constructed using plywood painted with white colour and measuring 72 × 72 cm with 36 cm walls.

Red lines were drawn on the floor with a marker for visibility on the floor. The lines divided the floor into sixteen  $18 \times 18$  cm squares. A central square ( $18 \text{ cm} \times 18 \text{ cm}$ ) was drawn in the middle of the open field (Kehinde et al., 2018). The central square was used because some rat strains have high locomotor activity and could cross the lines of the test chambers many times during a test session. The central square had sufficient space surrounding it to give meaning to the central location as being distinct from the outer locations.

### ***Procedures***

Rats were carried to the test room in their home cages and were handled by the base of their tails at all times. Rats were placed into the center, or one of the four corners of the open field and allowed to explore the apparatus for 5 minutes. After the 5-minute test, rats were returned in their home cages and the open field maze was disinfected with 70% ethyl alcohol and permitted to dry between tests (Kehinde et al., 2018).

### ***Behavioral parameters***

The behaviors that were scored include:

1. Line crossing: Frequency with which each of the rats crossed one of the grid lines with all four paws in 5 minutes.
2. Center square entries: Frequency with which each rat crossed one of the red lines with all four paws into the central square (Number of times each rat entered the center square).
3. Hinding: Frequency with which the rat stood on its hind limbs in the maze.
4. Walling: Number of times the rat touched the maze wall.
5. Urination: Number of puddles or streaks of urine in 5 minutes.
6. Defecation: Number of fecal boli produced in 5 minutes.

### **Histological studies**

Euthanasia was used to sacrifice the animals at the conclusion of this investigation, consistent with current best-practice recommendations for the humane killing of laboratory rodents (Clarkson et al., 2022). The animals were pinned through the front and rear paws while lying supine on the dissecting board. Using bone forceps, the animals' skulls were dissected, and each organ was meticulously removed and weighed. In order to examine changes in cellular morphology, the tissue

for histological studies were fixed in freshly made formal saline for 72 hours before being processed for routine histological examination and stained with haematoxylin and eosin (H&E) (Golberg et al., 2024). Also, phosphotungstic acid-haematoxylin (PTAH) technique (Golberg et al., 2024), Cresyl Violet (CFV) staining (Bolon et al., 2025), glial fibrillary acidic protein (GFAP) immunohistochemistry (Luijckx et al., 2024) and Masson's trichrome (MT) stain (Golberg et al., 2024) were employed.

### **Determination of Oxidative Stress Markers, Phosphatase and Glucose-Metabolising Enzymes, and Inflammatory/Apoptotic Markers**

Immediately after dissection, the required brain tissue was rinsed free of blood in ice-cold normal saline and weighed. A 10% (w/v) tissue homogenate was then prepared by homogenising the tissue in ice-cold 0.1 M phosphate buffer (pH 7.4) using a mechanical tissue homogeniser, keeping the sample on ice throughout to minimise enzyme degradation and oxidative artefact. The resulting homogenate was centrifuged at  $10,000 \times g$  for 15 minutes at 4°C, and the clear supernatant obtained was collected and used for the biochemical estimation of carbohydrate metabolism enzymes, acid phosphatase, alkaline phosphatase, and caspase-3 (Elfakharany et al., 2024; Rajkumar et al., 2022).

Malondialdehyde (MDA) levels were determined based on the reaction of MDA with thiobarbituric acid (TBA) under acidic conditions to form a pink chromogen, measured spectrophotometrically, reflecting the extent of lipid peroxidation in brain tissue homogenate (Chen et al., 2025a). Superoxide dismutase (SOD) activity was assessed by its ability to inhibit the autoxidation of the substrate under alkaline conditions, generating a colour change measured spectrophotometrically and expressed as units of enzyme activity per milligram of protein (Chen et al., 2025b). Reduced glutathione (GSH) levels were determined by the reaction of free sulfhydryl groups with a colorimetric reagent to yield a yellow-coloured product measured spectrophotometrically (Chen et al., 2025b). Catalase (CAT) activity was measured based on the enzyme's ability to decompose hydrogen peroxide, with the rate of decomposition monitored spectrophotometrically (Chen et al., 2025b).

Alkaline phosphatase (ALP) and acid phosphatase (ACP) activities were determined using a kinetic colorimetric method based on the hydrolysis of p-nitrophenyl phosphate to p-nitrophenol, a yellow-coloured product whose rate of formation was measured spectrophotometrically under

alkaline (ALP) or acidic (ACP) conditions (Chen et al., 2025). Glucose-6-phosphate dehydrogenase (G6PDH) activity was assayed by monitoring the rate of NADPH generation from NADP<sup>+</sup> during the oxidation of glucose-6-phosphate, measured spectrophotometrically at 340 nm (Dawi et al., 2024a). Lactate dehydrogenase (LDH) activity was determined by measuring the rate of NADH oxidation during the conversion of pyruvate to lactate, monitored spectrophotometrically at 340 nm (Dawi et al., 2024).

Caspase-3, interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF- $\alpha$ ) levels in brain tissue homogenate were quantified using commercially available rat-specific sandwich enzyme-linked immunosorbent assay (ELISA) kits, according to the manufacturer's instructions. Briefly, standards and samples were added to microtiter plates pre-coated with the relevant capture antibody, followed by incubation with a biotinylated detection antibody and a horseradish peroxidase-streptavidin conjugate; the enzymatic reaction was visualized using a tetramethylbenzidine (TMB) substrate, and absorbance was read at 450 nm and referenced against a standard curve to calculate analyte concentration (Chen et al., 2025).

### **Photomicrography**

Photomicrography of histological sections of the Frontal cortices was taken with an Olympus Microscope (New York, United State of America) coupled with camera at Department of Human Anatomy, Mulungushi University School of Medicine and Health Sciences, Livingstone Campus, Zambia.

### **Data analysis plan**

Data was presented as mean  $\pm$  standard error of the mean (mean $\pm$ SEM); analysed using one way ANOVA. All graphs will be drawn using Excel (Microsoft Corporation, U.S.A). P values less than 0.05 ( $p < 0.05$ ) will be taken to be statistically significant.

### **Ethical considerations**

Ethical and regulatory approval was obtained from Mulungushi University School of Medicine and Health Sciences Research Ethics and the National Health Research Authority with approval number: NHRA-1832/27/12/2024.

## Results

### Median lethal dose (LD<sub>50</sub>)

The acute toxicity test results for watermelon seed extract (Table 2) revealed that administering lower doses of 100, 400, and 1000 mg/kg of these extracts did not produce any signs of toxicity in the animals. Higher doses of 2000, 3000, and 4000 mg/kg BW showed no toxicity symptoms, and no fatalities were observed.

**Table 2 Median lethal dose (LD<sub>50</sub>) for watermelon seed extract**

| Dosage (mg/kg BW) | Number of animals | Mortality |
|-------------------|-------------------|-----------|
| Phase one         |                   |           |
| 100               | 3                 | 0/3       |
| 400               | 3                 | 0/3       |
| 1000              | 3                 | 0/3       |
| Phase two         |                   |           |
| 2000              | 3                 | 0/3       |
| 3000              | 3                 | 0/3       |
| 4000              | 3                 | 0/3       |

### Qualitative Analysis of Phytochemicals of Water Melon Seed

The quantitative analysis revealed presence tannins, phenols, alkaloids, flavonoids, steroids, terpenoids, cardiac glycosides, and reducing sugars

**Table 3 Qualitative analysis of watermelon seeds**

| Phytochemical | Presence of phytochemicals |
|---------------|----------------------------|
| Tannin        | +                          |
| Phenol        | +                          |
| Phlobatannin  | —                          |
| Alkaloid      | +                          |
| Saponin       | —                          |
| Flavonoid     | +                          |
| Steroid       | +                          |
| Anthraquinone | —                          |
| Terpenoid     | +                          |

| Phytochemical      | Presence of phytochemicals |
|--------------------|----------------------------|
| Cardiac glycosides | +                          |
| Reducing sugar     | +                          |

### Quantitative Analysis of Phytochemicals of watermelon seeds

The quantitative phytochemical analysis of watermelon seeds showed tannins as the most abundant constituent (45 mg/100 g), followed closely by phenols (43 mg/100 g), alkaloids (37 mg/100 g), reducing sugars (30 mg/100 g), flavonoids (20 mg/100 g), and cardiac glycosides (12 mg/100 g).

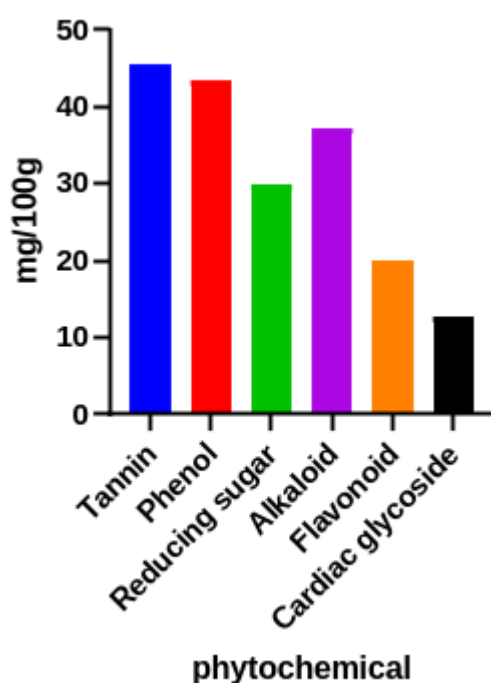


**Fig. 1 Showing** quantitative phytochemical analysis of watermelon seeds.

### In vitro biological activity results

Figure 1 illustrates the quantitative assessment of the flavonoid and total phenolic content of watermelon seed. Ferric Reducing Antioxidant Power (FRAP) demonstrates that watermelon seed extract had significant ferric-reducing activity at all doses, however it was still less than ascorbic acid. In Figure 2, watermelon seed extract showed potent 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, at higher doses it was still less than that of ascorbic acid. The nitric oxide (NO) scavenging action of watermelon seed is also displayed in Figure 3, and at greater doses, it was less than that of ascorbic acid. Figure 4 shows the hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)

scavenging activity in watermelon seed, which was good but still less the activity of ascorbic acid at greater doses. Finally, watermelon seed extract showed significant protection against lipid oxidation (fig 5).

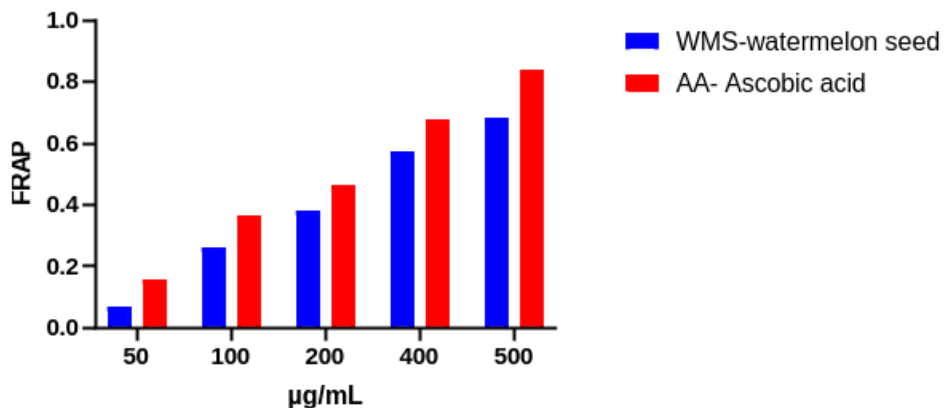


**Fig. 2 Graph** showing the effect of watermelon seed extract, compared with ascorbic acid, at various concentrations on ferric reducing antioxidant power.

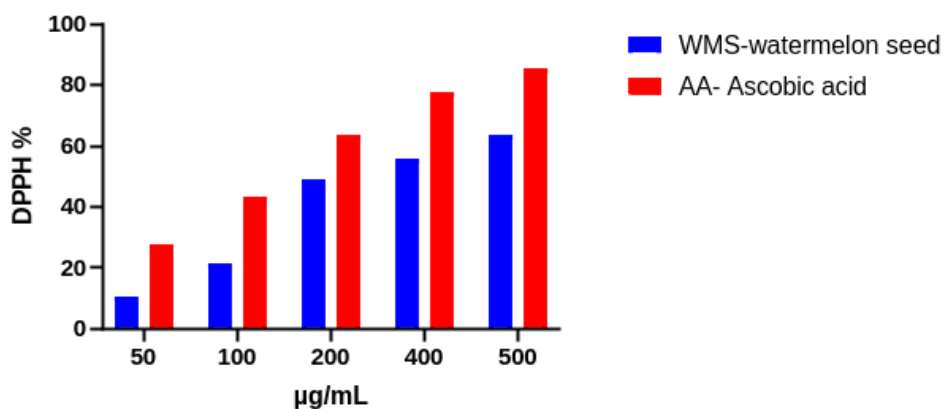


**Fig. 3 Graph** showing the effect of watermelon seed extract, compared with ascorbic acid, at various concentrations on inhibiting DPPH.

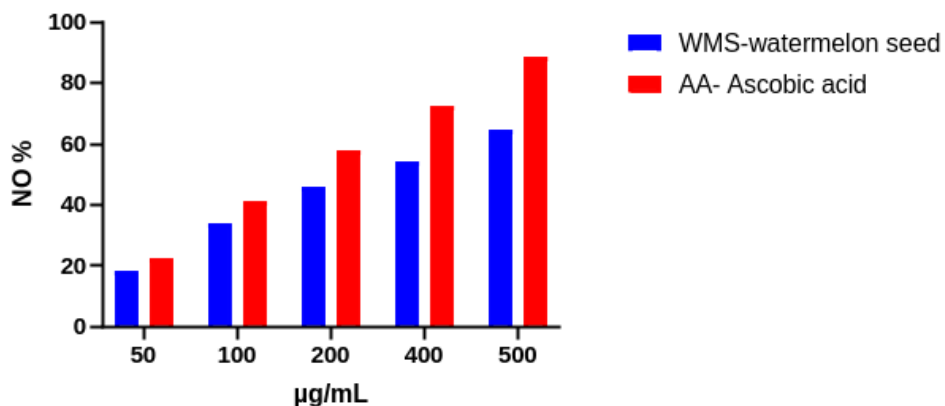


**Fig. 4 Graph** showing the effect of watermelon seed extract, compared with ascorbic acid, at various concentrations on inhibiting NO%.

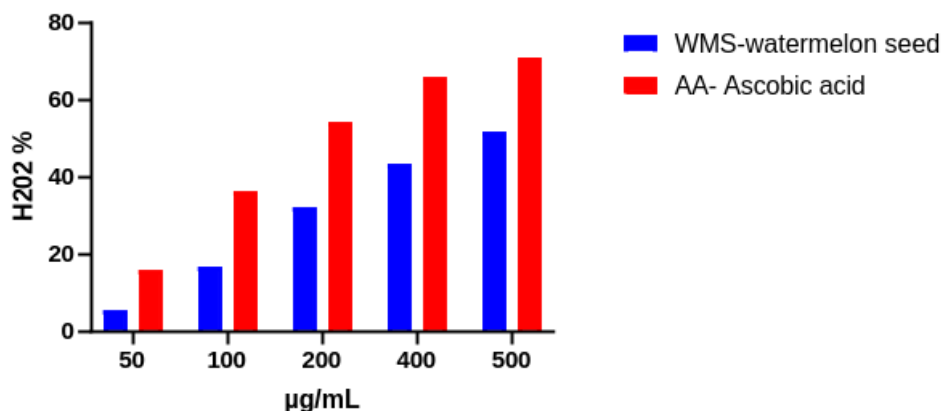


**Fig. 5 Graph** showing the effect of watermelon seed extract, compared with ascorbic acid, at various concentrations on inhibiting H2O2%.

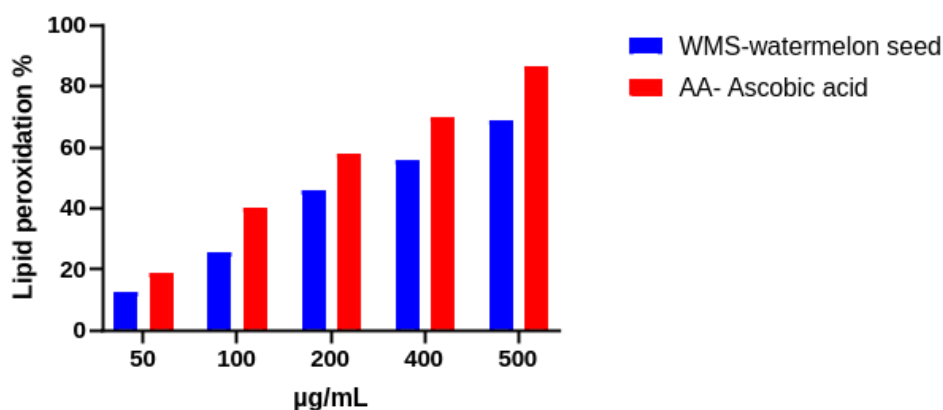


**Fig. 6 Graph** showing the effect of watermelon seed extract, compared with ascorbic acid, at various concentrations on inhibiting lipid peroxidation%.

### Phytochemical constituents in watermelon seed

GC-MS analysis of the watermelon seed extract identified a wide range of phytochemical compounds with different retention times and concentrations. The predominant constituent detected was Octadecanoic acid, ethyl ester with a remarkably high peak area percentage of 61.35% at a retention time of 13.931 min, indicating that it was the major phytochemical present in the extract. Other notable compounds included n-Hexadecanoic acid (14.11%), 5-Hydroxymethylfurfural (3.91%), and Naphthalene derivatives (3.3%). Additional constituents identified were Caryophyllene, Methyl salicylate, Cinnamaldehyde, Tumerone, Neointermedeol, Tetradecanoic acid, Pentadecanoic acid, Ethyl oleate, and naturally occurring sugars such as D-Allose and  $\beta$ -D-Glucopyranose.

**Table 4 Results of GC-MS analysis of watermelon seed extract**

| Retention time (RT) | Area (%) | Compound detected                                                                                      |
|---------------------|----------|--------------------------------------------------------------------------------------------------------|
| 5.240               | 1.9      | Caryophyllene, Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-                               |
| 6.242               | 0.11     | Humulene, 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-                                        |
| 6.283               | 0.05     | 2-Furanmethanol, Methylene cyclopropanecarboxylic acid                                                 |
| 6.719               | 3.3      | Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)] |
| 7.357               | 0.30     | Methyl salicylate                                                                                      |
| 8.286               | 0.24     | 5-Octadecene, (E)-, 1-Octadecene, Bromoacetic acid, tetradecyl ester                                   |
| 9.479               | 0.21     | 1,4-Benzenediol, 2-methyl-, Benzaldehyde, 4-fluoro-, 1,4-Benzenediol, 2-methyl-                        |
| 9.739               | 0.13     | 3-Furancarboxylic acid, methyl ester, Cyclohexanol, 2-methylene-6-methyl                               |
| 10.034              | 0.39     | Cinnamaldehyde, (E)-, 2-Propenal, 3-phenyl-, (Z)-3-Phenylacrylaldehyde                                 |
| 10.226              | 0.24     | 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, Nerolidol                                                  |
| 10.294              | 0.14     | Cyclotetradecane, 9-Eicosene, (E)-, 5-Eicosene, (E)-                                                   |
| 11.254              | 0.28     | Thymine                                                                                                |
| 11.399              | 0.30     | Tumerone, (Z)- $\gamma$ -Atlantone                                                                     |
| 11.555              | 0.36     | 2-Methoxy-4-vinylphenol, 3-Methoxyacetophenone, 4-Hydroxy-3-methylacetophenone                         |
| 11.840              | 0.17     | Hexadecanoic acid, methyl ester, Pentadecanoic acid, 14-methyl-, methyl ester                          |
| 11.949              | 0.14     | Curlone, 2-Methyl-6-(4-methylenecyclohex-2-en-1-yl)hept-2-en-4-one                                     |
| 12.084              | 0.63     | Neointermedeol                                                                                         |
| 12.188              | 0.36     | Hexadecanoic acid, ethyl ester, Octadecanoic acid, ethyl ester                                         |
| 12.266              | 0.95     | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-                                                    |
| 12.369              | 0.18     | 2-Propen-1-ol, 3-phenyl-                                                                               |
| 12.691              | 0.32     | 2,4-Di-tert-butylphenol                                                                                |
| 13.288              | 0.33     | Benzofuran, 2,3-dihydro-, N-Benzyl-2-phenethylamine, 1,2-Benzenedimethanol                             |
| 13.931              | 61.35    | Octadecanoic acid, ethyl ester, Undecanoic acid, ethyl ester                                           |
| 14.076              | 0.28     | Ethyl oleate                                                                                           |
| 14.274              | 3.91     | 5-Hydroxymethylfurfural                                                                                |
| 15.783              | 0.13     | Tetradecanoic acid                                                                                     |
| 16.567              | 0.11     | Pentadecanoic acid                                                                                     |
| 17.506              | 14.11    | n-Hexadecanoic acid                                                                                    |
| 17.636              | 0.28     | Z-10-Tetradecen-1-ol acetate                                                                           |
| 17.973              | 0.16     | Hexanoic acid, 4-methyl-                                                                               |

| Retention time (RT) | Area (%) | Compound detected                                                        |
|---------------------|----------|--------------------------------------------------------------------------|
| 18.072              | 0.11     | Heptadecanoic acid                                                       |
| 20.562              | 0.47     | Cyclooctene, 3-ethenyl-, 1-Hexadecyne, E,E-6,11-Tridecadien-1-ol acetate |
| 21.050              | 0.26     | D-Allose, $\beta$ -D-Glucopyranose, 1,6-anhydro-                         |

### **Effect of an ethanolic extract of Watermelon seed extract on the average body weight (g)**

Weekly changes in body weight in Wistar rats after diabetes mellitus induction are depicted in Figure 1. During acclimatization and diabetes induction (weeks -2 to 0), the body weights of all groups were similar. Compared to the NC group, the DC group showed significant and progressive body weight reduction following diabetes induction from week 1 ( $p < 0.05$ ). In comparison to the DC group, the DW and DM maintained body weights that were significantly higher ( $p < 0.05$ ).

### **Effect of an ethanolic extract of Watermelon seed extract on relative brain weight (%)**

The impact of an ethanolic extract of watermelon seeds on relative brain weight is shown in Figure 3. When compared to the NC group, the DC group's relative brain weight was considerably lower ( $P < 0.05$ ). The relative brain weight of the treated groups DW and DM was greater than that of the DC group ( $P < 0.05$ ). There was no discernible difference between the extract-only groups (WSO) and the NC ( $P > 0.05$ ).

### **Effect of an ethanolic extract of Watermelon seed extract on average blood glucose level (mg/dl)**

The long-term impact of an ethanolic extract of watermelon seeds on average blood glucose levels is depicted in Figure 2. After diabetes induction, blood glucose levels were considerably higher in the DC group than in the NC group ( $P < 0.05$ ). Over time, blood glucose levels were significantly lower in the treated groups DW and DM ( $P < 0.05$  versus DC), returning to nearly normal levels by week 6 ( $P > 0.05$  vs NC). The extract-only group (WSO) did not significantly differ from the NC group ( $P > 0.05$ ).

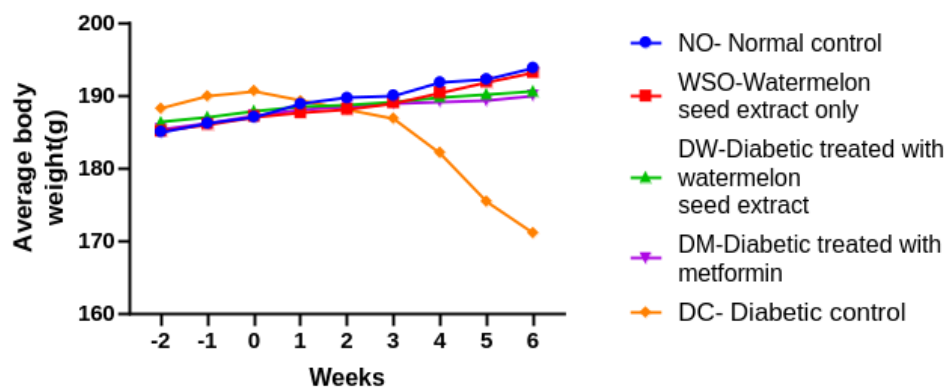


**Fig. 7** Effect of an ethanolic extract of watermelon seed on weekly average body weight (g). Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

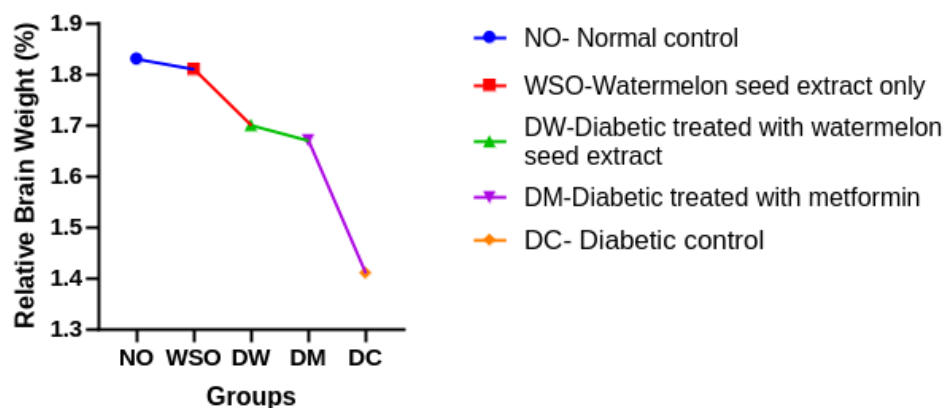


**Fig. 8** Effect of an aqueous extract of watermelon seed on relative brain weight (%). Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

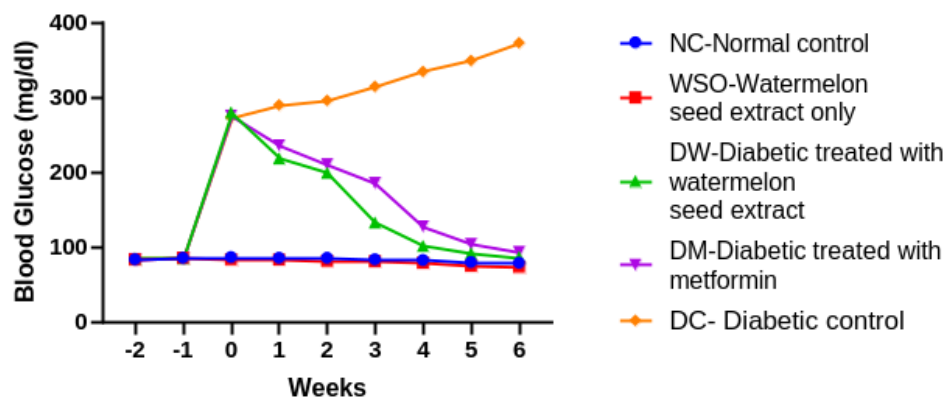


**Fig. 9** Effect of an ethanolic extract of watermelon seed on weekly blood glucose level (mg/dl). Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

### **Anxiety/exploratory and locomotor activities**

Figure 4 demonstrates that all groups showed comparable line-crossing activity throughout acclimatization ( $P > 0.05$ ). The DC group's locomotor activity significantly decreased after diabetes induction ( $P < 0.05$  vs. NC). When compared to DC ( $P < 0.05$ ) and NC ( $P > 0.05$ ), the treated groups DW and DM significantly increased line crossing.

Weekly variations in center-square entries are seen in Figure 5. All groups displayed similar center-square entries throughout acclimatization ( $P > 0.05$ ). The DC group showed a significant reduction ( $P < 0.05$  vs NC) following diabetes induction. When compared to DC, treated groups DW and DM significantly improved center-square entries ( $P < 0.05$ ), while extract-treated groups showed values near NC ( $P > 0.05$ ).

Figure 6 illustrates how the rats' hindings alter every week. The graph demonstrates that all groups displayed comparable hinding frequencies throughout acclimatization ( $P > 0.05$ ). The DC group's hinding behavior significantly decreased during diabetes induction ( $P < 0.05$  vs. NC). Hinding frequency was substantially higher in treated groups DW and DM than in DC ( $P < 0.05$ ), while extract-only groups displayed values near NC ( $P > 0.05$ ).

Variations in walling frequency for each group are shown in Figure 7. All groups displayed comparable walling frequencies throughout acclimatization, with no significant variations ( $P > 0.05$ ). Walling activity significantly increased in the DC group following diabetes induction ( $P < 0.05$  vs. NC). Walling frequency was considerably lower in treatment groups DW and DM than in DC ( $P < 0.05$ ), with extract-treated groups showing values near NC ( $P > 0.05$ ).

Figure 8 shows the weekly differences in micturition frequency for each of the study's groups. All groups displayed comparable micturition frequencies throughout acclimatization ( $P > 0.05$ ). The DC group showed a substantial increase in micturition during diabetes induction ( $P < 0.05$  vs NC). When compared to DC, treatment groups DW and DM significantly decreased the frequency of micturition ( $P < 0.05$ ), while extract-only-treated groups displayed values near NC ( $P > 0.05$ ).

Weekly variations in defecation between the research groups are seen in Figure 9. All groups displayed comparable defecation frequencies throughout acclimatization ( $P > 0.05$ ). The DC group showed a substantial increase in defecation after diabetes induction ( $P < 0.05$  vs NC). Defecation

frequency was considerably lower in treated groups DW and DM than in DC ( $P < 0.05$ ), while extract-only-treated groups had values near NC ( $P > 0.05$ ).

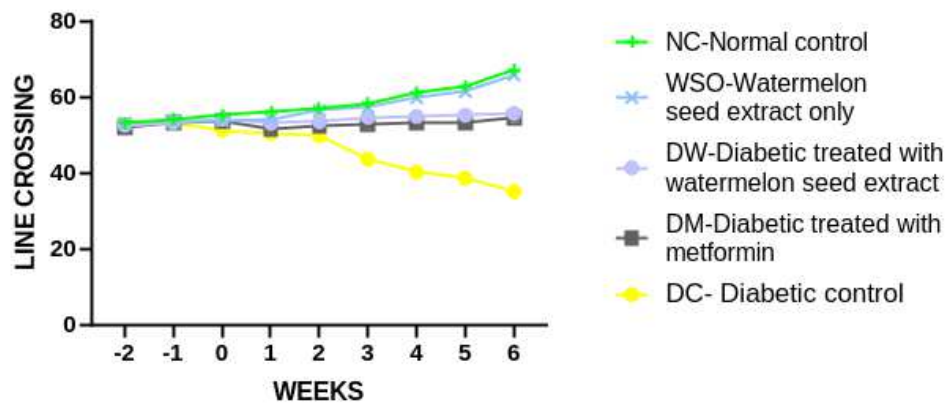


Fig. 10 Number of lines crossed per trial. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

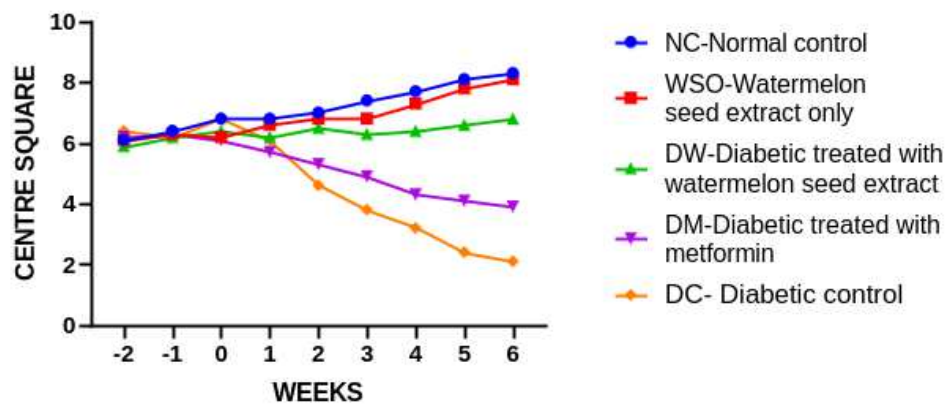


Fig. 11 Number of entrances in the central square. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

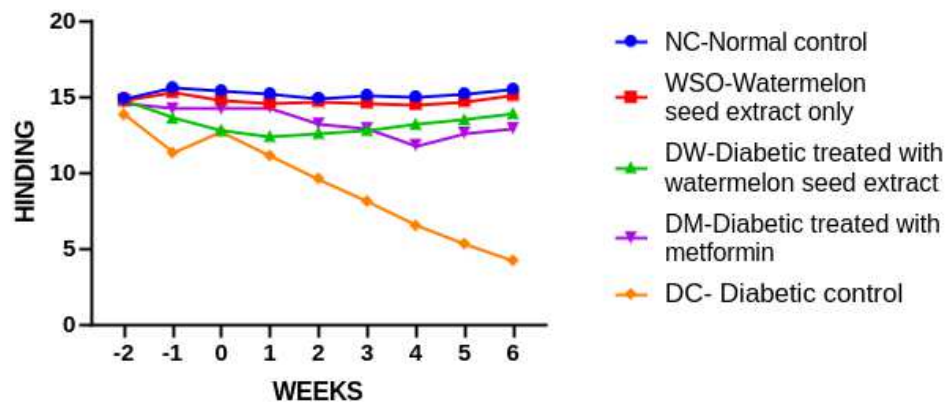


Fig. 12 Number of hindings. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

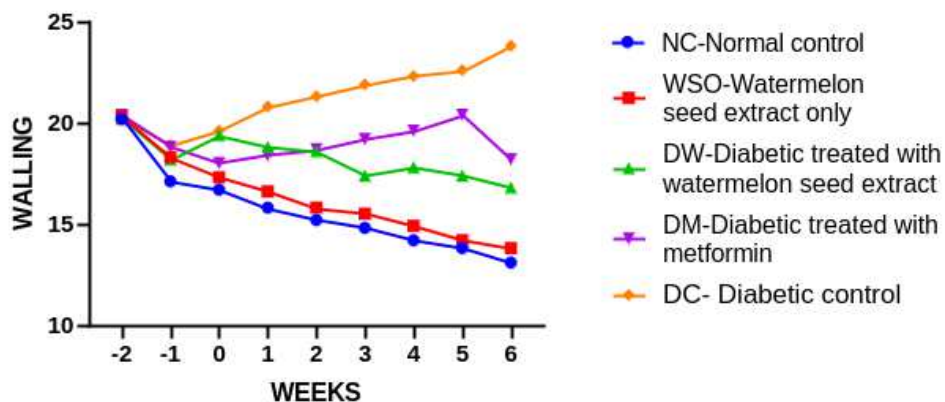


Fig. 13 Number of walling. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

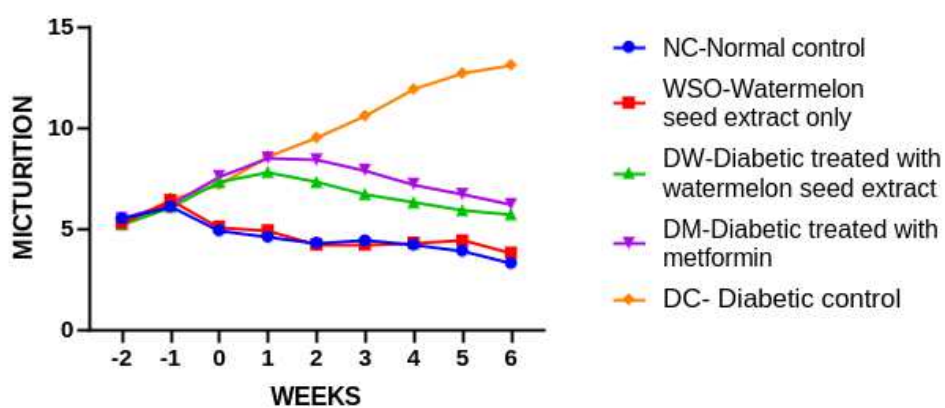


Fig. 14 Number of micturition. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

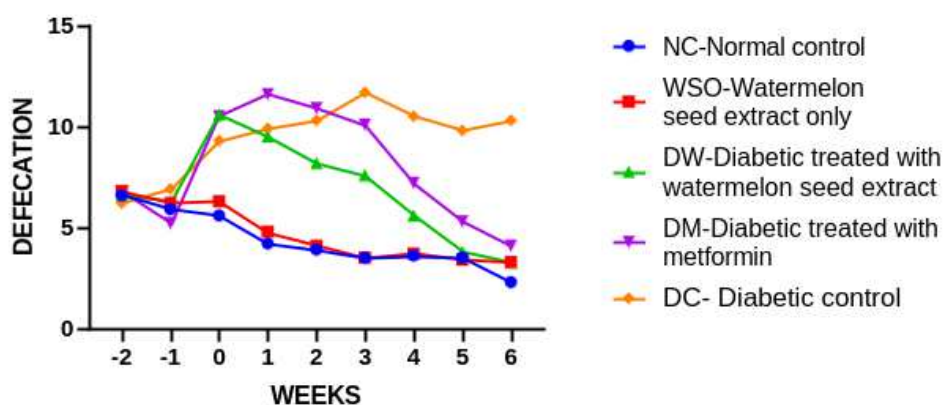


Fig. 15 Number of pellets. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

## **Histology results**

### ***Hematoxylin and Eosin (H&E) stain***

In figure 10, the normal control and water melon seed only groups showed pyramidal cells that appeared normal. These cells were densely populated, and the histoarchitecture of the frontal cortices remained intact (fig.10: NC and WSO). In the diabetic+ watermelon seed and diabetic+ metformin groups, some cells were necrotic while others appeared normal (fig. 10: DIA + WS and DIA + MET). The diabetic group predominantly had necrotic cells, with few normal cells remaining (fig. 10: DC).

### ***Phosphotungstic acid haematoxylin (PTAH) stain***

Figure 11 showed that the rats in the control and water melon seed only groups exhibited a normal distribution of astrocytes, with typical histoarchitecture observed (fig. 11: NC and WSO). In contrast, the diabetic groups treated with watermelon seed or metformin showed some neurons that were degenerated, while others appeared normal with astrocytes (fig. 11: DIA+WS and DIA+MET). Additionally, the diabetic group alone had astrocytes alongside degenerated neurons (fig. 11: DC).

### ***Cresyl Fast Violet (CFV) stain***

In figure 12, the control and water melon seed only groups exhibited a normal distribution of astrocytes with typical histoarchitecture (fig.12: NC and WSO). In contrast, the diabetic groups treated with watermelon seed or metformin showed some neurons degenerating while others appeared normal with astrocytes (fig.12: DIA+WS and DIA+MET). The diabetic group also demonstrated significant astrocyte degeneration (fig 12: DC).

### ***Glia Fibrillary Acidic Protein (GFAP) stain***

Under figure 13 control and water melon seed only groups showed intact frontal cortical structures with little GFAP immunoreactivity (fig. 13: NC and WSO). Conversely, diabetic groups treated with watermelon seed or metformin maintained much of their neuronal architecture with only limited astrocytic activation (fig. 13: DIA+WS and DIA+MET). Additionally, the diabetic group exhibited increased GFAP immunoreactivity (fig. 13: DC).

### ***Masson's Trichrome (MT) stain***

In figure 14 the rats in the control and watermelon seed only groups showed no abnormal interstitial collagen and maintained normal histoarchitecture (fig. 14: NC and WSO). In contrast, the diabetic+ watermelon seed displayed significantly increased collagen deposition compared to the normal control (fig.14: DIA+WS). Moreover, the diabetic+ metformin and diabetic groups exhibited extensive collagen buildup (fig. 14: DIA+MET and DC).

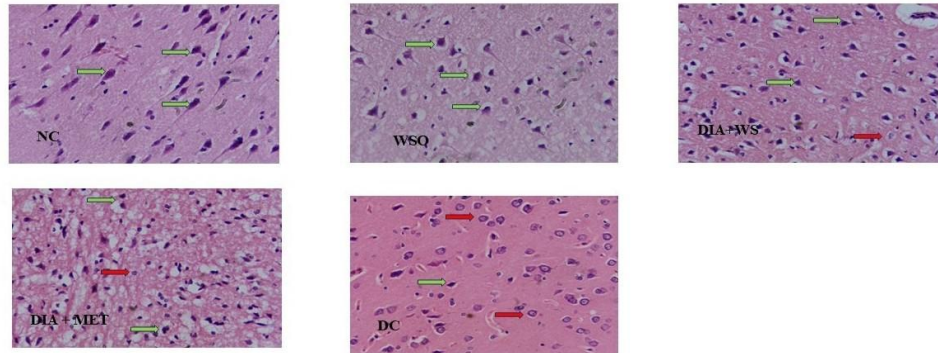


**Fig. 16** Photomicrograph showing the frontal cortex at week 6. NC- Normal control, WSO- Watermelon seeds only, DIA+WS- Diabetic+watermelon seed, DIA+MET- Diabetic+metformin and DC- Diabetic control. H&E stain X400. Green arrow = Normal pyramidal neuron; Red arrow = Necrotic cell.

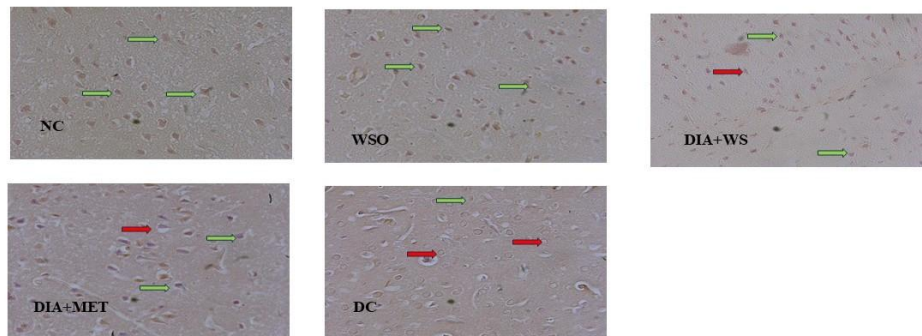


**Fig. 17** Photomicrograph showing the frontal cortex at week 6. NC- Normal control, WSO- Watermelon seeds only, DIA+WS- Diabetic+watermelon seed, DIA+MET- Diabetic+metformin and DC- Diabetic control. PTAH stain X400. Green arrow = Normal astrocyte; Red arrow = Necrotic cell.

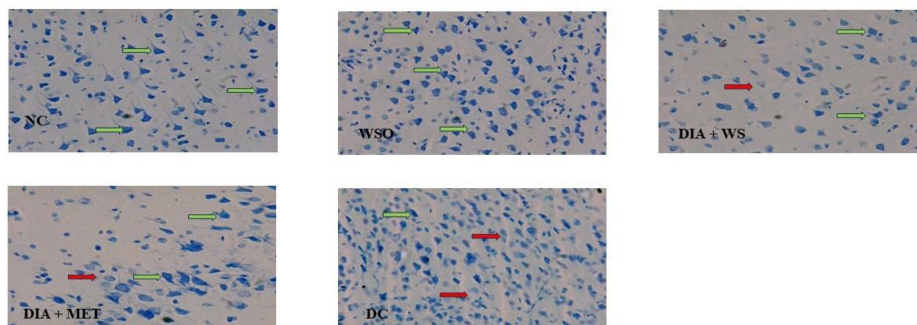


**Fig. 18** Photomicrograph showing the frontal cortex at week 6. NC- Normal control, WSO- Watermelon seeds only, DIA+WS- Diabetic+watermelon seed, DIA+MET- Diabetic+metformin and DC- Diabetic control. CFV stain X400. Green arrow = Normal astrocyte; Red arrow = Necrotic cell.

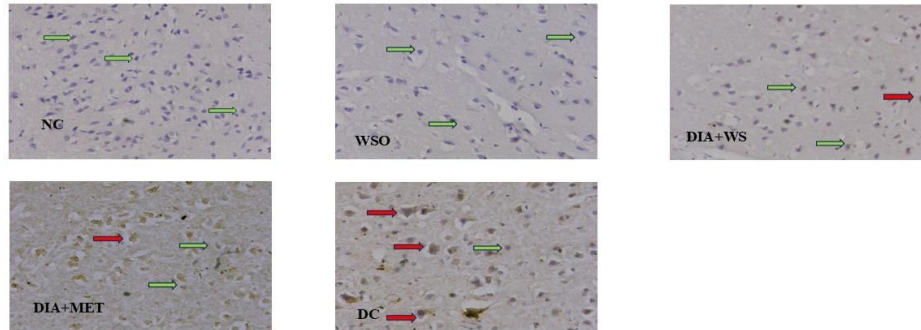


**Fig. 19** Photomicrograph showing the frontal cortex at week 6. NC- Normal control, WSO- Watermelon seeds only, DIA+WS- Diabetic+watermelon seed, DIA+MET- Diabetic+metformin and DC- Diabetic control. GFAP stain X400. Green arrow = Normal astrocyte; Red arrow = Necrotic cell.

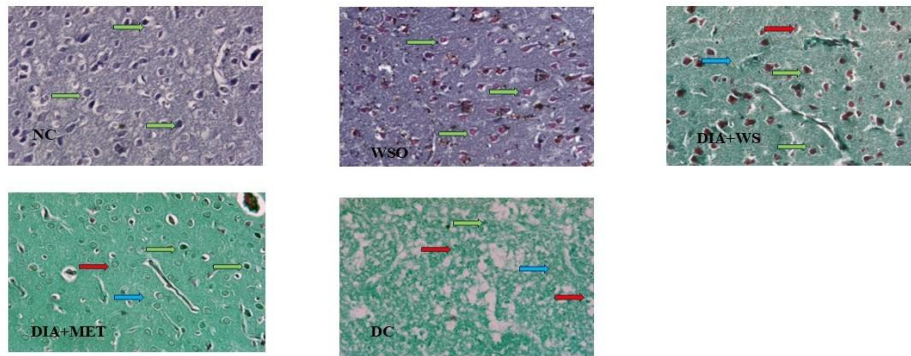


**Fig. 20** Photomicrograph showing the frontal cortex at week 6. NC- Normal control, WSO- Watermelon seeds only, DIA+WS- Diabetic+watermelon seed, DIA+MET- Diabetic+metformin and DC- Diabetic control. MT stain X400. Green arrow = Normal astrocyte; Red arrow = Necrotic cell; Blue arrow = Collagen deposition.

## Oxidative stress markers

Malondialdehyde (MDA) levels were significantly elevated (fig. 15) in the DC group compared with NC ( $P < 0.05$ ). Treatment groups DW and DM significantly reduced MDA levels compared with DC ( $P < 0.05$ ). The extract-only group (WSO) showed no significant difference from NC ( $P > 0.05$ ).

A significant reduction in superoxide dismutase (SOD) activity and levels was seen in the DC group (fig. 16) compared with the NC ( $P < 0.05$ ). Treatment groups DW and DM significantly increased SOD levels compared with DC ( $P < 0.05$ ). The extract-only groups (WSO) showed no significant difference from NC ( $P > 0.05$ ).

Glutathione (GSH) levels were significantly reduced in the DC group (fig. 17) compared with NC ( $P < 0.05$ ). Treated groups DW and DM significantly increased GSH levels compared with DC ( $P < 0.05$ ). The extract-only groups (WSO) showed no significant difference from NC ( $P > 0.05$ ).

Catalase (CAT) activity was significantly reduced in the DC group (fig. 18) compared with NC ( $P < 0.05$ ). Treated groups DW and DM significantly increased CAT levels compared with DC ( $P < 0.05$ ). The extract-only group (WSO) showed no significant difference from NC ( $P > 0.05$ ).

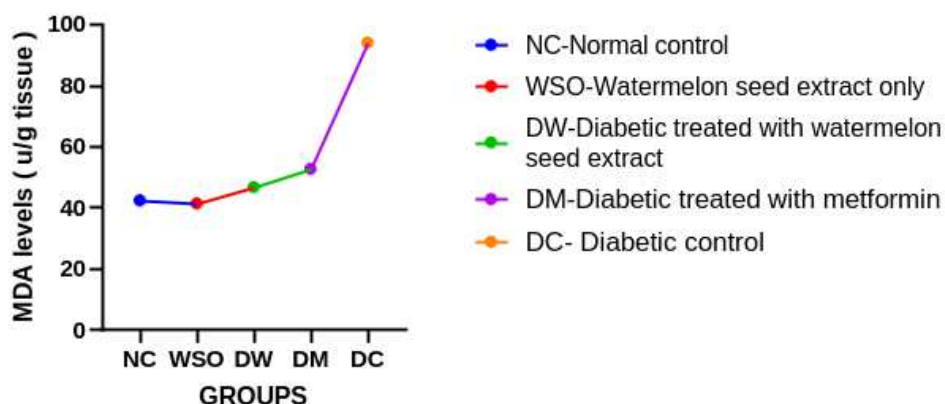


**Fig. 21** Effect of an ethanolic extract of watermelon seed on MDA levels. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

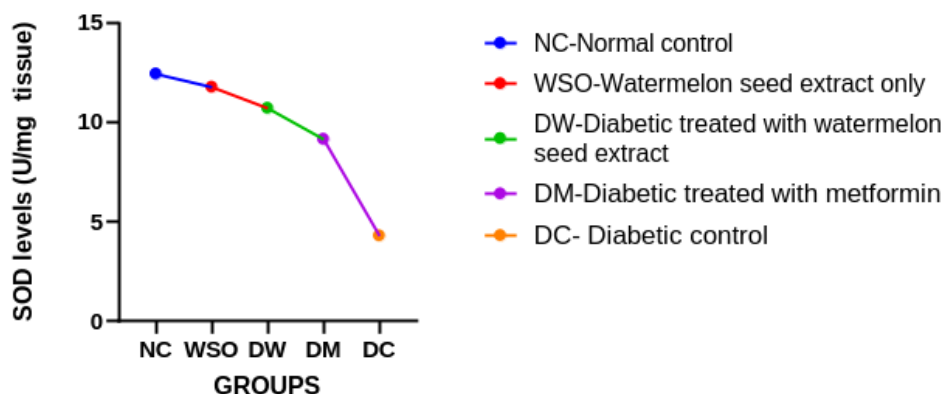


**Fig. 22** Effect of an ethanolic extract of watermelon seed on SOD levels. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

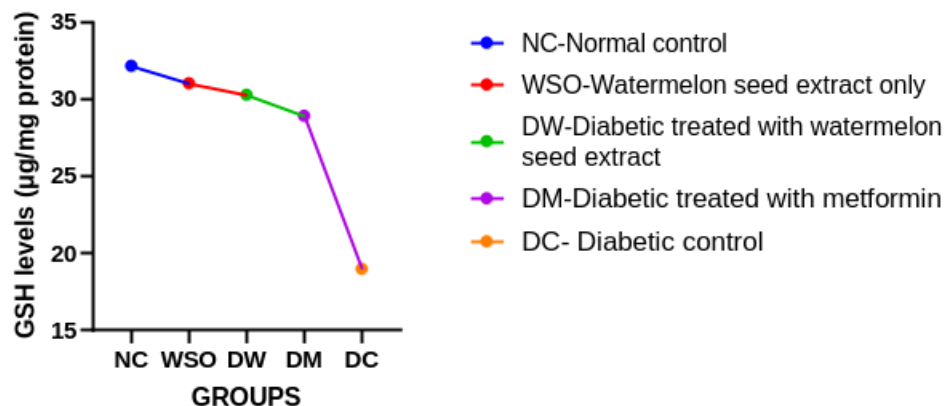


**Fig. 23** Effect of an ethanolic extract of watermelon seed on GSH levels. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

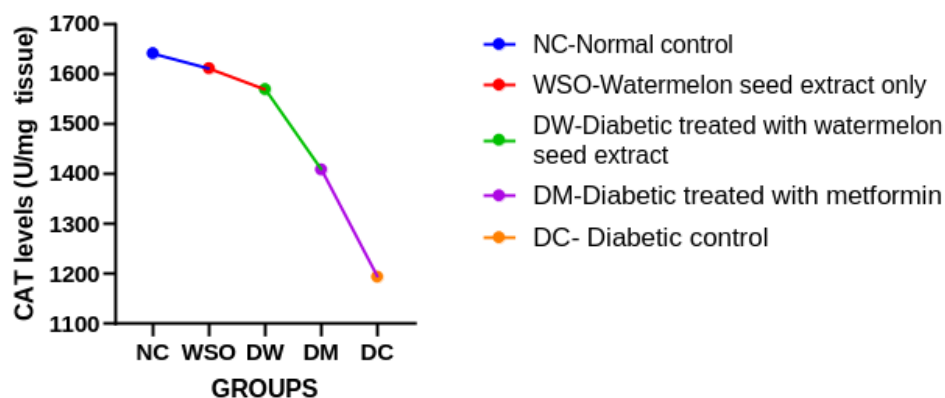


**Fig. 24** Effect of an ethanolic extract of watermelon seed on CAT levels. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

## Enzymes of glucose metabolism results

Alkaline phosphatase (ALP) levels were significantly elevated (fig. 19) in the DC group compared with the NC ( $P < 0.05$ ). Treated groups DW and DM significantly reduced ALP levels compared with DC ( $P < 0.05$ ). The extract-only groups (WSO) showed no significant difference from NC ( $P > 0.05$ ).

Acid phosphatase (ACP) levels (fig. 20) were significantly elevated in the DC group compared with NC ( $P < 0.05$ ). Treated groups DW and DM significantly reduced ACP levels compared with DC ( $P < 0.05$ ). The extract-only groups (WSO) showed no significant difference from NC ( $P > 0.05$ ).

The levels of Glucose-6-phosphate dehydrogenase (G6PDH) (fig. 21) were significantly lower in the DC group than in NC ( $P < 0.05$ ). Treated groups DW and DM significantly increased G6PDH

levels compared with DC ( $P < 0.05$ ). The extract-only group (WSO) showed no significant difference from NC ( $P > 0.05$ ).

The effects of watermelon seeds ethanolic extract on lactate dehydrogenase (LDH) levels (fig. 22) were significantly higher in the DC group than in the NC ( $P < 0.05$ ). Treated groups DW and DM significantly reduced LDH levels compared with DC ( $P < 0.05$ ). The extract-only group (WSO) showed no significant difference from NC ( $P > 0.05$ ).

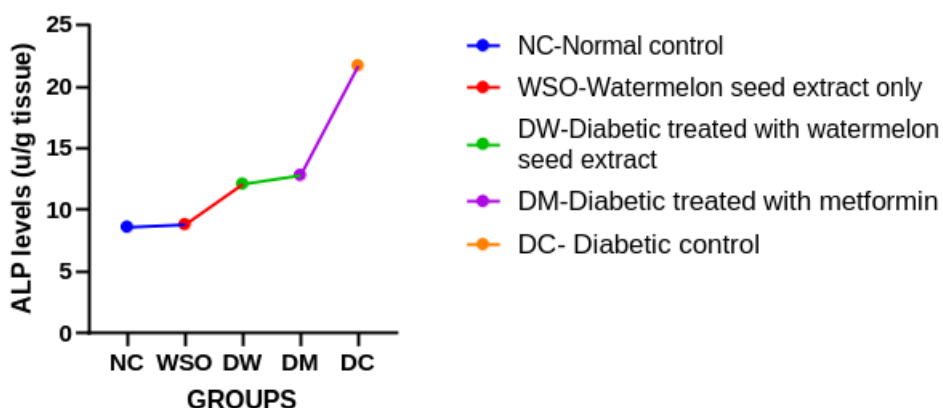


**Fig. 25** Effect of an ethanolic extract of watermelon seed on ALP levels. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

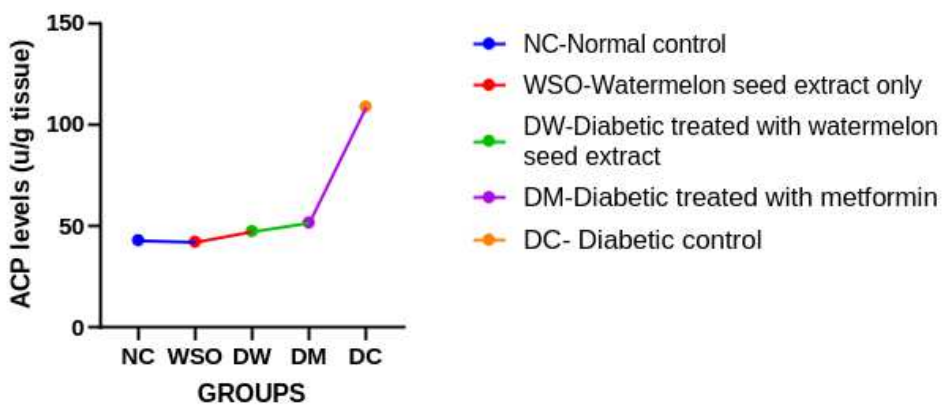


**Fig. 26** Effect of an ethanolic extract of watermelon seed on ACP levels. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

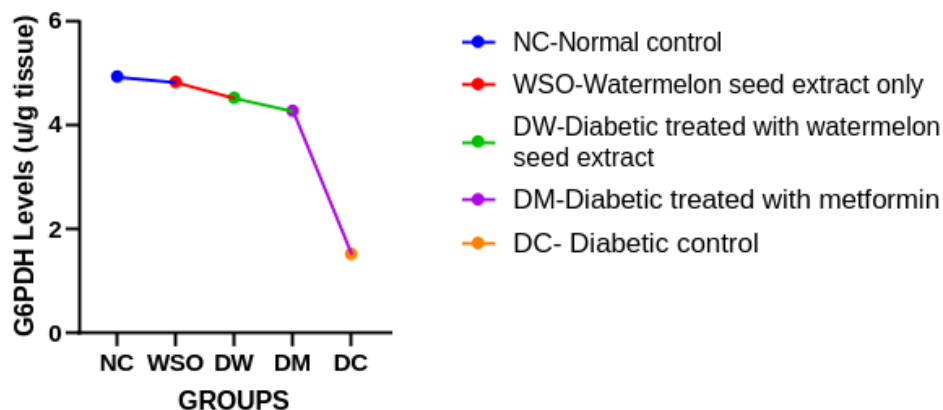


**Fig. 27** Effect of an ethanolic extract of watermelon seed on G6PDH levels. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

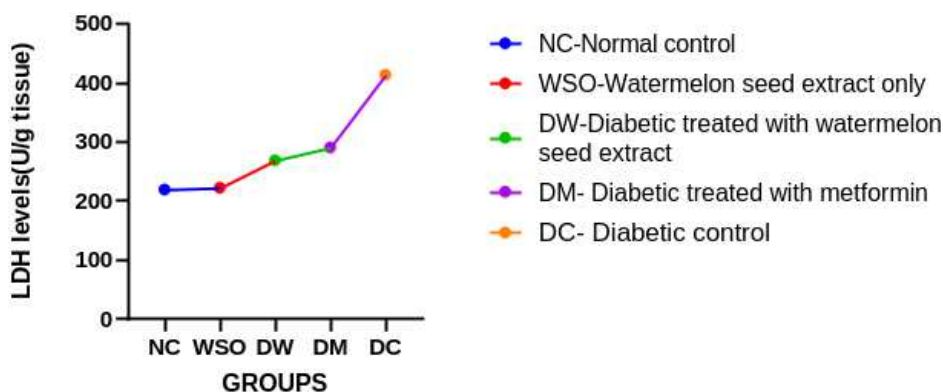


**Fig. 28** Effect of an ethanolic extract of watermelon seed on LDH levels. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

### Anti-inflammatory markers

Caspase-3 levels were significantly higher in the DC group (fig. 23) than in the NC group ( $P < 0.05$ ). Treatment with watermelon seed extract and metformin (DW and DM) significantly reduced caspase-3 levels ( $P < 0.05$ ). Watermelon seed extract-only (WSO) group showed no significant difference from the NC group

IL-6 levels were significantly higher in the DC group (fig. 24) than in the NC group ( $P < 0.05$ ). Treatment with watermelon seeds and metformin (DW and DM) significantly reduced IL-6 levels ( $P < 0.05$ ). Extract-only group (WSO) showed no significant difference from NC.

Tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) levels were significantly higher in the DC group (fig. 25) than in the NC group ( $P < 0.05$ ). Treatment with watermelon seeds and metformin (DW and DM)

significantly reduced TNF- $\alpha$  levels ( $P < 0.05$ ), whereas extract-only group (WSO) did not differ significantly from the NC group ( $P > 0.05$ ).

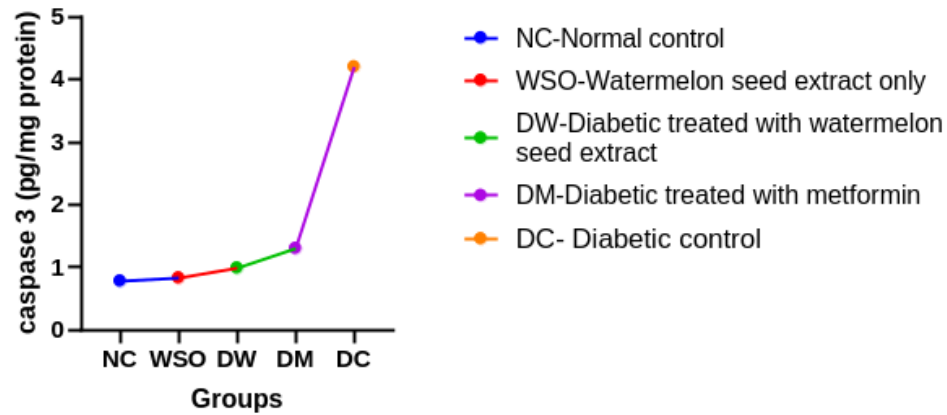


**Fig. 29** Effect of an ethanolic extract of watermelon seeds on Caspase-3. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

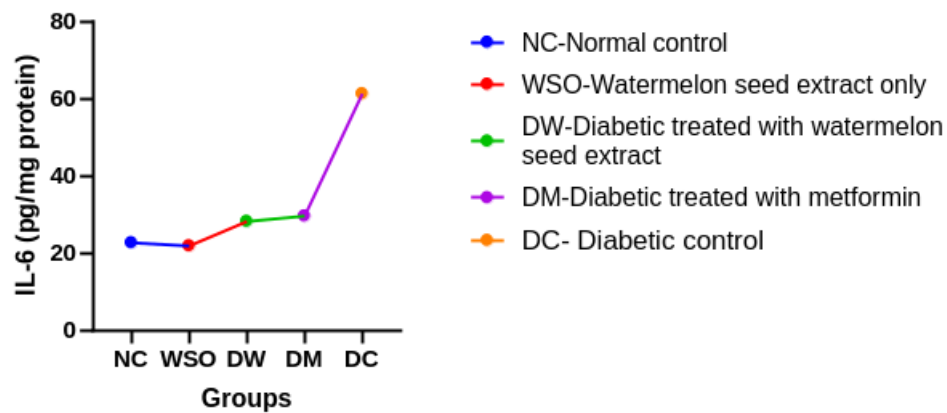


**Fig. 30** Effect of an ethanolic extract of watermelon seeds on IL-6 levels. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

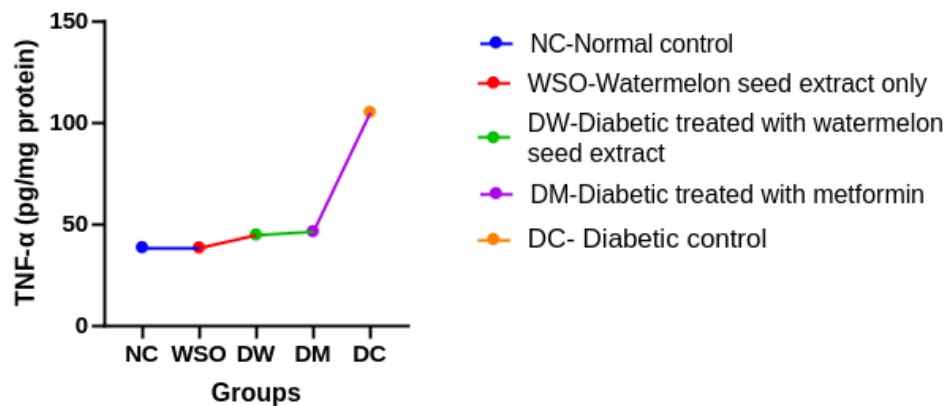


**Fig. 31** Effect of an ethanolic extract of watermelon seeds on TNF- $\alpha$  levels. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ .

## Discussion

The acute oral toxicity study showed no mortality or overt signs of toxicity at any dose tested (100–4000 mg/kg), indicating an LD<sub>50</sub> exceeding 4000 mg/kg body weight. Following the Globally Harmonised System and OECD guidelines (OECD, 2022), this places the extract in the practically non-toxic category. This finding aligns with recent literature reporting wide safety margins (LD<sub>50</sub> >2000–5000 mg/kg) for ethanolic extracts of edible or traditionally consumed plant parts.

Qualitative screening of the ethanolic extract of *Citrullus lanatus* seeds revealed the presence of tannins, phenols, alkaloids, flavonoids, steroids, terpenoids, cardiac glycosides and reducing sugars, whereas phlobatannins, saponins and anthraquinones were absent. This profile aligns with earlier reports identifying these classes as major constituents of the species (Ismael et al., 2022). The absence of saponins and anthraquinones, in contrast to some previous findings, may be attributable to cultivar, geographic or extraction-condition variability. The presence of flavonoids, alkaloids, tannins and terpenoids provides a phytochemical foundation for the extract's antioxidant, toxicological and antidiabetic potential, as these classes have been independently linked to glucose-lowering and cytoprotective mechanisms (Roy et al., 2024; Shamsudin et al., 2022).

Quantification showed tannins as the most abundant constituent (45 mg/100 g), followed by phenols (43 mg/100 g), alkaloids (37 mg/100 g), reducing sugars (30 mg/100 g), flavonoids (20 mg/100 g) and cardiac glycosides (12 mg/100 g). The high abundance of tannins and phenolics is mechanistically significant, as polyphenolic compounds are primary drivers of antioxidant and  $\alpha$ -amylase/ $\alpha$ -glucosidase inhibitory activities, with hydroxylation patterns determining the strength of antidiabetic and anti-inflammatory responses (Shamsudin et al., 2022). The flavonoid content (20 mg/100 g), though comparatively lower, remains biologically meaningful, as even modest concentrations can inhibit carbohydrate-digesting enzymes, enhance insulin secretion and improve peripheral glucose uptake (Shamsudin et al., 2022). Overall, this quantitative distribution mirrors the general pattern reported for *Citrullus lanatus* by-products, where phenolics and flavonoids are principal contributors to antidiabetic and antioxidant potential (Ermawati et al., 2024).

The extract demonstrated dose-dependent radical-scavenging and reducing activity across FRAP, DPPH, nitric oxide, hydrogen peroxide and lipid peroxidation inhibition assays, although potency was lower than that of the ascorbic acid standard. This pattern is appreciable but sub-standard activity relative to a pure reference, is typical of crude plant extracts, where antioxidant capacity is distributed across a mixture of phenolic, flavonoid and terpenoid constituents (Solanki et al., 2023). Consistent activity across mechanistically distinct assays suggests that the antioxidant effect reflects combined hydrogen-donating, metal-chelating and radical-quenching properties conferred by the tannin, phenolic and flavonoid fractions. This is directly relevant to diabetes pathophysiology, where hyperglycaemia-driven oxidative stress contributes to pancreatic  $\beta$ -cell dysfunction and tissue injury (Roy et al., 2024). The comparatively lower  $\text{H}_2\text{O}_2$  and lipid peroxidation inhibitory activity relative to FRAP/DPPH performance may reflect the differing polarity and reactivity requirements of these assays, which respond preferentially to specific phenolic subclasses rather than total phenolic content alone (Ermawati et al., 2024).

GC-MS analysis identified octadecanoic acid, ethyl ester as the predominant constituent (61.35% peak area), followed by n-hexadecanoic acid (14.11%), 5-hydroxymethylfurfural (3.91%), naphthalene derivatives (3.3%), and minor constituents including caryophyllene, methyl salicylate, cinnamaldehyde, tumerone, neointermedeol, tetradecanoic and pentadecanoic acids, ethyl oleate, D-allose and  $\beta$ -D-glucopyranose. The dominance of long-chain fatty acid ethyl esters is consistent with reports identifying these compounds as antioxidant, anti-inflammatory and hepatoprotective agents, properties mechanistically relevant to protecting metabolically active organs from diabetic insults. The presence of 5-hydroxymethylfurfural is noteworthy, as this furan derivative activates the Keap1/Nrf2 antioxidant pathway and upregulates superoxide dismutase-2 in pancreatic  $\beta$ -cells, contributing to  $\beta$ -cell protection against oxidative stress (Fletcher et al., 2026). Its co-occurrence with D-allose and  $\beta$ -D-glucopyranose corroborates the qualitative detection of reducing sugars. The minor terpenoid constituents (caryophyllene, tumerone, neointermedeol) further corroborate the qualitative screening and add mechanistic weight, given the documented capacity of plant-derived terpenoids to improve insulin sensitivity, modulate glucose metabolism and mitigate oxidative stress via redox-sensitive pathways (Roy et al., 2024).

The progressive, significant reduction in body weight ( $p < 0.05$ ) observed in the diabetic control (DC) group compared with the normal control (NC) from the first week after diabetes induction is

a well-recognised consequence of insulin deficiency, which promotes a catabolic state characterised by accelerated proteolysis, lipolysis and impaired glucose utilisation for energy. This reduction in average body weight can be attributed to the body's inability to use glucose for energy due to insulin deficiency or resistance, forcing it into a catabolic state in which fat and muscle are broken down for fuel (Harreiter & Roden, 2023). In contrast, the Diabetic treated with watermelon extract (DW) group maintained a significantly higher body weight than the Diabetic Control (DC) group ( $p < 0.05$ ) throughout the study period. This suggests that watermelon seed extract effectively countered the catabolic effects of diabetes, an effect that may be linked to its rich content of trace minerals and bioactive phytoconstituents that support glucose metabolism and reduce oxidative stress (Nadeem et al., 2022; Ogbeifun et al., 2023). By improving glycaemic control, the extract may reduce reliance on fat and protein catabolism for energy, thereby preserving body weight (Moiz et al., 2025). Watermelon seeds are rich in trace minerals, including zinc, selenium, manganese, and iron, which play crucial roles in glucose metabolism and insulin signalling; zinc in particular is essential for insulin synthesis, storage, and secretion, while selenium acts as a cofactor for antioxidant enzymes that protect pancreatic tissue from oxidative damage (Ogbeifun et al., 2023).

The relative brain weight was significantly reduced in the diabetic control (DC) group compared with the NC group ( $p < 0.05$ ). This reduction can be attributed to the loss of insulin and insulin-like growth factor signaling in the brain, which disrupts the processes that maintain brain protein content and leads to widespread cell degeneration, including loss of essential structures such as myelin (Choudhary, 2025). Conversely, the diabetic treated with watermelon seed extract (DW) and diabetic treated with metformin (DM) showed significantly higher relative brain weight than the DC group ( $p < 0.05$ ). The antioxidant properties of watermelon seed bioactive compounds may protect against cellular degeneration in the brain (Nadeem et al., 2022).

In this study, blood glucose levels were significantly higher in the diabetic control (DC) group ( $p < 0.05$ ). This elevation can be attributed to the destruction of pancreatic  $\beta$ -cells, generation of reactive oxygen species, and consequent insulin deficiency and sustained hyperglycaemia, consistent with the complex interaction of impaired insulin secretion, insulin resistance, excessive hepatic glucose production, defective peripheral glucose uptake, and increased renal glucose reabsorption that underlies uncontrolled diabetes mellitus (American Diabetes Association

Professional Practice Committee, 2025). Conversely, the treated groups; diabetic treated with watermelon seed extract (DW) and diabetic treated with metformin (DM) demonstrated significantly reduced blood glucose levels over time ( $p < 0.05$  vs DC), restoring values close to normal by week 6 ( $p > 0.05$  vs NC). The reduction in blood glucose in the DW group may be linked to the trace-mineral and phytochemical composition of watermelon seed extract: zinc supports insulin synthesis, storage, and secretion, while selenium functions as a cofactor for antioxidant enzymes that protect pancreatic tissue from oxidative damage (Ogbeifun et al., 2023). Flavonoids and other bioactive constituents present in the extract may further promote pancreatic  $\beta$ -cell proliferation and insulin secretion, contributing to reduced hyperglycaemia (Nadeem et al., 2022).

The diabetic control (DC) group exhibited a significant decline in locomotor activity compared with the normal control (NC) group ( $p < 0.05$ ). This reduction is attributable to uncontrolled hyperglycaemia, which impairs the brain's motor control centers and weakens the neural drive generating movement commands (Mazumdar & Singh, 2025). Conversely, treatment with watermelon seed extract (DW) and metformin (DM) significantly increased line-crossing frequency compared with DC ( $p < 0.05$ ), with values approaching those of NC ( $p > 0.05$ ). This restoration of locomotor activity towards near-normal levels suggests that the extract improves neuronal integrity and supports recovery of movement-associated function through restoration of antioxidant defences and protection of striatal neurons (Djientcheu Tientcheu et al., 2023). Diabetes-induced skeletal muscle weakness and atrophy are also recognised contributors to reduced locomotor activity, so treatments that counter these processes would be expected to support the observed recovery of movement (Shen et al., 2022). More broadly, watermelon has been shown to reduce blood glucose, improve lipid profiles, and enhance antioxidant enzyme activity, thereby alleviating the systemic burden of diabetes and enabling recovery of normal motor function (Moore & Okoduwa, 2026).

The diabetic control (DC) group exhibited a significant decline in center-square entries compared with normal control (NC) ( $p < 0.05$ ), reflecting increased anxiety attributed to persistent hyperglycaemia-induced central nervous system dysfunction, including brain atrophy and white matter injury affecting mood-regulating areas such as the hippocampus and amygdala (Choudhary, 2025). In contrast, the diabetic treated with watermelon seed extract (DW) and diabetic treated

with metformin (DM) groups significantly improved center-square entries compared with DC ( $p < 0.05$ ), with values close to NC ( $p > 0.05$ ). The restoration observed with watermelon seed extract is supported by evidence of its anxiolytic and neuroprotective properties, whereby antioxidant activity may restore monoamine neurotransmitter levels in anxiety-regulating brain regions by protecting serotonergic and dopaminergic neurons from diabetes-induced damage (Mohamad Salin et al., 2022). Studies on *Citrullus colocynthis*, a species closely related to watermelon, have demonstrated protective effects against cognitive impairments and anxiety-like behaviours in STZ-induced diabetic rats (Rajizadeh et al., 2023a). The presence of flavonoids, predominantly catechin, in watermelon seeds acts as a powerful antioxidant protecting neural tissues from free radicals (Obehioye, 2023).

The diabetic control (DC) group exhibited a significant decline in rearing behaviour compared with normal control (NC) ( $p < 0.05$ ), signifying increased anxiety attributable to dopaminergic dysfunction. Rearing is predominantly mediated by dopamine neurotransmission within the nigrostriatal and mesolimbic pathways, with D1 and D2 receptor activation playing critical roles in initiating and sustaining this exploratory motor pattern (Singh et al., 2025). Conversely, the diabetic treated with watermelon seed extract (DW) and diabetic treated with metformin (DM) groups significantly increased rearing frequency compared with DC ( $p < 0.05$ ), with extract-treated values close to NC ( $p > 0.05$ ). The increased rearing observed is supported by the neuroprotective and antioxidant properties of the treatment agents: the flavonoid-rich watermelon seed extract protects dopaminergic neurons from oxidative damage, preserving dopamine synthesis and receptor function (Ogbeifun et al., 2023). Obehioye (2023), further demonstrated that watermelon and related seed extracts at moderate doses present significant anxiolytic activity without affecting locomotion, confirming specific anxiety reduction without general motor suppression. Research has shown that watermelon seed extract significantly increases line crossing in the open-field test, indicating restoration of both exploratory and locomotor activity (Ogbeifun et al., 2023).

The diabetic control (DC) group exhibited a significant increase in walling activity compared with normal control (NC) ( $p < 0.05$ ), attributable to a diabetes-induced state of heightened anxiety due to serotonergic dysfunction. Walling behaviour is primarily modulated by serotonergic neurotransmission within anxiety-regulating brain regions, including the amygdala, hippocampus, and prefrontal cortex, and diabetes alters serotonin synthesis, release, and receptor function in

these regions (Harreiter & Roden, 2023). Dysregulation of serotonergic tone shifts the behavioural balance towards avoidance and thigmotaxis (Özcanlı Çay & Çalışkan, 2026). In contrast, the diabetes treated with watermelon seed extract (DW) and diabetes treated with metformin (DM) groups exhibited significantly reduced walling frequency compared with DC ( $p < 0.05$ ), with values close to NC ( $p > 0.05$ ). The reduced walling behaviour in the DW group is attributed to watermelon seed extract's ability to exert direct anxiolytic effects through modulation of GABAergic or serotonergic pathways; flavonoid components have been reported to possess anxiolytic properties through interaction with GABA-A receptors, reducing anxiety and promoting exploratory behaviour (Nadeem et al., 2022). Research on Cucurbitaceae seeds has demonstrated that ethanol seed extracts produce dose-dependent anxiolytic effects, significantly increasing open-arm entries in the elevated plus maze without affecting motor coordination (Belemkar & Shendge, 2021; Rajizadeh et al., 2023).

The diabetic control (DC) group exhibited a significant increase in micturition frequency compared with normal control (NC) ( $p < 0.05$ ), due to persistent hyperglycaemia resulting in glucose overflow into urine, creating an osmotic gradient that draws water into renal tubules and markedly increases urine production (Moneim et al., 2022). Conversely, the diabetic treated with watermelon seed extract (DW) and diabetic treated with metformin (DM) groups significantly reduced micturition frequency compared with DC ( $p < 0.05$ ), with extract-treated values close to NC ( $p > 0.05$ ). The significant reduction following watermelon seed extract treatment is attributed to its antihyperglycaemic activity, which reduces the osmotic load on the kidneys; by lowering blood glucose, the extract reduces glycosuria, thereby decreasing the osmotic gradient driving excessive urine production (Ogbeifun et al., 2023). The hypoglycaemic effects of watermelon seed extract are well documented, with studies demonstrating significant blood glucose reductions in alloxan-induced diabetic rats through phytochemical effects, particularly flavonoids, on pancreatic  $\beta$ -cells leading to proliferation and increased insulin secretion (Ogbeifun et al., 2023).

The diabetic control (DC) group exhibited a significant increase in defecation frequency compared with NC ( $p < 0.05$ ), attributable to diabetic enteropathy—damage to nerves controlling the digestive tract. Chronic high blood sugar damages autonomic nerves and the enteric nervous system that regulates gut motility (Cunningham et al., 2021), leading to uncoordinated contractions, rapid intestinal transit, and impaired absorption, resulting in frequent, loose stools.

In contrast, the diabetic treated with watermelon seed extract (DW) and diabetic treated with metformin (DM) groups significantly reduced defecation frequency compared with DC ( $p < 0.05$ ), with extract-treated values close to NC ( $p > 0.05$ ). The ability of watermelon seed extract to reduce defecation frequency is supported by evidence of its antispasmodic and antidiarrhoeal properties: bioactive compounds exert calcium channel-blocking activity, regulating smooth muscle contractile response and providing antiperistaltic and antisecretory effects (Banjo et al., 2024). The significant reduction in the DM group is linked to metformin's ability to stimulate GLP-1 secretion from gut tissue, which influences gastrointestinal motility and insulin secretion (Yaseen et al., 2025). Studies on *Citrullus colocynthis* have demonstrated that diabetic rats exhibit significantly higher fecal pellet numbers compared with controls, with extract administration significantly decreasing this parameter while also increasing time spent in the center of the open field, indicating concurrent anxiolytic effects (Rajizadeh et al., 2023).

Haematoxylin and Eosin (H&E) staining revealed that the diabetic control (DC) group predominantly exhibited necrotic cells with few remaining normal cells. This extensive necrosis is attributable to the brain's vulnerability to oxidative stress owing to its high oxygen consumption, abundant lipid content, and relatively low antioxidant capacity, which damages cellular membranes, proteins, and DNA, ultimately leading to neuronal necrosis or apoptosis (Szablewski, 2025). Conversely, the diabetic groups treated with watermelon seed extract (DIA+WS) or metformin (DIA+MET) displayed a partial neuroprotective effect, with some necrotic cells alongside apparently normal cells. This preservation is likely attributable to the antihyperglycaemic effects of both treatments, which reduce the metabolic stress imposed on neurons by chronic hyperglycaemia (Ogbeifun et al., 2023). Watermelon seeds contain bioactive phytochemicals and trace minerals that exert significant free-radical-scavenging activity and support the endogenous antioxidant enzyme system (Belemkar & Shendge, 2021). The neuroprotective effects of watermelon seed extract are plausibly mediated through synergistic antioxidant activity of phenolic acids, flavonoids, and trace minerals (zinc and selenium) that support antioxidant enzyme function, neutralise ROS, and maintain cellular redox balance, consistent with preservation of reduced glutathione (GSH) and prevention of elevated lactate dehydrogenase (LDH) activity (Belemkar & Shendge, 2021; Szablewski, 2025).

Phosphotungstic acid haematoxylin (PTAH) staining revealed that the diabetic control (DC) group exhibited astrocytes alongside degenerated neurons, attributable to significant structural changes (astrogliosis) in astrocytes owing to chronic uncontrolled hyperglycaemia (Salem et al., 2025). Studies have demonstrated that in STZ-induced diabetic rats, astrocytes exhibit marked ultrastructural changes, including reduced nuclear electron density, electro-lucent heterochromatin, and vacuolated cytoplasm (Nurkenov et al., 2021a). In contrast, the diabetic groups treated with watermelon seed extract (DIA+WS) or metformin (DIA +MET) showed some degenerated neurons alongside normal-appearing neurons and astrocytes, representing partial preservation. This is attributed to the antioxidant effects of watermelon seed extract, rich in phenolic compounds, flavonoids, and trace minerals with potent antioxidant properties (Belemkar & Shendge, 2021; Ogbeifun et al., 2023). The flavonoid components may act as anti-inflammatory agents by limiting pro-inflammatory cytokine expression (TNF- $\alpha$ , IL-1 $\beta$ , and IL-6). The trace mineral content of watermelon seeds, including zinc, magnesium, and selenium, may contribute to neuroprotective effects through roles in antioxidant enzyme function and neurotransmitter regulation (Belemkar & Shendge, 2021).

Cresyl Fast Violet (CFV) staining demonstrated that the diabetic group (DC) exhibited significant astrocyte degeneration, attributable to loss of astrocyte function and structural integrity due to chronic uncontrolled hyperglycaemia (Salem et al., 2025). This observation is consistent with previously reported ultrastructural changes in diabetic astrocytes, including reduced nuclear electron density, electro-lucent heterochromatin, and vacuolated cytoplasm (Nurkenov et al., 2021). In contrast, the diabetic groups treated with watermelon seed extract (DIA+WS) or metformin (DIA + MET) showed an intermediate histological picture with some degenerating neurons alongside normal-appearing neurons and astrocytes. The preservation seen in the extract-treated group is attributed to the extract's ability to reduce oxidative stress, likely protecting Nissl substance integrity and maintaining neuronal protein synthetic activity, as reflected by relatively strong CFV staining (Chavda et al., 2024). The anti-inflammatory properties of watermelon seed extract may further protect against microglial activation and the release of neurotoxic inflammatory mediators. The primary mechanism underlying partial preservation of Nissl substance in the watermelon-treated group likely involves antioxidant properties of phenolic and flavonoid content, which neutralise ROS and improve superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) activities (Belemkar & Shendge, 2021).

Glial Fibrillary Acidic Protein (GFAP) immunohistochemistry staining revealed that the diabetic control (DC) group exhibited increased GFAP immunoreactivity, attributable to hyperglycaemia-induced astrocyte activation (Chavda et al., 2024). Studies have demonstrated that in diabetic animal models, reactive astrocytes undergo morphological changes characterised by somatic hypertrophy, dendrite extension, an increased number of dendritic processes, and elevated GFAP immunoreactivity (Nurkenov et al., 2021). Conversely, the diabetic groups treated with watermelon seed extract (DIA + WM) or metformin (DIA + MET) maintained much of their neuronal architecture with only limited astrocytic activation. This preservation is attributed to the ability of watermelon seed extract to significantly reduce hyperglycaemia, thereby reducing the metabolic stress that drives astrocyte activation and limiting GFAP upregulation (Liu et al., 2025). While direct studies on watermelon seed extract and GFAP expression remain limited, research on *Momordica charantia*, which contains phenolics and alkaloids comparable to watermelon seeds, has shown significant reduction of elevated GFAP levels in diabetic rats through antioxidant and anti-inflammatory mechanisms (Abou-El-Naga et al., 2025).

Masson's Trichrome (MT) staining revealed that the diabetic + metformin (DIA + MET) and diabetic control (DC) groups exhibited extensive collagen buildup, attributable to hyperglycaemia-induced cerebral microangiopathy characterised by capillary fibrosis and collagen accumulation (Chavda et al., 2024). Studies have demonstrated that in STZ-induced diabetic rats, brain microvessels show thickening of the basement membrane with accumulation of collagen fibrils in arteriole and capillary walls (Khan et al., 2025). In contrast, the diabetic + watermelon (DIA + WM) group displayed significantly increased collagen deposition compared with the normal control, though this deposition was less extensive than in the DC and DIA+MET groups. Watermelon seed extract may support vascular antioxidant status and help limit oxidative vascular damage, which could partially attenuate progression to fibrosis (Belemkar & Shendge, 2021). The finding of residual collagen deposition in the extract-treated group indicates that, while the extract provided some attenuation of fibrosis, complete prevention of collagen accumulation was not achieved.

Malondialdehyde (MDA) levels were significantly elevated in the diabetic control (DC) group compared with normal control (NC) ( $p < 0.05$ ), attributable to oxidative stress resulting from the decomposition of lipid hydroperoxides derived from polyunsaturated fatty acids, which occurs

through  $\beta$ -scission of the hydroperoxide and cleavage of the carbon chain, generating MDA as an end-product (Asfandiyar et al., 2024; Qiu et al., 2025). Conversely, the diabetic treated with watermelon seed extract (DW) and diabetic treated with metformin (DM) groups significantly reduced MDA levels compared with DC ( $p < 0.05$ ), indicating that both interventions effectively attenuated diabetes-induced lipid peroxidation. The comparable efficacy of watermelon seed extract to metformin suggests substantial antioxidant potential (Bello et al., 2022). The reduction following extract treatment is ascribed to its rich polyphenolic content, including catechin-type flavonoids and phenolic acids, which scavenge free radicals and prevent the initiation and propagation of lipid peroxidation (Benmeziane & Derradji, 2023).

Superoxide Dismutase (SOD) activity and levels were significantly reduced in the diabetic control (DC) group compared with normal control (NC) ( $p < 0.05$ ), this is ascribed to impaired antioxidant defense caused by hyperglycaemia, with diabetic tissues expressing significantly lower amounts of extracellular SOD protein (Qiu et al., 2025). In contrast, the diabetic treated with watermelon seed extract (DW) and diabetic treated with metformin (DM) groups significantly increased SOD levels compared with DC ( $p < 0.05$ ). The increase following extract treatment is attributed to the flavonoid-rich extract's notable SOD-like activity and antioxidant properties, which significantly increase SOD and GPx levels while decreasing MDA (Benmeziane & Derradji, 2023). Research on watermelon seed oil has demonstrated significant enhancement of SOD and CAT levels while decreasing cholesterol, triglyceride, LDL, and MDA levels, with no sign of toxicity even at maximal doses (Eke et al., 2021).

Glutathione (GSH) levels were significantly reduced in the diabetic control (DC) group compared with normal control (NC) ( $p < 0.05$ ), resulting from massive ROS production forcing accelerated GSH utilisation to neutralise free radicals, becoming oxidised glutathione (GSSG) in the process, with ROS production continuously outstripping GSH regeneration capacity (Backeström et al., 2021). Conversely, the diabetic treated with watermelon seed extract (DW) and diabetic treated with metformin (DM) groups significantly increased GSH levels compared with DC ( $p < 0.05$ ). The increase following extract treatment is attributed to the antioxidant traits of watermelon seeds, with studies demonstrating significant increases in glutathione peroxidase and glutathione reductase activities, which play crucial roles in the glutathione redox cycle (Ogbeifun et al., 2023). These results are consistent with research showing that diabetes reduces glutathione reductase

activity, compromises GSH synthesis through impaired cysteine uptake, and reduces glutathione peroxidase activity (De Felice et al., 2022). The phytochemical composition of watermelon seeds, particularly catechin flavonoids, supports maintenance of GSH levels through antioxidant protection (Benmeziiane & Derradji, 2023).

Catalase (CAT) activity was significantly reduced in the diabetic control (DC) group compared with normal control (NC) ( $p < 0.05$ ), this is attributed to direct chemical modification of the enzyme by high blood sugar and subsequent oxidative damage, with non-enzymatic glycation causing structural alterations and aggregation that drastically reduce catalytic activity, often resulting in efficiency loss of more than 80% (Burillo et al., 2021; Deshmukh & Jain, 2021). In contrast, the diabetic treated with watermelon seed extract (DW) and diabetic treated with metformin (DM) groups significantly increased CAT levels compared with DC ( $p < 0.05$ ). The increase following watermelon seed extract treatment is attributed to the antioxidant potential of watermelon seeds, with studies showing that supplementing rats with watermelon seeds significantly maintains catalase activity relative to untreated diabetic controls (Rabiu et al., 2025). Research on watermelon juice and leaf extract has demonstrated that alloxan- and STZ-induced diabetic rats exhibit significant increases in catalase activity compared with untreated diabetic rats, with values approaching control levels (Jibril et al., 2022).

Alkaline Phosphatase (ALP) levels were significantly elevated in the diabetic control (DC) group compared with normal control (NC) ( $p < 0.05$ ), this elevation is attributed to damage to neuronal plasma membranes and intracellular organelles, resulting in increased membrane permeability and leakage of membrane-associated enzymes, including ALP (Chen et al., 2025). Conversely, the diabetic treated with watermelon seed extract (DW) and diabetic treated with metformin (DM) groups significantly reduced ALP levels compared with DC ( $p < 0.05$ ). The reduction following watermelon seed extract treatment is attributed to the hepatoprotective and antioxidant features of watermelon seed extract, effective in reducing AST, ALT, and ALP levels in hepatotoxic states through mitigation of hepatic oxidative stress and preservation of hepatocyte membrane integrity (Ogbeifun et al., 2023). The reduction in the DM group is attributed to metformin's ability to lower blood glucose through AMPK activation, suppressing mitochondrial ROS production, inhibiting NF- $\kappa$ B signalling, and improving endothelial function (Xing et al., 2024). Research on aqueous alfalfa extract, sharing similar phytochemical profiles with watermelon seed extract, significantly

decreases glucose, AST, and ALT levels, suggesting beneficial effects in preventing diabetic complications (Mehreen et al., 2024).

Acid Phosphatase (ACP) levels were significantly elevated in the diabetic control (DC) group compared with normal control (NC) ( $p < 0.05$ ), this elevation is ascribed to marked lysosomal dysfunction within the frontal cortex due to hyperglycaemia, with diabetes associated with increased activity of multiple lysosomal hydrolases, including acid phosphatase,  $\beta$ -glucuronidase, and N-acetyl- $\beta$ -glucosaminidase (Chen et al., 2025c; Okotie et al., 2023). Conversely, the diabetic treated with watermelon seed extract (DW) and diabetic treated with metformin (DM) groups significantly reduced ACP levels compared with DC ( $p < 0.05$ ). The reduction following watermelon seed extract treatment is linked to the extract's ability to reduce oxidative stress, protecting lysosomal membranes from oxidative damage and preventing acid phosphatase leakage into the circulation (Rabiu et al., 2025). The rich flavonoid content contributes to this membrane-stabilising effect. Studies have demonstrated that extracts of *Citrullus lanatus* exert significant impact in STZ-induced diabetic rats, affecting glucose levels and various biochemical parameters (Mehreen et al., 2024).

Glucose-6-Phosphate Dehydrogenase (G6PDH) levels were significantly lower in the diabetic control (DC) group than in normal control (NC) ( $p < 0.05$ ), this can be attributed to decreased expression of the G6PDH gene and increased phosphorylation of the enzyme due to elevated glucose levels (Szablewski, 2025). In contrast, the diabetic treated with watermelon seed extract (DW) and diabetic treated with metformin (DM) groups significantly increased G6PDH levels compared with DC ( $p < 0.05$ ). The increase following watermelon seed extract treatment is attributed to the extract's ability to increase the activity of hepatic hexokinase, phosphofructokinase, and glucose-6-phosphate dehydrogenase in diabetic rats compared with untreated diabetic controls, promoting glucose metabolism through the pentose phosphate pathway (Ogbeifun et al., 2023). Watermelon seeds contain flavonoids, alkaloids, saponins, phenolic acids, and essential minerals (zinc, magnesium, and selenium) that exhibit antioxidant properties protecting G6PDH from oxidative inactivation (Ogbeifun et al., 2023). In another study it was found that the antidiabetic potential of watermelon juice involves modulation of glucose transporters, anti-inflammatory activities, and enhancement of the antioxidant defence system: the administration of different doses of watermelon juice significantly reduced fasting blood glucose

levels, serum lipid profile, and lipid peroxidation while significantly increasing antioxidant enzyme activities and hexokinase activity in diabetic rats (Moneim et al., 2022).

Lactate Dehydrogenase (LDH) levels were significantly higher in the diabetic control (DC) group than in normal control (NC) ( $p < 0.05$ ), this is mechanistically attributed to hyperglycaemia-induced alterations in the  $\text{NAD}^+/\text{NADH}$  ratio, increased flux through the polyol pathway, AGE formation, and activation of protein kinase C isoforms, all leading to elevated  $\text{NADH}/\text{NAD}^+$  ratio and increased lactate production, reflecting metabolic adaptation to mitochondrial dysfunction but indicating substantial neuronal stress and impaired bioenergetic homeostasis (Dawi et al., 2024). Conversely, the diabetic treated with watermelon seed extract (DW) and diabetic treated with metformin (DM) groups significantly reduced LDH levels compared with DC ( $p < 0.05$ ). The reduction following watermelon seed extract treatment is due to the extract's ability to lower blood glucose levels, attenuating hyperglycaemia-induced glycolytic flux and associated LDH elevation (Bazabang et al., 2023). Furthermore, the bioactive phenolic and flavonoid compounds in watermelon seeds have demonstrated inhibitory effects against alpha-amylase and alpha-glucosidase, promoting improved glycaemic control and protecting cellular membranes from LDH leakage. Bioactive compounds in watermelon seeds protect tissue from diabetic damage and reduce serum biomarkers of tissue dysfunction, including LDH (Jibril et al., 2022).

Caspase-3 levels were significantly higher in the diabetic control (DC) group than in normal control (NC) ( $p < 0.05$ ), this can be attributed to convergence of both intrinsic (mitochondrial) and extrinsic (death receptor) apoptotic pathways, consistent with established literature documenting that diabetes induces apoptosis through caspase-3 activation (Jiang et al., 2025). In contrast, the diabetic treated with watermelon seed extract (DW) and diabetic treated with metformin (DM) groups significantly reduced caspase-3 levels ( $p < 0.05$ ). The reduction following watermelon seed extract treatment is attributed to the extract's antioxidant properties; UPLC/T-TOF-MS/MS analysis has identified phenolic acids such as chlorogenic acid, along with flavonoids, that possess antioxidant and anti-inflammatory properties capable of mitigating the oxidative stress driving caspase-3 activation (El Gizawy et al., 2022).

Interleukin-6 (IL-6) levels were significantly higher in the diabetic control (DC) group than in the normal control (NC) ( $p < 0.05$ ), this can be attributed to hyperglycaemia-induced oxidative stress activating the NF- $\kappa$ B pathway and resulting inflammatory cascade (Khaliq et al., 2025).

Conversely, the diabetic treated with watermelon seed extract (DW) and diabetic treated with metformin (DM) groups significantly reduced IL-6 levels ( $p < 0.05$ ). The reduction in the DW group is attributable to the rich bioactive-compound profile of watermelon, including phenolic acids and flavonoids, which exert anti-inflammatory effects by scavenging ROS, reducing oxidative stress, and limiting activation of pro-inflammatory transcription factors such as NF- $\kappa$ B (Bazabang et al., 2023b; El Gizawy et al., 2022). By reducing hyperglycaemia, the extract decreases metabolic stress driving IL-6 production, and the presence of cucurbitacins, particularly cucurbitacin E, further contributes to anti-inflammatory properties by neutralising reactive nitrogen-containing molecules and inhibiting cyclo-oxygenase enzymes (Mehreen et al., 2024).

Tumour Necrosis Factor-alpha (TNF- $\alpha$ ) levels were significantly higher in the diabetic control (DC) group than in normal control (NC0 ( $p < 0.05$ ), this can be attributed to hyperglycaemia-induced ROS overproduction stimulating TNF- $\alpha$  synthesis; ROS trigger I $\kappa$ B kinase (IKK), which phosphorylates and degrades I $\kappa$ B, allowing NF- $\kappa$ B to enter the nucleus and activate transcription of the TNF- $\alpha$  gene (Khaliq et al., 2025). In contrast, the diabetic treated with watermelon (DW) and diabetic treated with metformin (DM) groups significantly reduced TNF- $\alpha$  levels ( $p < 0.05$ ). This result in the DW group is attributable to metabolites derived from watermelon seed and rind extracts, which suppress NF- $\kappa$ B-driven inflammatory pathways, alleviating oxidative stress and apoptosis, with suppression of NF- $\kappa$ B directly reducing TNF- $\alpha$  transcription and secretion (El Gizawy et al., 2022). *Citrullus lanatus* seed flavonoids have been demonstrated to substantially reduce TNF- $\alpha$  and IL-1 $\beta$  levels in ethanol-treated rats ( $p < 0.05$ ), accompanied by decreased oxidative stress markers and improved renal function, concluding that this flavonoid fraction may act as a plant-based natural therapy to minimise organ toxicity through its antioxidant and anti-inflammatory properties (Bazabang et al., 2023).

## Conclusion

The ethanolic extract of *Citrullus lanatus* (watermelon) seed, rich in tannins, phenolics, alkaloids and flavonoids, normalised blood glucose, preserved body and brain weight, and reversed anxiety-like and hypoactive behaviours in STZ-induced diabetic rats, with efficacy comparable to metformin. The extract restored oxidative stress markers (MDA, SOD, GSH, CAT), glucose-metabolising enzymes (ALP, ACP, G6PDH, LDH), and suppressed inflammatory/apoptotic

markers (caspase-3, IL-6, TNF- $\alpha$ ), while providing partial histological protection against neuronal necrosis, astrogliosis, and Nissl substance loss in the frontal cortex. However, collagen deposition was only partially attenuated, indicating incomplete reversal of cerebral microvascular fibrosis within the six-week treatment window. The extract exhibited no toxicity in non-diabetic animals, consistent with its wide safety margin. These findings position watermelon seed extract as a promising, low-cost nutraceutical adjunct to conventional antidiabetic therapy, particularly in resource-limited settings.

## **Declarations**

### **Disclaimers**

The opinions and findings presented in this article are those of the authors alone and may not be representative of those of the organizations with which they are affiliated. Although the writers are in charge of information's correctness and comprehensiveness, they disclaim all obligations for any losses, whether direct or indirect, that may arise from using this content.

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### **Conflict of interest/Competing interests**

Authors declare that they had no competing interests.

### **Ethics approval and consent to participate**

Ethical and regulatory approval was obtained from the Mulungushi University School of Medicine and Health Sciences Research Ethics Committee and the National Health Research Authority (approval number: NHRA-1832/27/12/2024). All animal procedures were carried out in accordance with institutional and national guidelines for the care and use of laboratory animals.

### **Consent for publication**

The study was approved by Ethical Committee of Mulungushi University school of Medicine and Health Sciences, Livingstone, Zambia and The National Research Authority.

### **Availability of data and materials**

Raw data is uploaded separately

### **Authors' contributions**

Isabel Namfukwe Luambia: Conceptualization, Methodology, supervised the project, Writing – Original Draft, review & editing; Yusuf Uthman Ademola: Conceptualization, Methodology, Analysis; Jamia Milanzi: Data Collection, Analysis, Project Administration; Precious Kapambwe: Data Collection, Analysis & Visualization; Fridah Mutalife: Data Collection, Analysis & Visualization; Albertina Ng'andu: Data Collection, Analysis & Visualization. All authors reviewed the work.

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### **References**

- Abou-El-Naga, A. M., Mansour, H. A. E.-H., El-Sawi, M. R., El-Dein, M. A., Tag, Y. M., Ghanem, R. A., & Shawki, M. A. (2025). Restorative effects of *Momordica charantia* extract on cerebellar GFAP and NGF expression in pregnant diabetic rats and their offspring. *PloS One*, 20(4), e0321022. <https://doi.org/10.1371/journal.pone.0321022>
- Alrefaei, A. F., & Elbeeh, M. E. (2025). Alpha-Lipoic Acid and Metformin Combination Therapy Synergistically Activate Nrf2-AMPK Signaling Pathways to Ameliorate Cognitive Dysfunction in Type 2 Diabetic Encephalopathy: A Preclinical Study. *Biology*, 14(7), 885. <https://doi.org/10.3390/biology14070885>
- Ameen, O., Samaka, R. M., & Abo-Elvoud, R. A. A. (2022). Metformin alleviates neurocognitive impairment in aging via activation of AMPK/BDNF/PI3K pathway. *Scientific Reports*, 12(1), 17084. <https://doi.org/10.1038/s41598-022-20945-7>
- American Diabetes Association Professional Practice Committee. (2025). 2. Diagnosis and Classification of Diabetes: Standards of Care in Diabetes-2025. *Diabetes Care*, 48(1 Suppl 1), S27–S49. <https://doi.org/10.2337/dc25-S002>
- Asfandiyar, null, Hadi, N., Ali Zaidi, I., Kamal, Z., Ashraf, null, Ullah Khan, R., Rumman, null, Hashim Khan, M., & Omair, F. (2024). Estimation of Serum Malondialdehyde (a Marker of Oxidative Stress) as a Predictive Biomarker for the Severity of Coronary Artery Disease (CAD) and Cardiovascular Outcomes. *Cureus*, 16(9), e69756. <https://doi.org/10.7759/cureus.69756>

- Backeström, A., Papadopoulos, K., Eriksson, S., Olsson, T., Andersson, M., Blennow, K., Zetterberg, H., Nyberg, L., & Rolandsson, O. (2021). Acute hyperglycaemia leads to altered frontal lobe brain activity and reduced working memory in type 2 diabetes. *PloS One*, 16(3), e0247753. <https://doi.org/10.1371/journal.pone.0247753>
- Banjo, S., Bassey, A., & Adelakin, L. (2024). Methanolic Extract of *Citrullus lanatus* Seed Ameliorates Sodium Benzoate-Induced Memory Impairment, Anxiety Disorder, and Histoarchitectural Disruptions in *Rattus norvegicus* Frontal-Lobe Cortex. *Medtigo Journal of Neurology and Psychiatry*, 1. <https://doi.org/10.63096/medtigo3084111>
- Bazabang, S. A., Makena, W., Onimisi, O. B., Rilwan, H. B., Buba, H. S., Jerome, V. K., & Yohana, A. (2023a). Flavonoid-rich extract from *Citrullus lanatus* (Watermelon) seed attenuated Ethanol-Induced Kidney injury in Wistar Rats. *Pharmacological Research - Modern Chinese Medicine*, 7, 100236. <https://doi.org/10.1016/j.prmcm.2023.100236>
- Bazabang, S. A., Makena, W., Onimisi, O. B., Rilwan, H. B., Buba, H. S., Jerome, V. K., & Yohana, A. (2023b). Flavonoid-rich extract from *Citrullus lanatus* (Watermelon) seed attenuated Ethanol-Induced Kidney injury in Wistar Rats. *Pharmacological Research - Modern Chinese Medicine*, 7, 100236. <https://doi.org/10.1016/j.prmcm.2023.100236>
- Belemkar, S., & Shendge, P. N. (2021). Toxicity profiling of the ethanolic extract of *Citrullus lanatus* seed in rats: Behavioral, biochemical and histopathological aspects. *Bioscience Reports*, 41(1), BSR20202345. <https://doi.org/10.1042/BSR20202345>
- Bello, O., Ayoola, M., Obembe, O., & Akinwunmi, K. (2022). Antidiabetic and Toxicity Studies of the Extract of Four Nigerian Medicinal Plants. *European Journal of Medicinal Plants*, 32–45. <https://doi.org/10.9734/ejmp/2022/v33i111107>
- Benmeziane, F., & Derradji, –. (2023). Composition, bioactive potential and food applications of watermelon (*citrullus lanatus*) seeds – a review. *Journal of Food Measurement and Characterization*, 17(5), 5045–5061. <https://doi.org/10.1007/s11694-023-02012-5>
- Bolon, B., Moser, A., & Chlipala, E. (2025). Myelin Methods: A Mini-Review. *Toxicologic Pathology*, 53(4), 345–354. <https://doi.org/10.1177/01926233241309332>
- Burillo, J., Marqués, P., Jiménez, B., González-Blanco, C., Benito, M., & Guillén, C. (2021). Insulin Resistance and Diabetes Mellitus in Alzheimer’s Disease. *Cells*, 10(5), 1236. <https://doi.org/10.3390/cells10051236>
- Chavda, V., Yadav, D., Patel, S., & Song, M. (2024). Effects of a Diabetic Microenvironment on Neurodegeneration: Special Focus on Neurological Cells. *Brain Sciences*, 14(3), 284. <https://doi.org/10.3390/brainsci14030284>
- Chen, Y., Li, P., Yue, Y., Du, W., Zhang, X., Ren, Y., Lyu, G., Wang, X., & Liu, J. (2025a). Sweet guardian: The role of glucose oxidase-catalase cascade in diabetes care: A review. *International Journal of Biological Macromolecules*, 329(Pt 1), 147865. <https://doi.org/10.1016/j.ijbiomac.2025.147865>

- Chen, Y., Li, P., Yue, Y., Du, W., Zhang, X., Ren, Y., Lyu, G., Wang, X., & Liu, J. (2025b). Sweet guardian: The role of glucose oxidase-catalase cascade in diabetes care: A review. *International Journal of Biological Macromolecules*, 329, 147865. <https://doi.org/10.1016/j.ijbiomac.2025.147865>
- Chen, Y., Li, P., Yue, Y., Du, W., Zhang, X., Ren, Y., Lyu, G., Wang, X., & Liu, J. (2025c). Sweet guardian: The role of glucose oxidase-catalase cascade in diabetes care: A review. *International Journal of Biological Macromolecules*, 329(Pt 1), 147865. <https://doi.org/10.1016/j.ijbiomac.2025.147865>
- Choudhary, A. K. (2025). Neuroinflammation and Cognitive health in Type 2 Diabetes. *Medical Research Archives*, 13(8). <https://doi.org/10.18103/mra.v13i8.6866>
- Clarkson, J. M., Martin, J. E., & McKeegan, D. E. F. (2022). A review of methods used to kill laboratory rodents: Issues and opportunities. *Laboratory Animals*, 56(5), 419–436. <https://doi.org/10.1177/00236772221097472>
- Cunningham, A. L., Stephens, J. W., & Harris, D. A. (2021). Intestinal microbiota and their metabolic contribution to type 2 diabetes and obesity. *Journal of Diabetes and Metabolic Disorders*, 20(2), 1855–1870. <https://doi.org/10.1007/s40200-021-00858-4>
- Dawi, J., Misakyan, Y., Affa, S., Kades, S., Narasimhan, A., Hajjar, F., Besser, M., Tumanyan, K., & Venketaraman, V. (2024a). Oxidative Stress, Glutathione Insufficiency, and Inflammatory Pathways in Type 2 Diabetes Mellitus: Implications for Therapeutic Interventions. *Biomedicines*, 13(1), 18. <https://doi.org/10.3390/biomedicines13010018>
- Dawi, J., Misakyan, Y., Affa, S., Kades, S., Narasimhan, A., Hajjar, F., Besser, M., Tumanyan, K., & Venketaraman, V. (2024b). Oxidative Stress, Glutathione Insufficiency, and Inflammatory Pathways in Type 2 Diabetes Mellitus: Implications for Therapeutic Interventions. *Biomedicines*, 13(1), 18. <https://doi.org/10.3390/biomedicines13010018>
- De Felice, F. G., Gonçalves, R. A., & Ferreira, S. T. (2022). Impaired insulin signalling and allostatic load in Alzheimer disease. *Nature Reviews. Neuroscience*, 23(4), 215–230. <https://doi.org/10.1038/s41583-022-00558-9>
- Deshmukh, C., & Jain, A. (2021). *Diabetes Mellitus: A Review*. 3, 224–230.
- Djientcheu Tientcheu, J. P., Nguenguim Tsofack, F., Gounoue, R. K., Fifen, R. N., Dzeufiet, P. D., & Dimo, T. (2023). The Aqueous Extract of *Sclerocarya birrea*, *Nauclea latifolia*, and *Piper longum* Mixture Protects Striatal Neurons and Movement-Associated Functionalities in a Rat Model of Diabetes-Induced Locomotion Dysfunction. *Evidence-Based Complementary and Alternative Medicine: eCAM*, 2023, 7865919. <https://doi.org/10.1155/2023/7865919>
- Dumon, C., Pisella, L., Diabira, D., Belaidouni, Y., Wayman, G. A., & Gaiarsa, J.-L. (2019). Developmental Switch of Leptin Action on Network Driven Activity in the Neonatal Rat Hippocampus. *Frontiers in Cellular Neuroscience*, 13, 254. <https://doi.org/10.3389/fncel.2019.00254>

Eke, R., Ejiofor, E., Oyedemi, S., Onoja, S., & Omeh, N. (2021). Evaluation of nutritional composition of *Citrullus lanatus* Linn. (Watermelon) seed and biochemical assessment of the seed oil in rats. *Journal of Food Biochemistry*, 45(6), e13763. <https://doi.org/10.1111/jfbc.13763>

El Gizawy, H. A., El-Haddad, A. E., Attia, Y. M., Fahim, S. A., Zafer, M. M., & Saadeldeen, A. M. (2022). In Vitro Cytotoxic Activity and Phytochemical Characterization (UPLC/T-TOF-MS/MS) of the Watermelon (*Citrullus lanatus*) Rind Extract. *Molecules*, 27(8), 2480. <https://doi.org/10.3390/molecules27082480>

Elfakharany, S. A., Eskaros, S. S., Azhary, N. M. E., Abdelmonsif, D. A., Zeitoun, T. M., Ammar, G. A. G., & Hatem, Y. A. (2024). Neuroprotective Role of Selenium Nanoparticles Against Behavioral, Neurobiochemical and Histological Alterations in Rats Subjected to Chronic Restraint Stress. *Molecular Neurobiology*, 61(12), 10159–10181. <https://doi.org/10.1007/s12035-024-04196-3>

Ermawati, Ahmad, A., Sartini, S., Hasan, N., Permana, A. D., Leman, Y., Duppa, M. T., Karim, H., Fajriah, S., Sapar, A., & Atun, S. (2024). Evaluation of Antidiabetic Effects of Watermelon Rind Extract: Integrative Computational Simulations and In Vitro Studies. *Tropical Journal of Natural Product Research*, 8(10), 8629–8639. <https://doi.org/10.26538/tjnpr/v8i10.3>

Fialho, T. C., Abrantes, L. C. S., Martins, K. V. C., Carvalho, R. P. R., Ramírez-López, C. J., Sousa, A. F. R. de, Guimarães-Ervilha, L. O., Oliveira, L. A., Lacerda, G. F., Moreira, A. J. B., Costa, S. F. F., Lana, V. S. de, Machado-Neves, M., Natali, A. J., Forte, P., Leite, L. B., Carvalho, I. M. M., Martino, H. S. D., da Silva, R. C., & Lucia, C. M. D. (2025). Resistance Exercise Associated with Camu-Camu (*Myrciaria dubia*) and Creatine Supplementation Modulates Antioxidant Response and Cardiac Parameters in Wistar Rats. *Nutrients*, 17(22), 3587. <https://doi.org/10.3390/nu17223587>

Fletcher, J. D., Maurer, H. L., Eschenfelder, M. A., Smith, B. R., Nayakanti, S., Guergues, J., Wolf, T., Stevens, S. M., & Burkhardt, B. R. (2026). NMR and proteomic analysis of *Cornus officinalis* extract reveals abundance of 5-hydroxymethylfurfural and induced expression of Nrf2 and superoxide dismutase-2 in 1.1B4 human pancreatic  $\beta$ -cells and murine islets. *Molecular and Cellular Endocrinology*, 611, 112675. <https://doi.org/10.1016/j.mce.2025.112675>

Genitsaridi, I., Salpea, P., Salim, A., Sajjadi, S. F., Tomic, D., James, S., Thirunavukkarasu, S., Issaka, A., Chen, L., Basit, A., Luk, A. O. Y., Ma, R. C. W., Mbanya, J.-C., Ramachandran, A., Wild, S. H., Duncan, B. B., Boyko, E. J., & Magliano, D. J. (2026). 11th edition of the IDF Diabetes Atlas: Global, regional, and national diabetes prevalence estimates for 2024 and projections for 2050. *The Lancet Diabetes & Endocrinology*, 14(2), 149–156. [https://doi.org/10.1016/S2213-8587\(25\)00299-2](https://doi.org/10.1016/S2213-8587(25)00299-2)

Golberg, M., Kobos, J., Clarke, E., Bajaka, A., Smędra, A., Balawender, K., Wawrzyniak, A., Seneczko, M., Orkisz, S., & Żytkowski, A. (2024). Application of histochemical stains in anatomical research: A brief overview of the methods. *Translational Research in Anatomy*, 35, 100294. <https://doi.org/10.1016/j.tria.2024.100294>

- Harreiter, J., & Roden, M. (2023). Diabetes mellitus – Definition, Klassifikation, Diagnose, Screening und Prävention (Update 2023). *Wiener Klinische Wochenschrift*, *135*, 7–17. <https://doi.org/10.1007/s00508-022-02122-y>
- He, K.-J., Wang, H., Xu, J., Gong, G., Liu, X., & Guan, H. (2024). Global burden of type 2 diabetes mellitus from 1990 to 2021, with projections of prevalence to 2044: A systematic analysis across SDI levels for the global burden of disease study 2021. *Frontiers in Endocrinology*, *15*, 1501690. <https://doi.org/10.3389/fendo.2024.1501690>
- Ismael, R., Mustafa, Y., & Al-Qazaz, H. (2022). Citrullus lanatus, a Potential Source of Medicinal Products: A Review. *Journal of Medicinal and Chemical Sciences*, *5*, 607–618. <https://doi.org/10.26655/JMCHEMSCI.2022.4.16>
- Jiang, Z., Liu, B., Lu, T., Liu, X., Lv, R., Yuan, K., Zhu, M., Wang, X., Li, S., Xu, S., Wang, X., Wang, Y., Gao, Z., Zhao, P., Zhang, Z., Hao, J., Lu, L., & Yin, Q. (2025). SGK1 drives hippocampal demyelination and diabetes-associated cognitive dysfunction in mice. *Nature Communications*, *16*(1), 1709. <https://doi.org/10.1038/s41467-025-56854-2>
- Jibril, M. M., Haji-Hamid, A., Abas, F., Karrupan, J., Mohammed, A. S., Jaafar, A. H., Pak Dek, M. S., & Ramli, N. S. (2022). Watermelon (Citrullus lanatus) leaf extract attenuates biochemical and histological parameters in high-fat diet/streptozotocin-induced diabetic rats. *Journal of Food Biochemistry*, *46*(2), e14058. <https://doi.org/10.1111/jfbc.14058>
- Kehinde, A. T., Ademola, Y. U., Atilade, A. O., Aderinola, A. A., Samuel, M. B., & Segun, O. O. (2018). *Neurobehavioural Study on the Effect of Aqueous Extract of Citrus medica Leaf on Prefrontal Cortex of Hyperglycemia Wistar Rats*. *3*(1).
- Khaleghi-Mehr, M., Delshad, A.-A., Shafie-Damavandi, S., & Roghani, M. (2023). Metformin mitigates amyloid  $\beta$ 1-40-induced cognitive decline via attenuation of oxidative/nitrosative stress and neuroinflammation. *Metabolic Brain Disease*, *38*(4), 1127–1142. <https://doi.org/10.1007/s11011-023-01170-1>
- Khaliq, A. S., Kumar, A., Khan, M., Rodoshi, Z. N., Khaliq, A. S., Kumar, A., Khan, M., & Rodoshi, Z. N. (2025). Diabetes Mellitus and Cognitive Decline: A Systematic Review Exploring the Link to Dementia and Neurodegenerative Diseases. *Cureus*, *17*(3). <https://doi.org/10.7759/cureus.80415>
- Khan, M. S., Jeon, Y., Lee, C.-H., Yang, J. M., & Kim, S. G. (2025). Dysregulation of Extracellular Matrix in Diabetic Complications: Clinical Impacts and New Molecular Targets. *Innovations in Acupuncture and Medicine*, *18*(1), 3. <https://doi.org/10.1186/s44424-025-00005-3>
- Knez, E., Kadac-Czapska, K., & Grembecka, M. (2025). Evaluation of Spectrophotometric Methods for Assessing Antioxidant Potential in Plant Food Samples—A Critical Approach. *Applied Sciences*, *15*(11), 5925. <https://doi.org/10.3390/app15115925>
- Liu, G., Li, H., Feng, L., Li, M., Gao, P., & Wang, F. (2025). O-GlcNAcylation promotes astroglial-mesenchymal transition via the connexin43 pathway under high-glucose conditions. *Experimental Eye Research*, *251*, 110206. <https://doi.org/10.1016/j.exer.2024.110206>

Luambia, I. N., Siabwacha, A., & Ngosa, M. (2024). *Evaluation of the Effect of an Aqueous Extract of Psidium guajava (Guava) Leaves on the Frontal Cortex of Diabetic Wistar Rats.*

Luambia, I. N., Siabwacha, A., Ngosa, M., Kaundu, S., Kombe, M., Siame, L. M., Malata, M., & Yusuf, U. A. (2024). Evaluation of the Effect of an Aqueous Extract of Psidium guajava (Guava) Leaves on the Frontal Cortex of Diabetic Wistar Rats. *Asian Journal of Biological Sciences*, 17(2), 243–253. <https://doi.org/10.3923/ajbs.2024.243.253>

Luijckx, L., Rodriguez, M., & Machaalani, R. (2024). Quantifying GFAP immunohistochemistry in the brain—Introduction of the Reactivity score (R-score) and how it compares to other methodologies. *Journal of Neuroscience Methods*, 402, 110025. <https://doi.org/10.1016/j.jneumeth.2023.110025>

Mazumdar, D., & Singh, S. (2025). Understanding diabetic encephalopathy: A model-based approach to explore cognitive and motor impairments through glutamatergic dysregulation. *Molecular Biology Reports*, 52(1), 945. <https://doi.org/10.1007/s11033-025-11022-z>

Mehreen, A., Iqbal, M. A., Ashfaq, Z., Anwar, R., Mahmood, M., Jameel, F., & Khan, M. A. (2024). Effects of Cucumis melo and Citrullus lanatus extracts on glucose level, lipid profile and hepato-renal performance of streptozotocin-induced diabetic albino rats. *Pakistan Journal of Pharmaceutical Sciences*, 37(1), 71–78.

Mohamad Salin, N. S., Md Saad, W. M., Abdul Razak, H. R., & Salim, F. (2022). Effect of Storage Temperatures on Physico-Chemicals, Phytochemicals and Antioxidant Properties of Watermelon Juice (Citrullus lanatus). *Metabolites*, 12(1), 75. <https://doi.org/10.3390/metabo12010075>

Moiz, A., Filion, K. B., Tsoukas, M. A., Yu, O. H., Peters, T. M., & Eisenberg, M. J. (2025). Mechanisms of GLP-1 Receptor Agonist-Induced Weight Loss: A Review of Central and Peripheral Pathways in Appetite and Energy Regulation. *The American Journal of Medicine*, 138(6), 934–940. <https://doi.org/10.1016/j.amjmed.2025.01.021>

Moneim, A., Sulieman, A. M., & Ibrahim, S. (2022). *Antioxidant and pharmacological activity of watermelon (Citrullus lanatus) seed oil.* <https://doi.org/10.1016/B978-0-12-824135-6.00027-1>

Moore, A. G. S., & Okoduwa, S. I. R. (2026). Modulation of PDX1 gene expression and glycemic control by Citrullus lanatus in experimental type 2 diabetes. *Scientific Reports*, 16(1), 3793. <https://doi.org/10.1038/s41598-025-33982-9>

Nadeem, M., Navida, M., Ameer, K., Iqbal, A., Malik, F., Nadeem, M. A., Fatima, H., Ahmed, A., & Din, A. (2022). A comprehensive review on the watermelon phytochemical profile and their bioactive and therapeutic effects. *Food Science and Preservation*, 29(4), 546–576. <https://doi.org/10.11002/kjfp.2022.29.4.546>

Noor, A. I. O., & Terefe, E. M. (2025). Evaluation of the antidiarrheal activity of dichloromethane-methanol crude extract of the aerial parts of Croton kinondoensis (Euphorbiaceae) in Mice. *PLOS One*, 20(10), e0333527. <https://doi.org/10.1371/journal.pone.0333527>

- Nugrahani, N., Anggraeni, C. N., Widyastuti, N., & Kholifa, M. (2025). Analyzing Aloe vera and Avocado Seed Extracts for Antioxidants, Saponins, Tannins, Flavonoids, and Alkaloids Using the UV-VIS Spectrophotometric Method. *F1000Research*, 14, 36. <https://doi.org/10.12688/f1000research.157091.2>
- Nurkenov, T., Tsoy, A., Olzhayev, F., Abzhanova, E., Turgambayeva, A., Zhussupova, A., Avula, B., Ross, S., Aituarova, A., Kassymova, D., Zhusupova, G., Shalakhmetova, T., Tokay, T., Lee, J. C., & Askarova, S. (2021a). Plant Extract of Limonium gmelinii Attenuates Oxidative Responses in Neurons, Astrocytes, and Cerebral Endothelial Cells In Vitro and Improves Motor Functions of Rats after Middle Cerebral Artery Occlusion. *Antioxidants*, 10(11), 1814. <https://doi.org/10.3390/antiox10111814>
- Nurkenov, T., Tsoy, A., Olzhayev, F., Abzhanova, E., Turgambayeva, A., Zhussupova, A., Avula, B., Ross, S., Aituarova, A., Kassymova, D., Zhusupova, G., Shalakhmetova, T., Tokay, T., Lee, J. C., & Askarova, S. (2021b). Plant Extract of Limonium gmelinii Attenuates Oxidative Responses in Neurons, Astrocytes, and Cerebral Endothelial Cells In Vitro and Improves Motor Functions of Rats after Middle Cerebral Artery Occlusion. *Antioxidants*, 10(11), 1814. <https://doi.org/10.3390/antiox10111814>
- Nyadanu, D., Lowor, S. T., Akpertey, A., Tchokponhoué, D. A., Pobee, P., Dogbatse, J. A., Okyere, D., Amon-Armah, F., & Brako-Marfo, M. (2020). Genetic variability of bioactive compounds and selection for nutraceutical quality in kola [*Cola nitida* (Vent) Schott. And Endl.]. *PLoS ONE*, 15(12), e0242972. <https://doi.org/10.1371/journal.pone.0242972>
- Obehioye, L. (2023). *ANTIDIABETIC EFFECTS OF WATER MELON AND PAWPAW SEED POWDERS AND OILS IN ALLOXAN- INDUCED DIABETIC RATS*.
- OECD. (2022). *Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure*. OECD. <https://doi.org/10.1787/9789264071049-en>
- Ogbeifun, H., Peters, D., & Monanu, M. (2023). *Ameliorative Effect of Citrullus lanatus (Water Melon) Seeds on Alloxan Induced Hepato and Nephro Toxicity*. <https://doi.org/10.9734/AJARR/2020/v9i430223>
- Okotie, H. O., Anjuwon, T. M., Okonkwo, O. L., Ameh, D. A., & James, D. B. (2023). Antidiabetic, Antioxidant and Anti-Inflammatory Activities of Residual Aqueous Fraction of *Ethulia conyzoides* in Induced Type 2 Diabetic Rats. *Tropical Life Sciences Research*, 34(1), 121–138. <https://doi.org/10.21315/tlsr2023.34.1.8>
- Olubodun, S. O., Iyamu, P. E., Henry, E. U., Idris, J. A., & Osemwengie, T. I. (2024). Toxicology of the renal indices of Wistar rats induced with leaf extracts of *Acalypha wilkesiana*. *Journal of Biological Research and Biotechnology*, 22(3), 2522–2531.
- Özcanlı Çay, Ö., & Çalışkan, H. (2026). Anxiety-like behaviors increased in juvenile rats in the streptozotocin-induced diabetes model during the early stage. *World Journal of Psychiatry*, 16(7). <https://doi.org/10.5498/wjp.118260>

Qiu, C., Li, S., Jia, S., Yu, F., Wei, C., Wang, X., & Guo, B. (2025). The effects of exercise on oxidative stress MDA and SOD in patients with type 2 diabetes: A systematic review and meta-analysis. *PeerJ*, 13, e19814. <https://doi.org/10.7717/peerj.19814>

Rabiu, K., Nura, L., & Aderounmu, G. (2025). Ameliorative Effects of Supplements of Papaya Seed, Watermelon Seed, and Clove buds on Testosterone-DMBA Induced Prostate Cancer in Wistar Rats: Oxidative Stress, Inflammation, and Histological Analysis. *The Nigerian Health Journal*, 25(3), 1106–1124. <https://doi.org/10.71637/tnhj.v25i3.1150>

Rajizadeh, M. A., Aminizadeh, A. H., Esmaeilpour, K., Bejeshk, M. A., Sadeghi, A., & Salimi, F. (2023a). Investigating the effects of *Citrullus colocynthis* on cognitive performance and anxiety-like behaviors in STZ-induced diabetic rats. *The International Journal of Neuroscience*, 133(4), 343–355. <https://doi.org/10.1080/00207454.2021.1916743>

Rajizadeh, M. A., Aminizadeh, A. H., Esmaeilpour, K., Bejeshk, M. A., Sadeghi, A., & Salimi, F. (2023b). Investigating the effects of *Citrullus colocynthis* on cognitive performance and anxiety-like behaviors in STZ-induced diabetic rats. *The International Journal of Neuroscience*, 133(4), 343–355. <https://doi.org/10.1080/00207454.2021.1916743>

Rajkumar, M., Sakthivel, M., Senthilkumar, K., Thangaraj, R., & Kannan, S. (2022). Galantamine tethered hydrogel as a novel therapeutic target for streptozotocin-induced Alzheimer's disease in Wistar rats. *Current Research in Pharmacology and Drug Discovery*, 3, 100100. <https://doi.org/10.1016/j.crphar.2022.100100>

Roy, S., Ghosh, A., Majie, A., Karmakar, V., Das, S., Dinda, S. C., Bose, A., & Gorain, B. (2024). Terpenoids as potential phytoconstituent in the treatment of diabetes: From preclinical to clinical advancement. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, 129, 155638. <https://doi.org/10.1016/j.phymed.2024.155638>

Salem, E. A., Alqahtani, S. M., El-Shoura, E. A. M., Zaghloul, S. S., Abdelzaher, L. A., Mohamed, S. A. M., Alalhareth, I. S., & Sheref, A. A. M. (2025). Neuroprotective effects of semaglutide and metformin against rotenone-induced neurobehavioral changes in male diabetic rats. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 398(9), 11919–11931. <https://doi.org/10.1007/s00210-025-03920-7>

Shah, A. A., Gupta, A., Rehman, A.-U., Kapoor, S., Kaur, H., Rohilla, B., Rashmi, K., & Bajpai, A. (2023). GC-MS Analysis of Phytoactive Compounds, Antioxidant and Antibacterial Activity of *Citrullus lanatus* Seeds. *Biomedical and Pharmacology Journal*, 16(1), 73–86. <https://doi.org/10.13005/bpj/2589>

Shamsudin, N. F., Ahmed, Q. U., Mahmood, S., Shah, S. A. A., Sarian, M. N., Khattak, M. M. A. K., Khatib, A., Sabere, A. S. M., Yusoff, Y. M., & Latip, J. (2022). Flavonoids as Antidiabetic and Anti-Inflammatory Agents: A Review on Structural Activity Relationship-Based Studies and Meta-Analysis. *International Journal of Molecular Sciences*, 23(20), 12605. <https://doi.org/10.3390/ijms232012605>

Shen, Y., Li, M., Wang, K., Qi, G., Liu, H., Wang, W., Ji, Y., Chang, M., Deng, C., Xu, F., Shen, M., & Sun, H. (2022). Diabetic Muscular Atrophy: Molecular Mechanisms and Promising Therapies. *Frontiers in Endocrinology*, 13, 917113. <https://doi.org/10.3389/fendo.2022.917113>

Singh, P., Walia, V., & Verma, P. K. (2025). Effect of Combined Treatment with Levofloxacin and Metformin on Diabetes-the Diabetes Related Behavioral and Biochemical Alterations. *Turkish Journal of Pharmaceutical Sciences*, 21(6), 528. <https://doi.org/10.4274/tjps.galenos.2024.26786>

Solanki, H. K., Dabhade, K. A., Bupesh, G., Arumugam, V. A., Visvanathan, P., Bhaskar, M., & Saravanan, K. M. (2023). Phytoconstituent screening and in vitro hypoglycemic and antioxidant properties of terpenoid fraction of Kaempferia pulchra extracts in Indian traditional medicine. *Journal of Herbmed Pharmacology*, 12(2), 194–201. <https://doi.org/10.34172/jhp.2023.20>

Szablewski, L. (2025). Associations Between Diabetes Mellitus and Neurodegenerative Diseases. *International Journal of Molecular Sciences*, 26(2), 542. <https://doi.org/10.3390/ijms26020542>

Xing, C., Liu, S., Wang, L., Ma, H., Zhou, M., Zhong, H., Zhu, S., Wu, Q., & Ning, G. (2024). Metformin enhances endogenous neural stem cells proliferation, neuronal differentiation, and inhibits ferroptosis through activating AMPK pathway after spinal cord injury. *Journal of Translational Medicine*, 22(1), 723. <https://doi.org/10.1186/s12967-024-05436-9>

Yaseen, B., Alkotaji, M., & Khalaf, M. (2025). Metformin and Body Weight: A Narrative Review of Clinical Evidence and Mechanistic Insights. *Iraqi Journal of Pharmacy*. <https://doi.org/10.33899/iraqij.p.2025.165044.1171>

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