ACUTE TOXICITY ASSAY AND ANTI-DIABETIC ACTIVITIES OF SHEMMECO EXTRACT IN *Drosophila melanagaster*

Abstract

A chronic metabolic condition of insulin resistance or insulin insensitivity known as diabetes type 2 is characterized by persistent hyperglycemia with microvascular and macrovascular consequences. Adults with diabetes are two to three times more likely to experience heart attacks, strokes, neuropathy, foot ulcers and eventually amputation of the leg. Due to its genetic similarity to humans, *Drosophila melanogaster* (fruit fly) has been used as a model to study human diseases. The acute toxicity assay and anti-diabetic properties of Shemmeco extract were examined in this study. The LC50 in this study was conducted using the following concentrations: 5mg, 10mg, 25mg, 50mg, 100mg, 250mg, 500mg, 1000mg, 2500mg and 5000mg of extract, each per 10g fly diet. For ten days, *Drosophila melanogaster* was fed a high diet of sucrose (2.5g/10g diet) to elicit insulin resistance type 2 diabetes. The diabetic flies were treated with 5mg, 10mg, 25mg, 50mg and 250mg per 10g diet each of Shemmeco extract being safe concentrations from result of acute toxicity and 16mg of metformin/10g diet as standard drug. Glucose content significantly elevated ($p<0.05$) in diabetes untreated compared to control group. Metformin significantly declined ($p<0.05$) the glucose content compared to diabetes untreated. However, there was a slight decline of glucose content of fruit flies treated with 5mg (S1) of Shemmeco extract compared to diabetic untreated and metformin treated. The slight decline of glucose content of fruit flies treated with 5mg (S1) of Shemmeco extract therefore indicates that the extract is promising in the treatment of diabetes type 2 if developed further.

1.0 Introduction

Herbal medicine has been growing steadily and if properly harnessed can be put to much better use. Currently, several herbal formulations which are used for curative purposes have not been put to scientific investigation and this has continued to keep these formulations (some of which may actually be therapeutic) away from the public acknowledgement and reception as it should have been (Jagrai and Sarkar, 2022). Furthermore, most herbal formulation are kept as secrets with most persons not willing to acknowledge that they actually turn to them for curative need and practitioners not being able to explain the mechanisms by which the formulations carry out their curative activities (Eshete and Molla, 2021). Package and safety concerns of herbal formulations is another factor which has affected the growth of the sector, although some progress has been made in this area, it is still open Shemmeco Nobel Resources has identified this problem and aims to elucidate the positive role herbal formulation may have in the treatment and management of diabetes type 2 with the goal to improve the fight against this disease. Shemmeco Nobel Resources also hopes that actual mechanisms of action of Shemmeco formulation will be made available to the scientific community and pave the way for other researches too. Shemmeco Nobel Resources reserves the right to keep unpublished the procedures of formulation for Shemmeco herbal extract and result of its phytochemical composition until the patent is obtained.

The fruit fly, *Drosophila melanogaster* has been extensively studied for decades. It was introduced as a decisive method in biology about a century ago. Application of the *Drosophila* model is empowered by the fundamental fact that it shares basic biological, biochemical, neurological and physiological similarities with mammals. It is documented that about 75% of human disease causing genes have functional homologs in *D. melanogaster* (Adesola *et al.,* 2021).

Drosophila processes systems which control nutrient uptake, storage and metabolism; and these systems have been reported to be analogous to those of humans. *Drosophila* is well known for its high sensitivity to toxic substances and is thus considered a useful model for toxicity studies as well as evaluating the biological action of pharmacological agents (Adedara, I.A; Abolaji, A.O; Rocha, JBT (2016)

The LC50 is the concentration of toxicant that is lethal to 50% of sampled fly population, and it is primarily conducted to determine the safe concentration of the toxicant that the flies can survive on during treatment (Rizzo *et al.,* 2021).

2.0 Materials and methods

Method of sample collection

The flies used for this study was collected from the Federal Capital Territory (FCT), Abuja, Nigeria following the procedures below.

2.1 Installation of trap bait

Clean plastic bottles of about 750 mL were used to set trap for the flies. Ripe banana was sliced into the bottle with a pinch of yeast. A razor blade was used to open the middle of the bottles to allow the flies access food and bottles were placed in a damp and moist environment with minimal human interference. Trap was recovered after 48 hours of installation to collect flies.

2.2 Rearing and culturing

The collected samples were transferred from bait bottles into a collection tube. In moving flies away from the bottles to collection tubes, opening of the bottle was kept under bright light directing the flies towards the light. The flies were kept at room temperature (25°C) at 70% relative humidity with a 12 hr light/dark clock cycle in vials containing standard corn meal medium.

2.3 Identification of fly sex

The flies were given anesthesia (CO_2) that made them unconscious for about 25 mins. to sort the male and female. From recent studies, it is noted that female possess larger body size and have swollen abdomen thus, this was the basis for identification in this study.

2.4 Determination of acute toxicity for Shemmeco extract (LC50)

The LC50 in this study was conducted following the methods described by Mohammad & Singh (2009); and Charpentier *et al.,* (2014) with some modifications. Thus, the following concentrations were applied: (5mg, 10mg, 25mg, 50mg, 100mg, 250mg, 500mg, 1000mg, 2500mg and 5000mg of extract each per 10g fly diet. Twenty flies per group were used. Thereafter, they were separated into eleven groups as follows:

Group 1: Control

Group 2: 5mg/10g

Group 3: 10mg/10g

Group 4: 25mg/10g

Group 5: 50mg/10g

Group 6: 100mg/10g

Group 7: 250mg/10g

Group 8: 500mg/10g

Group 9: 1000mg/10g

Group 10: 2500mg/10g

Group 11: 5000mg/10g

2.5 Induction and treatment of diabetes in the flies.

The methods described by Omale *et al.,* (2021) were used to induce type 2 diabetes in the *D. melanogaster*. Briefly, sucrose (2.5g sucrose/10g diet) was incorporated into a regular fly diet to induce diabetes type 2. All other ingredients of the standard fly food (1% agar, 3.4% yeast and 8.3% corn mill were kept contant). The flies were then observed after 10 days for symptoms of diabetes, which include delayed egg production, delayed emergence of L3 larvae, decreased body size for both larvae (L3) and adult flies and decreased locomotor activities. The glucose concentration of the fly-homogenate was quantified using the glucose oxidase method. Fifty flies each (both gender) divided into eight groups were used as follows:

Group 1 Control: None diabetic flies in 10g diet

Group 2: Diabetic flies untreated

Group 3: Diabetic flies treated with 16mg of metformin/10g diet

Group 4: Diabetic flies treated with S1/10g diet

Group 5: Diabetic flies treated with S2/10g diet

Group 6: Diabetic flies treated with S3/10g diet

Group 7: Diabetic flies treated with S4/10g diet

Group 8: Diabetic flies treated with S6/10g diet

Key: S signifies the code for Shemmeco extract while S1-S6 is increasing concentration of the extract.

3.0 Results

Table 1: Result of acute toxicity assay for Shemmeco extract (LC50) in *Drosophila melanogaster.*

Concentration of Shemmeco extract per 10g diet			Start population Day 0	Cummulative Mortality Record						
				Day 1	2	3	4	5	6	7
CONTROL	C	C1	20	0	0	0	0	0	0	0
		C2	20	0	0	0	0	0	0	0
		C3	20	0	0	0	0	0	0	0
5mg/10g	S1	S11	20	0	0	0	0	0	0	0
		S12	20	0	1	1	1	1	1	1
		S13	20	0	0	0	0	0	0	0
10mg/10g	S2	S21	20	0	0	1	2	2	2	2
		S22	20	0	0	0	1	1	1	1
		S23	20	0	0	1	2	2	2	2
25mg/10g	S3	S31	20	0	0	0	0	1	1	3
		S32	20	1	1	1	1	1	1	1
		S33	20	0	0	0	0	0	1	1
50mg/10g	S4	S41	20	0	0	0	0	1	1	1
		S42	20	0	0	0	0	0	0	1
		S43	20	0	0	0	0	1	1	1
100mg/10g	S5	S51	20	0	0	2	2	2	2	2
		S52	20	0	1	1	1	1	1	1
		S53	20	0	0	0	1	2	2	2
250mg/10g	S6	S61	20	0	0	0	1	1	1	1
		S62	20	0	0	0	1	1	1	1
		S63	20	0	0	0	0	1	1	1
500mg/10g	S7	S71	20	0	0	1	1	1	1	1
		S72	20	0	0	2	2	2	3	3
		S73	20	0	0	1	1	2	2	3
1000mg/10g	S8	S81	20	0	1	2	2	9	16	20
		S82	20	1	1	2	4	5	15	19
		S83	20	0	1	1	3	3	9	20
2500mg/10g	S9	S91	20	1	1	1	3	11	18	20
		S92	20	1	2	2	4	9	13	20
		S93	20	2	3	3	3	11	19	20
5000mg/10g	S10	S101	20	1	4	6	8	14	20	20
		S102	20	3	6	9	11	13	18	20
		S103	20	3	9	11	13	20	19	20

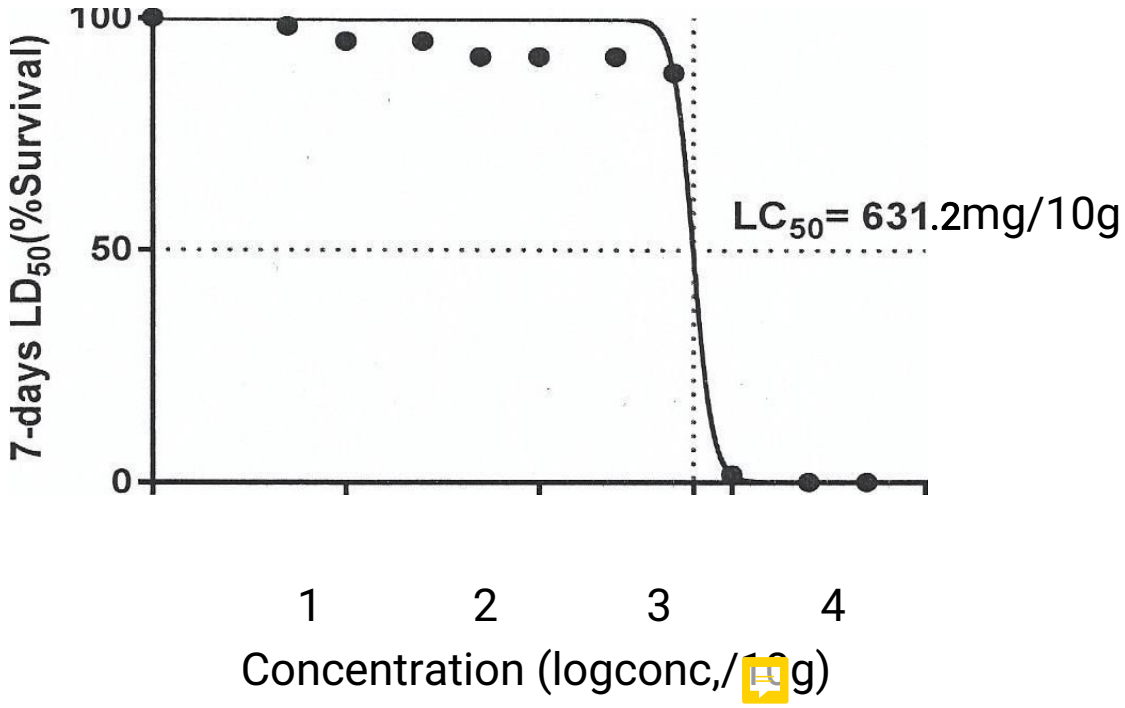

Fig. 1: LC50 curve obtained from the result of acute toxicity assay of Shemmeco extract

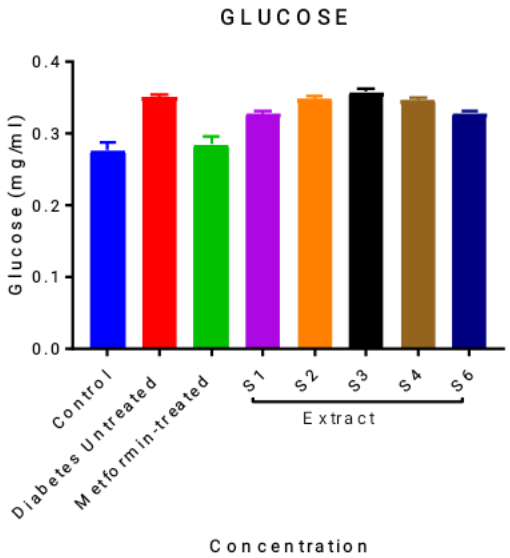

Fig. 2: The glucose content of fruit flies (diabetic) treated with Shemmeco extract

Table 2: Glucose (mg/ml) concentration of *D. melanogaster*

Group	Glucose concentration
Group 1 (Control)	0.28±0.02
Group 2 (Diabetic, untreated)	0.25±0.01
Group 3 (Diabetic, Metformin Treated)	0.28±0.02
Group 4 (S1)	0.33±0.01
Group 5 (S2)	0.35±0.01
Group 6 (S3)	0.36±0.01
Group 7 (S4)	0.35±0.01
Group 8 (S6)	0.33±0.00

Values are presented as mean ± standard deviation (n=5)

4.0 Discussion

In comparison to the control group, flies fed with a diet high in sucrose (2[illegible]g/10g diet) developed insulin resistant diabetes, which was characterized by a reduction in the size of both adult and larval flies. The relatively sluggish movements of both adult flies and the roving L3 larvae were also noticed, and this was explained by a neurovascular abnormality in type 2 diabetes. By measuring the glucose content of the flies using the glucose oxidase method, it was discovered that the flies fed with a high-sucrose diet on their own had higher glucose levels than the control group when given Shemmeco extract doses of 5, 10, 25, 50, and 250 mg along with 16 mg of metformin.

This experiment used metformin, an oral hypoglycemic medication and insulin sensitizer that is frequently used in the treatment of insulin-resistant diabetes. Shemmeco extract has a good potential for treating type 2 diabetes based on the observation in this study that it caused a small drop in glucose content.

The findings of this investigation demonstrated the safety of the various doses. Amidst the doses applied; 5mg, 10mg, 25mg, 50mg, 100mg, 250mg, 500mg, 1000mg, 2500mg and 5000mg in toxicity assay, it was observed that doses 5mg, 10mg, 25mg, 50mg, and 250mg was considered safe in that the mortality rate recorded are highly negligible to treat the flies. This study's limitation is in its inability to pinpoint the precise mechanism underlying the extract's anti-diabetic effects. The investigation was unable to identify the active ingredient that was responsible for the anti-diabetic effects. Therefore, further analysis and characterization of the active ingredients in Shemmeco extracts is advised as a potential basis for enhancing its efficacy development and synthesis.

5.0 Conclusion

It is important to draw the conclusion that Shemmeco extract had anti-diabetic effects in *Drosophila melanogaster* based on the findings of this study. Shemmeco extract is also risk-free at the various dosages used for treatment in this study.

6.0 Significance statement.

This study's support of an extract's anti-diabetic and acute toxicity properties may help scientists create new medications for different conditions using the *Drosophila melanogaster* model which many researchers of ethnopharmacology were not as now able to explore. The study also provides an open opportunity to investors, donor agencies and philanthropists worldwide who wants to invest into the efficacy development and synthesis of Shemmeco extract as part of their contribution and fight against diabetes type 2.

References

1. Adedara, I.A; Abolaji, A.O; Rocha,J.B.T(2016) Diphenyl Diselenide Protects Against Mortality, Locomotor Deficits and Oxidative Stress in *Drosophila melanogaster* models of Manganese-Induced Neurotoxicity. Neurochem Res.41, 1430-1438

2. Adesola, R.O., Lawal, J.T., & Oladele, O.E. (2021). *Drosophila melanogaster* (Meigen, 1830): A potential model for human diseases. *World News Nat. Sci. Int. Sci.* J, 36, 42-59.

3. Charpentier,G; Louat,F; Bonmatin,J.M(2014) Lethal and Sub-Lethal Effects of Imidacloprid After Chronic Exposure on the Insect Model, *Drosophila melanogaster.* Environmental Science Technolofy:2014.

4. Coogan CM (2013). Diagnosis and Prevention of Metabolic Diseases in *Drosophila melanogaster* . Engaged Learning Collection, 27.

5. Eshete, M.A., & Molla, E.L. (2021). Cultural significance of medicinal plants in healing human ailments among Guji semi-pastoralist people, Suro Barguda District, Ethiopia. *Journal of ethnobiology and ethnomedicine* , 17(1), 1-18

6. Garai, A., & Sarkar, B (2022). Economically independent reverse logistics of customer-centric closed-loop supply chain for herbal medicines and biofuel. *Journal of Cleaner Production* , 334, 129977.

   http.//scholar.sinu.edu/upjournal_research/27

7. Mohammad,F & Singh, P.A(2009) Drosophila Systems Model of Pentleneterazole Induced Locomotor Plasticity Responsive to Antiepileptic Drugs. Doi 10.1186/1752-0509-3-1

8. Rizzo, R., Pistillo, M., Germinara, G. S., Lo Verde, G., Sinacori, m., Maggi, F.,… & Benelli, G. (2021). Bioactivity of Carlina acaulis essential oil and its main component towards the olive fruit fly, Bactrocera oleae: Ingestion toxicity, electrophysiological and behavioural insights. *Insects* , 12(10), 880.

9. S.O Omale, J.C.Aguiyi, O.G. Adejunle, T.O Johnson, S.O. Ochala, M.A. Etuh and M.C.Eze (2021). Evaluation of the antidiabetic effects of the stem bark extract of Barinari curatellifolia (planch. Ex Benth) in Drosophila melanogaster. Journal of

pharmacology and toxicology: volume 16 (1): 9-21.

10. Tennessen JM, Barry W, Cox J Thummei CS (2014). Methods for Studying Metabolism in Drosophila. Methods 68(1):105-115.