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* **Corresponding author**.

yusupharm123@gmail.com

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Evaluation of the Ameliorative Effects of *Persea americana* Seeds on the Biochemical and Hematological Parameters in Type 2 Diabetic Rats

Mukasa Yusuf[1]*

1 Principal Investigator, Department of Pharmacology and Therapeutics, Makerere University, P.O Box 4004, Kampala, Uganda

Abstract

Background: Diabetes Mellitus (DM) is a chronic hyperglycemic condition with various types, however, Type 2 DM or Non-Insulin Dependent Diabetes Mellitus (NIDDM) accounts for 90% of all DM cases. Type 2 DM thus poses the biggest burden, necessitating a search for sustainable alternatives to its costly conventional management. Medicinal plant extracts are key to addressing the cost management related challenges, especially for resource limited settings. We studied the effects of *Persea americana* seed extracts on biochemical and hematological indices because these get deranged in NIDDM, indicative of a worsening prognosis. Studies on seed extracts of *P. americana* showed beneficial effects in NIDDM, however, these focused majorly on Fasting Blood Glucose (FBG) and lipids. We further explored the effects of the methanolic (ME) and total crude (TCE) extracts on the hematological indices in NIDDM rats. **Materials and Methods:** Type 2 DM was induced in the study animals using streptozotocin. The diabetic control and glibenclamide (GLB) groups orally received water and 10mg/kg B.W.T of GLB respectively, whereas other groups orally received either 200mg or 400mg doses of both extracts per kg B.W.T. **Results :** Only the higher dose of the TCE (TCE400) had a comparable FBG lowering effect to GLB ($p=0.309$), however, all extract groups except ME200 had ameliorative effects ($p=0.09$, 1.00 and 0.01 for ME400, TCE200 and TCE400 respectively). The effects of all extracts on total cholesterol (TC) and LDL unlike on triglycerides and HDL were significantly ($p<0.05$) better than glibenclamide's. Only the TCEs had ameliorative effects on TC levels ($p=0.18$ and 0.48 for TCE200 and TCE400 respectively). All extracts except ME200, had significantly ($p<0.05$) better activity on mean MCH and MCV levels in comparison to GLB. Only TCE400 caused a more significant improvement in the mean RBC than GLB ($p=0.004$) and only TCE400 and ME400 had more significant improvement in Hct levels than GLB ($p=0.04$). **Conclusion:** Total crude extracts of *Persea americana* seed at doses of about 400mg/kg body weight can improve the treatment outcomes

of DM in diabetic animals.

Keywords: Traditional medicine; Medicinal plant; Maceration; Total crude extract; Diabetes Mellitus; Ameliorative; Antidiabetic; Oral hypoglycemic agents (OHAs)

1 Introduction

1.1 Background

Diabetes Mellitus (DM) is one of the fastest-growing global health emergencies of the 21st century, affecting more than half a billion people today.[1] Of its various types, Type 2 DM, or Non-Insulin Dependent Diabetes Mellitus (NIDDM) accounts for 90% of all cases and was estimated in 2017 to have a global prevalence of 425 million in adults aged 20 to 79 years.[2] This poses a huge public health burden since the prevalence is expected to reach 783 million by 2045.[1]

Type 2 DM is caused by a combination of resistance to insulin action (predominant) and varying degrees of inadequate compensatory insulin secretory response or increased glucose production.[2] Excessive nutritional states favor insulin resistance whereas excess free fatty acids and hyperglycemia result into reduced insulin secretion through inducing endoplasmic reticulum stress thereby causing β-cell dysfunction.[3] Hyperglycemia also induces the formation of reactive oxygen species (ROS) and the activation of inflammatory mediators.[4] Complications of NIDDM are believed to be due to alterations in both biochemical and hematological parameters.[4,5]

The management of NIDDM includes both pharmacological and non-pharmacological interventions, however, the use of OHAs, i.e., the former approach, is the main stay of NIDDM management.[6,7] The anti-inflammatory properties of the conventional OHAs independent of their blood glucose lowering property is, however, unclear.[8,9] These medications are also costly and therefore impose a substantial economic burden especially to low-income countries.[1,10]

Various anti-diabetic medicinal plants are sought as alternatives to OHAs, and their use is recommended by WHO with extracts obtained from different plant parts reported to manage NIDDM.[11] *P. americana* seed extracts possess hypoglycemic, antioxidant and anti-inflammatory properties with a potential to reverse or halt further progress of NIDDM.[12,13] The required cost-effective therapies can be obtained from seed extracts of *P. americana*. Furthermore, since the seeds are usually discarded after consumption of the fruit, their utilization for medicinal purposes can minimize resource wastage as well as ecological problems that can likely arise from poor disposal. This therefore justified the selection of *P. americana* seed extracts in the evaluation of the ameliorative effects in NIDDM animal models.

This study objectively evaluated the ameliorative effects of *P. americana* seed extracts in NIDDM with findings supporting earlier studies on the efficacy in NIDDM with respect to fostering improvements in FBG and lipid profile.

The ameliorative effects of *P. americana* seed extracts on hematological parameters were also assessed in this study and findings can be used to make recommendations on how to effectively utilize extracts of *P. americana* seeds to manage NIDDM as well as lay a platform for additional research.

1.2 Research Objectives

1. Do the methanolic and total crude extracts of *P. americana* seeds ameliorate biochemical derangements in Type 2 Diabetes Mellitus?
2. Do the methanolic and total crude extracts of *P. americana* seeds ameliorate hematological derangements in Type 2 Diabetes Mellitus?

2 Materials and Methods

2.1 Study type and area

This was an experimental laboratory based in-vivo preclinical study conducted at the Department of Pharmacology & Therapeutics, School of Biomedical Sciences, Makerere University College of Health Sciences located at Mulago Hospital complex, Kampala, Uganda.

2.2 Selection of plant materials

Convenient selection of source of *P. americana* fruits (avocado) was done and 124 ripe fruits were procured from a garden in Luzira located in Kampala, Central Uganda, GPS coordinates (26.3008° S, 27.9482° E).

2.3 Preparation of plant materials (Identification, Collection and Drying)

A leaf of the avocado plant from which the fruits were obtained was examined by a taxonomist to identify the specific species by its local name, common name, scientific name and morphological descriptions at the Makerere University herbarium. A voucher specimen was prepared and a reference number (MY 001M), was given for future reference with the specimen kept in Makerere University herbarium.

The succulent fleshy parts of the avocado fruit were removed using a clean stainless-steel knife to obtain the seeds which were then sliced into smaller pieces using a knife and air-dried under a shed to a constant weight.

2.3.1 ***Extraction Process***

The dry slices of *P. americana* seeds were ground in a wooden mortar and pestle to obtain a fine powder which was stored in a clean glass container. Extraction was done by maceration process by soaking 515g of the powder in 2.5 liters of 99% methanol i.e., the methanolic extract, (ME) and 520g of the powder serially, first in 2.5 liters of diethyl ether, followed by 2.5 liters of 99% methanol and finally, distilled water, i.e., the total crude extract, (TCE). The mixtures were shaken regularly and were filtered after the third day using a universal filter paper and funnel. The filtrate for the different solvents was then evaporated using a Heidolph rotary evaporator (Model number R-210/R) at a temperature of 60°C to obtain semi dry extracts. Equal volumes of the semi dry extracts obtained using the three different solvents for the TCE were mixed together. The semi dry extracts were then placed in an oven at 30°C until they dried. The mass obtained after drying was weighed using a digital weighing scale (KERN EW 600-2M, Kern & Sohn, Germany), and the percentage yield was calculated using the formula:

Percentage yield = Weight of the concentrated extract obtained (g) / (Weight of the plant powder used (g)) * 100.

The weighed mass of *P. americana* extracts was then dissolved independently in distilled water to achieve the desired doses prior to each administration.

2.4 Experimental animals

2.4.1 ***Sample size***

This was calculated using Mead's resource equation. The minimum number of rats per group, n = (10/6) +1 ≈ 3 rats per group. The maximum number of rats per group, n = (20/6) +1 ≈ 5 rats per group. Therefore 5 rats were selected per group.

2.4.2 ***Inclusion criteria***

Wistar albino male rats of 8 ($\pm$1) weeks old were included into the study following assessment of their FBG level on the 24th day of the study with the cut off set at $\geq$ 250 mg/dL.

2.4.3 ***Exclusion Criteria***

The sickly looking, malnourished appearing Wistar albino rats and those out of the 8 $\pm$1 weeks and 100 to 150 g, age and weight ranges respectively were excluded from this study.

2.4.4 ***Animal handling***

The rats were randomly assigned to six different groups using a simple random sampling technique. These rats were kept in wooden cages of ≈450 square cm floor area under standard conditions of 12 hours dark and light cycle, at a temperature of 25 $\pm$2 ^{0}C and relative humidity of about 40-70% for 7 days to enable acclimatization.

2.4.5 ***Induction of DM in the rats***

Streptozotocin (99% potency, Med Chem Express-USA) solution for injection was freshly prepared by dissolving 232.738mg of the powder in 11.65 ml of cold normal saline solution to make a stock solution at a concentration of 20mg/ml. Each rat intraperitoneally received 60mg/kg body weight of this solution with the aid of a 2ml, 23-gauge needle following a fasting period of 6 hours. Each rat was then orally given 1ml of a distilled water mixed with glucose. Initial assessment for the development of DM following routine monitoring of the rats was done 48 hours later and confirmation of the development of DM was made after another 24 hours, on day 24 of the study. Note that day 1 of the study was considered as the day the rats were obtained before they were acclimatized. Assessment was done by drawing

blood from the rat tails and taking a reading using an On call plus glucometer and On call plus glucose strips (Acon Labs Inc, USA) to assess the blood glucose concentration following a 6-hour fasting period. Only rats whose FBG levels was or exceeded 250mg/dL were considered diabetic. Dosing of these rats with the various study interventions including distilled water and glibenclamide as the negative and positive controls as well as the lower (200mg/kg body weight) and higher (400mg/kg body weight) doses of both the methanolic and total crude extracts was started on the same day (day 24 of the study) of confirmation of their diabetic status and continued for another 7 days following the assessment of their baseline biochemical and hematological parameters i.e., parameters from blood samples taken on day 24 after confirmation of DM status.

2.4.6 ***Biochemical and Hematological parameter assessment***

These in addition to each rat's body weight were assessed as follows; two separate blood samples of approximately 0.5 to 1ml were collected in separate vacutainers from a pricked tail following a fasting period of 6 hours. The rats were, however, given access to drinking water during the 6 hours of fasting.

- **For hematological parameters**

Vacutainers containing Ethylene Diamine Tetra Acetate (EDTA) were used to collect the blood samples of about 0.8 to 1ml which were then mixed by inversion and then analyzed using an automatic hematology analyzer (SYS MIX, Japan) at St Jude laboratories located in Luzira, Kampala, Uganda.

- **For biochemical parameters**

Blood samples of about 0.8 to 1ml were collected in vacutainers which were then centrifuged (Eppendorf® Minispin® model SPIN 1.000, Hamburg, Germany) for 5 minutes at a g force of 769m/s^2 and then processed using a spectrophotometer (Shimadzu, Japan) at the same laboratory for the assessment of the levels of triglycerides, total cholesterol, LDL and HDL parameters.

- **For Body Weight**

Each rat was individually weighed using a calibrated weighing scale (KERN EW 600- 2M, Kern & Sohn, Germany). The healthy rats were then continued on the pellet diet, but the daily meal was doubled to increase calorie intake for two weeks.

2.4.7 ***Administration of study interventions***

The study interventions were orally administered using an oral intragastric tube with the dose per unit body weight. The diabetic control group, each rat received 1ml of distilled water per day for the entire 7 days.

For the ME200 group, ≈716mg of the ME was dissolved in 52ml of distilled water to make a concentration of ≈14mg/ml and each rat received a given volume of the mixture based on its baseline body weight per day for the 7 days.

For the ME400 group, ≈1,567mg of the ME was dissolved in 113ml of distilled water to make a concentration of ≈14mg/ml and each rat received a given volume of the mixture based on its baseline body weight per day for the 7 days.

For the TCE200 group, ≈706mg of the TCE was dissolved in 66ml of distilled water to make a concentration of ≈11mg/ml and each rat received a given volume of the mixture based on its baseline body weight per day for the 7 days.

For TCE400 group, ≈1611mg of the TCE was dissolved in 151ml of distilled water to make a concentration of ≈ 11mg/ml and each rat received a given volume of the mixture based on its baseline body weight per day for the 7 days.

For the GL10 group, 8 tablets of glibenclamide, 5mg strength were finely crashed and dissolved in 32ml of distilled water to make a concentration of ≈1.25mg/ml. Each rat received a given volume of the mixture based on its baseline body weight per day for the 7 days.

Following the 7-day treatment period i.e., on day 31 of the study, the biochemical and hematological parameters as well as body weight were reassessed. The rats were then euthanized on the same day by intraperitoneally injecting each rat with a combination of Ketamine and xylazine at doses of 300 mg and 30 mg/kg body weight respectively followed by cervical dislocation. The doses of the euthanizing agents were calculated based on the body weights measured on day 31 of the study.

2.5 Variables

The dependent variables included the biochemical and hematological parameters measured from each rat in each group whereas independent variables included the interventions that were orally administered to the study animals.

2.6 Data Management and Analysis

Data was entered excel and the mean, standard deviation as well as the standard error of the mean were calculated. Changes during the study course, including the 3 different sampling intervals i.e., Days, 7, 24 and 31, for each group were independently compared using paired (dependent) T-tests. Multiple group comparisons were performed for the extract groups alone (4 group comparisons) as well as with glibenclamide (5 group comparisons) using Welch's ANOVA and finally, comparisons between the various groups considering the change in the mean levels of the selected parameters between day 24 and day 31 were done using independent T-test, assuming unequal variances. Statistical

significance was considered at a p-value of 0.05.

2.7 Ethical Considerations

This work was presented to the Department of Pharmacology and Therapeutics which approved it for ethical consideration under the school of biomedical Sciences research and ethics committees. The proposed protocol was also submitted to the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB) located at Makerere University Kampala, Uganda for approval prior to the conduct of the study.

3 Results

3.1 Effects of interventions on fasting blood glucose (FBG) levels

The mean FBG levels in the negative diabetic control (DC) group exhibited persistent significant elevation throughout the study. Both doses of the two extract types like glibenclamide significantly improved the FBG by lowering it from the elevated levels recorded on day 24, following induction with streptozotocin (STZ). The improvement in FBG levels by both doses of the ME and TCE200 was significantly less than that of glibenclamide, however, the higher doses of both extracts were significantly better than the lower doses (Table 1).

3.2 Effects of interventions on lipid parameters

The mean HDL inversely correlated with LDL, TC and TG, with the mean TC, TG and LDL levels in the negative DC group exhibiting persistent significant increase throughout the study. All extracts significantly improved the TC levels when compared to glibenclamide but ME400 was significantly better than ME200.

The effects of glibenclamide on the mean TG levels unlike that of the extracts was not significant.

Glibenclamide, like the extracts caused significant improvements in the LDL levels, however, the effects of the TCEs were significantly better than that of glibenclamide.

The improvement in HDL levels by all extracts unlike glibenclamide was significant but still comparable to that of the latter as well as to each other (Table 2).

3.3 Effects of interventions on hematological parameters

The correlations among the various hematological parameters were significant with the strongest being between MCV and Hct (r=0.99) and the weakest being between RBCs and MCHC (r=0.76).

The mean RBC, MCH, MCHC, MCV, Hbg and Hct levels in the negative DC group on day 31 were significantly (p=0.00) lower than the levels recorded on day 7 with the TCEs exhibiting a remarkable improvement in the mean RBC counts. The effect of all extracts on MCH levels were significantly better than that of glibenclamide but only the higher doses of both extract types caused a significant improvement in the mean MCHC (p=0.001 and p=0.01) for ME and TCE respectively. All extracts significantly improved the mean MCV levels with ME400 and the TCEs having a significantly better activity than glibenclamide. All extracts unlike glibenclamide significantly improved the mean Hbg and Hct levels and their effect was significantly better than that of glibenclamide (Table 3).

3.4 Comparison of the different extract doses of the mean FBG levels

The effects on FBG levels for comparisons among the extract treated groups alone and with glibenclamide on day 31 were significant with p-values, 0.001 and 0.00* respectively. TCE400 exhibited the greatest and comparable effects to glibenclamide (p=0.31) and ME400 (p=0.08) but significantly better activity than TCE200 (p=0.01) and ME200 (p=0.003). The FBG effect for TCE200 was comparable to both doses of the ME200 (p=0.09) and ME400 (p=0.08).

3.5 Comparison of the different extract doses on the lipid profile

Comparisons in the mean TG levels among the extract treated groups were significant (p=0.00). TCE400 exhibited the greatest but comparable effects to TCE200 (p=0.08) but a significantly greater activity than ME400 (p=0.001) and ME200 (p=0.00). TCE200 exerted a significantly better effect than both doses of the ME. Group comparisons regarding the mean TC levels were significant (p=0.03). TCE200 exhibited the greatest but comparable effect to ME400 (p=0.71) as well as a significantly greater activity than TCE400 (p=0.04) and ME200 (p=0.03). ME400 exerted a significantly better effect than TCE400 (p=0.02).

Group comparisons regarding the mean LDL levels were significant (p=0.003). TCE (400) exhibited the greatest effect which was comparable to ME400 (p=0.37) and TCE200 (p=0.05) but significantly greater than ME200 (p=0.001). TCE 200 exerted a significantly better effect than ME200 (p=0.01), however, its activity was comparable to that of ME400 (p=0.36).

3.6 Comparison of the different extract doses on the hematological indices

Extract group comparisons for the mean RBC levels were significant (p=0.04) but only TCE400 showed significant

Table 1. Changes in Fasting Blood Glucose during the Study Course

Group/ Dose (mg/kg)	Changes in fasting blood glucose (mean ± SEM, mg/dL) during the study			Day 7 vs Day 24 (p-value)	Day 31 vs Day 7 (p-value)	Day 24 vs Day 31 (p-value)
	Day 7 (Pre-diabetic, Baseline)	Day 24 (post-STZ, pre-treatment)	Day 31 (post treatment), (% change)			
DC (Water)	133.44 ± 3.76	295.15 ± 5.87	322.84 ± 2.84 (9.4%↑)	*0.00	*0.00	*0.04
ME200	129.76 ± 1.31	298.36 ± 6.05	141.65 ± 2.19 (52.5%↓)	*0.00	*0.04	*0.00
ME400	126.07 ± 1.22	317.07 ± 5.20	133.26 ± 1.65 (60%↓)	*0.00	0.09	*0.00
TCE200	130.57 ± 1.43	287.23 ± 6.02	128.65 ± 1.64 (55.2%↓)	*0.00	1.00	*0.00
TCE400	131.96 ± 2.43	302.43 ± 6.12	114.69 ± 4.72 (62.1%↓)	*0.00	*0.01	*0.00
GL10	128.73 ± 0.76	310.11 ± 7.06	109.37 ± 2.98 (64.7%↓)	*0.00	*0.01	*0.00

DC: Diabetic Control, ME: Methanolic Extract, TCE: Total Crude Extract, GL10: Glibenclamide [Values are expressed as mean ± SEM (n=5), Percentage increase (↑) or decrease (↓), *significant at p<0.05]

Table 2. Changes in the Lipid Parameters during the Study Course

Group/ Dose (mg/kg)	Changes in Lipid Profiles (mean ± SEM, mg/dL) during the study			Day 7 vs Day 24 (p-value)	Day 31 vs Day 7 (p-value)	Day 24 vs Day 31 (p-value)
	Day 7 (Pre-diabetic, Baseline)	Day 24 (Post-STZ, pre- treatment)	Day 31 (Post treatment) (% change)			
			Total Cholesterol			
DC (Water)	68.43 ± 4.04	131.88 ± 1.30	159.43 ± 1.28 (20.9%↑)	*0.00	*0.00	*0.00
ME200	65.65 ± 0.58	125.24 ± 1.91	98.14 ± 1.80 (21.6%↓)	*0.001	*0.001	*0.02
ME400	69.66 ± 1.83	128.56 ± 1.52	82.42 ± 0.70 (35.9%↓)	*0.00	*0.001	*0.00
TCE200	69.37 ± 3.98[d]	130.11 ± 1.87	78.80 ± 1.11 (39.4%↓)	0.001	0.18	*0.00
TCE400	70.22 ± 2.34	127.48 ± 1.17	74.06 ± 0.92 (41.9%↓)	*0.00	0.48	*0.00
GL10	65.28 ± 1.24	125.13 ± 2.22	101.84 ± 3.09 (18.6%↓)	*0.00	*0.003	0.09
			Triglycerides			
DC (Water)	84.11 ± 1.17	108.45 ± 2.95	123.88 ± 4.97 (14.2%↑)	*0.003	*0.003	0.06
ME200	83.67 ± 0.76	105.04 ± 2.37	88.22 ± 0.66 (16.0%↓)	*0.003	0.15	*0.01
ME400	87.01 ± 1.35	110.81 ± 2.90	85.62 ± 1.80 (22.7%↓)	*0.01	0.81	**0.001
TCE200	85.75 ± 1.01	114.05 ± 4.07	86.82 ± 1.25 (23.9%↓)	*0.003	0.66	*0.003
TCE400	84.32 ± 1.37	100.65 ± 0.46	83.09 ± 1.06 (17.5%↓)	*0.003	1.00	*0.00
GL10	86.54 ± 1.60	109.24 ± 3.19	93.18 ± 2.30 (14.7%↓)	*0.03	*0.03	0.09
			Low Density Lipoprotein			
DC (Water)	37.12 ± 1.08	94.60 ± 1.23	106.88 ± 3.28 (13.0%↑)	*0.00	*0.00	*0.01
ME200	38.54 ± 1.74	87.14 ± 1.94	45.82 ± 1.76 (47.4%↓)	*0.00	0.06	*0.00
ME400	35.34 ± 1.22	88.33 ± 1.11	33.62 ± 1.62 (61.9%↓)	*0.00	0.24	*0.00
TCE200	38.96 ± 1.79	90.22 ± 2.63	36.78 ± 1.03 (59.2%↓)	*0.00	0.96	*0.00
TCE400	36.22 ± 0.51	93.48 ± 2.79	33.06 ± 1.06 (64.6%↓)	*0.00	0.06	*0.00
GL10	36.38 ± 1.27	95.27 ± 4.12	58.98 ± 1.81 (38.1%↓)	*0.00	*0.001	*0.001
			High Density Lipoprotein			
DC (Water)	36.51 ± 0.65	30.61 ± 1.34	29.88 ± 1.05 (2.4%↓)	0.09	*0.02	0.66
ME200	37.50 ± 1.78	30.56 ± 0.40	35.99 ± 1.00 (17.8%↑)	*0.003	1.00	*0.03
ME400	37.88 ± 1.23	32.40 ± 0.70	39.62 ± 1.04 (22.3%↑)	0.06	1.00	*0.02
TCE200	36.96 ± 0.69	29.98 ± 0.87	36.81 ± 1.03 (22.8%↑)	*0.001	1.00	*0.03
TCE400	34.27 ± 1.16	31.11 ± 0.38	38.04 ± 1.17 (22.3%↑)	0.12	0.18	*0.003
GL10	35.37 ± 0.55	31.75 ± 0.40	34.22 ± 1.31 (14.1%↑)	*0.003	*0.04	0.06

DC: Diabetic Control, ME: Methanolic Extract, TCE: Total Crude Extract, GL10: Glibenclamide [Values are expressed as mean ± SEM (n=5), Percentage increase (↑) or decrease (↓), *significant at p<0.05].

Table 3. Changes in the Hematological Parameters during the Study Course

Group/ Dose (mg/kg)	Changes in Hematology profiles (mean ± SEM, units)			Day 7 vs Day 24 (p-value)	Day 31 vs Day 7 (p-value)	Day 24 vs Day 31 (p-value)
	Day 7 (Pre-diabetic, baseline)	Day 24 (post-STZ, pre-treatment)	Day 31 (post treatment, % change)			
			Red Blood Cell count (mean ± SEM, 10^6 /μL)			
DC (Water)	7.13 ± 0.14	5.90 ± 0.23	5.00 ± 0.16 (15.3%↓)	*0.01	*0.00	0.06
ME200	6.92 ± 0.24	5.43 ± 0.05	6.12 ± 0.31 (12.7%↑)	*0.01	0.21	0.21
ME400	7.05 ± 0.21	6.04 ± 0.18	6.93 ± 0.48 (14.7%↑)	0.14	1.00	0.42
TCE200	7.16 ± 0.52	5.75 ± 0.20	7.01 ± 0.60 (21.9%↑)	0.15	1.00	0.24
TCE400	7.02 ± 0.25	5.22 ± 0.29	7.40 ± 0.67 (41.8%↑)	0.06	1.00	*0.03
GL10	7.21 ± 0.21	5.48 ± 0.17	6.00 ± 0.18 (10.5%↑)	*0.001	*0.001	0.21
			MCH (mean ± SEM, mmg)			
DC (Water)	19.34 ± 0.46	16.2 ± 0.52	11.09 ± 0.93 (31.5%↓)	*0.03	*0.001	*0.03
ME200	19.03 ± 0.48	16.92 ± 0.84	18.82 ± 0.75 (11.2%↑)	0.12	1.00	0.42
ME400	18.44 ± 0.68	17.33 ± 0.81	19.61 ± 0.30 (13.2%↑)	1.00	0.21	0.12
TCE200	19.15 ± 0.41	16.49 ± 0.82	17.80 ± 0.66 (7.9%↑)	0.24	1.00	0.30
TCE400	18.50 ± 0.47	17.08 ± 0.59	19.66 ± 1.61 (15.1%↑)	0.45	1.00	0.33
GL10	17.89 ± 0.64	18.10 ± 0.87	16.02 ± 0.48 (11.5%↓)	1.00	0.48	0.15
			MCHC (mean ± SEM, mg/dL)			
DC (Water)	30.14 ± 0.33	26.34 ± 0.95	19.46 ± 1.77 (26.1%↓)	0.06	*0.01	0.06
ME200	31.32 ± 0.56	27.56 ± 0.93	30.54 ± 0.58 (10.8%↑)	0.06	0.45	0.27
ME400	30.74± 0.47	27.21 ± 0.711	32.60 ± 1.04 (19.8%↑)	*0.03	0.45	*0.001
TCE200	31.22 ± 0.32	25.38 ± 0.67	30.66 ± 0.21 (21.0%↑)	*0.01	1.00	0.09
TCE400	30.98 ± 0.35	26.85 ± 1.12	33.40 ± 1.73 (24.3%↑)	0.06	1.00	*0.01
GL10	31.11 ± 0.44	27.44 ± 0.85	30.62 ± 2.14 (11.6%↑)	*0.03	1.00	0.6
			MCV (mean ± SEM, μm^3)			
DC (Water)	71.21 ± 0.68	62.17 ± 1.11	47.26 ± 0.82 (24%↓)	*0.01	*0.00	*0.003
ME200	72.43 ± 0.70	60.33 ± 0.77	67.14 ± 1.31 (11.3%↑)	*0.01	*0.03	*0.03
ME400	70.60 ± 1.06	59.87 ± 1.40	71.27 ± 1.87 (19.0%↑)	*0.003	1.00	*0.03
TCE200	73.29 ± 1.06	61.05 ± 0.39	71.46 ± 1.31 (17.1%↑)	*0.002	0.03	*0.01
TCE400	72.61 ± 0.47	60.79 ± 0.59	73.22 ± 0.86 (20.5%↑)	*0.001	1.00	*0.003
GL10	72.18 ± 0.40	60.00 ± 0.62	63.62 ± 0.96 (6.0%↑)	*0.00	0.002	0.21
			Hbg (mean ± SEM, g/dL)			
DC (Water)	15.33 ± 0.29	12.06 ± 0.54	9.20 ± 0.25 (23.7%↓)	0.05	*0.00	*0.03
ME200	15.24 ± 0.53	11.21 ± 0.38	15.76 ± 1.08 (40.6%↑)	*0.03	1.00	*0.02
ME400	17.01 ± 0.86	11.64 ± 0.43	16.91 ± 0.92 (45.3%↑)	*0.003	1.00	*0.01
TCE200	15.43 ± 0.34	12.36 ± 0.53	16.02 ± 0.69 (29.6%↑)	*0.01	1.00	*0.02
TCE400	16.03 ± 0.49	11.68 ± 0.35	17.04 ± 0.51 (45.9%↑)	*0.03	1.00	*0.00
GL10	16.50 ± 0.22	11.37 ± 0.46	15.05 ± 0.34 (32.4%↑)	*0.001	0.16	0.09
			Hct (mean ± SEM, %)			
DC (Water)	45.22 ± 0.99	34.50 ± 0.48	27.66 ± 1.10 (19.8%↓)	*0.003	*0.001	*0.01
ME200	44.38 ± 0.56	33.81 ± 0.83	42.14 ± 1.49 (24.6%↑)	*0.003	0.48	*0.03
ME400	45.14 ± 0.94	33.78 ± 1.02	45.63 ± 1.68 (35.0%↑)	*0.003	1.00	*0.003
TCE200	45.57 ± 0.78	34.54 ± 0.83	43.46 ± 1.14 (25.8%↑)	*0.003	0.63	*0.001
TCE400	46.01 ± 0.71	34.33 ± 1.19	46.20 ± 1.60 (34.6%↑)	*0.01	1.00	*0.01
GL10	45.27 ± 1.04	36.58 ± 1.45	40.22 ± 2.80 (10.0%↑)	*0.01	0.20	0.84

DC: Diabetic Control, ME: Methanolic Extract, TCE: Total Crude Extract, GL10: Glibenclamide [Values are expressed as mean ± SEM (n=5), Percentage increase (↑) or decrease (↓), *significant at p<0.05].

(p=0.03) improvement in the RBC count with its activity comparable to TCE200 (p=0.13) but a significantly better than that of ME400 (p=0.03) and ME200 (p=0.01). The effect of TCE200 was comparable to that of the MEs.

Comparisons for the mean MCH levels among the extract treated groups were significant (p=0.03). TCE400 exhibited a comparable effect to both ME400 (p=0.95) and ME200 (p=0.83). TCE200 also showed a comparable activity to both doses of the MEs. Only ME400 and TCE400 demonstrated significant improvement in the mean MCHC levels with (p=0.001 and 0.01) respectively. TCE200 showed a significantly better activity than both doses of the ME200 (p=0.02) and ME400 (p=0.04). Extract group comparisons regarding the mean MCV levels were significant (p=0.03). The effect of TCE400 was significantly (p=0.03) greater than that of ME200, however, the effects of all extracts on the mean Hbg and Hct levels were comparable i.e., ($0.08 \leq p \leq 0.92$) and ($0.12 \leq p \leq 0.92$) respectively.

4 Discussion

4.1 Extraction yield

The yield of *P. americana* seed extract like in other studies was very low probably due to the low quantity of medicinal plant soluble bioactive components.[14] Several factors such as extraction method determine the yield of extract which varies based on the method employed.[15]

The total crude extract obtained a comparatively higher yield than the methanolic and this may be attributed to its wider extraction potential for both polar and non-polar components of the plant material.[16] This is also because different solvents and methods employed extract different levels of phytochemical compounds.[17]

4.2 Induction of Diabetes Mellitus

The chemical used, STZ induced DM through the destruction of the pancreatic islet β-cell which reduced the population of functional beta cells eventually resulting in hyperglycemia and hence DM.[1,18]

4.3 Effects of P. *americana* seed extracts on biochemical parameters

Both extracts of *P. americana* seeds significantly (p<0.05) improved FBG by a mechanism which is unclear, however, attributed to improved pancreatic beta cell function through enhancing the secretion of insulin and c- peptide in addition to reducing insulin resistance.[19] Other studies attribute it to the presence of various elements that ensure blood glucose homeostasis through the regulation of the key enzymes involved in gluconeogenesis within the liver with subsequent blockade of gluconeogenesis and enhanced glucose utilization in the body in addition to the insulin stimulatory properties of the various phytochemicals including, flavonoids, saponins, steroids, terpenoids, tannins and alkaloids.[20]

Higher doses of both extract types performed better than the lower ones, a concept of dose dependent potency observed in other studies.[21,22]

Streptozotocin induces changes in lipid profiles as early as after 24 hours just as was observed in this study.[23] The abnormal change in lipids is due to DM induced dyslipidemia arising from disturbances in the regulation and expression of hormone-sensitive lipase, apoB100, microsomal triglyceride transfer protein, lipoprotein lipase, and hepatic triglyceride lipase because of insulin resistance.[24] In general, the higher doses of both extracts exerted better lipid lowering activity than the respective corresponding lower doses, as observed previously.[25,26]

The TCEs and ME400 in most instances exhibited significantly better lipid profile improvements than glibenclamide, findings consistent with another study.[26] The ethanolic extract of *P. americana* leaf exhibited better LDL lowering effects than glibenclamide in one study.[27] In another study, glibenclamide exhibited a significant improvement in the HDL but with no significant improvement for total cholesterol, triglycerides, LDL cholesterol and V-LDL cholesterol.[28] Metformin, acarbose, voglibose, rosiglitazone and pioglitazone had significant effects on the lipid profile among the various OHAs.[29]

The extracts of *P. americana* are believed to improve the lipid profile due to the presence of phytochemicals such as flavonoids that have potent antioxidant properties.[30] The mechanism by which flavonoids exert their antioxidant properties is through their ability to scavenge and reduce reactive oxygen species by interacting with them.[31] Flavonoids are also believed to lower lipids through the inhibition of cholesterol synthesis and increase of LDL receptor expression.[32]

4.4 Effects of *P. americana*seed extracts on hematological parameters

Almost all the various hematological indices showed a significant decrease after STZ administration possibly due to the increased destruction of RBC membranes with subsequent rupture of the cells under the influence of NIDDM induced oxidative stress.[33,34]

The administration of the *P. americana* seed extracts variably induced positive changes in these indices, even demonstrating ameliorative effects in some instances. The positive effects of the seed extracts could be attributed to their ability to halt or retard further break down of RBCs courtesy of their potent antioxidative properties.[35] Furthermore, the present flavonoids are believed to both stimulate erythropoietin production and prevent free radical induced hemolysis of RBCs.[36,37]

Findings from our study are like those of a study by Obiandu et al (2022) where some hematological indices,

specifically RBC count, Hbg and Hct were significantly improved following the administration of *P. americana* extracts.[38]

5 Conclusion

The two different doses of both extracts exhibited positive changes on both the biochemical and hematological parameters in the diabetic rats at variable magnitudes with the higher doses generally performing better. The ability of the extracts to improve the STZ induced derangements in these selected parameters to levels comparable to those of the healthy non-diabetic rats (baseline parameters taken on day 7) highlighted their ameliorative capability.

Appropriately selected doses of the seed extracts of *P. americana*, preferably of the total crude type can be used to manage DM by correcting changes in key prognostic markers, including biochemical and hematological parameters.

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