Phytochemical Characterization and Investigation of Anthelminthic, Antidiabetic, and Toxicological Effects of *Polysciasscutellaria*

ABSTRACT

The current study examined the properties of *Polysciasscutellaria*leaf, including its invitro anthelminthic, invivo antidiabetic, and toxicological effects, using a crude methanol extract. The leaf powder of the dried plant was extracted using methanol. After phytochemical screening, the methanolic extract (MEPSL) underwent additional testing to evaluate its anthelminthic, antidiabetic, and toxicological activities. During the phytochemical screening process, compounds such as carbohydrates, alkaloids, glycosides, saponins, flavonoids, and reducing sugars were found in MEPSL. The earthworm assay was utilized to conduct anthelminthic testing with various concentrations. A study was conducted on *Swiss albino*mice to evaluate the antidiabetic effects. The Blood Glucose Determination test was performed using doses of 150 mg/kg and 300 mg/kg. The toxicological tests for acute toxicity were conducted using cinnamon oil at doses of 3000, 5000, and 7000 mg/kg. According to the MEPSL findings, the earthworm met its demise after 30 minutes and 24 seconds during the 100 mg/mL anthelminthic test. In contrast, Albendazole took a significantly longer time of 38 minutes and 18 seconds to achieve the same result. MEPSL showed a remarkable reduction in blood glucose levels compared to the standard Glibenclamide. In toxicological testing, a dosage of 5000 mg/kg resulted in the mortality of 3 out of 5 mice, while the application of cinnamon oil led to the death of all 5 mice within 24 hours. Research findings indicate that MEPSL exhibits Very significant anthelmintic, antidiabetic, and moderate toxicological properties. They may endorse the plant's utilization in mainstream medicine for reducing drug intoxication, and managing diabetes and parasitic disorders.

Keywords:*Polysciasscutellaria, Antidiabetic, Anthelmentic, Toxicological, Swiss albino*

1. INTRODUCTION

Phytochemicals, plant substances with therapeutic potential, are being used in contemporary allopathic medicine. Phytochemicals' structural and functional variety helps create novel therapeutic medicines, and their abundance in medicinal plants has led to new lead structures. With recent advances in synergistic and high-throughput screening methods for testing a wide range of phytochemicals, industry interest has grown[1].The major goal of allopathic medicine, also referred to as contemporary or mainstream medicine, is to cure illnesses and their symptoms using drugs, surgery, radiation, and other treatments[2]. However, the investigation of alternative medications, such as those based on phytochemicals, has increased as a result of the present

allopathic drugs' inability to address certain ailments, such as neurodegenerative illnesses[3].Numerous illnesses have been discovered to respond well to the use of phytochemicals in therapy. For example, in modern allopathic treatment, traditional herbal medicines containing many phytoconstituents have been employed to manage diabetes problems[4]. Furthermore, phytochemicals have long been used to treat a wide range of illnesses and have been shown to prevent the transcription and reproduction of viruses. In reality, Western countries consume half of all medications that are produced from plants[5].Plant-derived products are used to create antivirals because they are less toxic and have a lower potential for resistance development. Moreover, various advantageous new drug delivery systems (NDDS) may be used to enhance the therapeutic potential of physiologically active phytochemicals[6].Research collaborations between allopathic medicine and medicinal systems like naturopathy and Ayurveda, which often use naturally produced compounds and phytochemicals, are also being promoted. It is anticipated that this partnership will improve knowledge of how to include natural materials in wound care and other therapeutic domains[7].In a broader sense, phytochemicals have made and will continue to make a substantial contribution to current allopathic medicine. By using these natural molecules' medicinal potential, researchers may create less harmful and more effective medicines for a range of illnesses.

Polysciasscutellaria, an indigenous plant from Indonesia, has been traditionally used to treat breast discomfort, wounds, urinary tract issues, and body odor. The historical use of *P. scutellaria* for body odor treatment indicates its antibacterial characteristics. The hypothesis is based on the discovery that human axilla microbiota transforms odorless body secretions into volatile odorous molecules that cause body odor[8]. *Polysciasscutellaria* leaves contain alkaloids, saponins, flavonoids, and polyphenols with diverse pharmacological properties[9]. This investigation aims to determine the Anthelmintic, Antidiabetic, and Toxicological properties of *Polysciasscutelaria* from its methanolic leaf extract and conduct phytochemical screening.

2. MATERIAL AND METHODS

2.1 Plant Material

The specimen of *Polysciasscutellaria* (Burm.f.) Fosberg was gathered in November 2022 from Ramna Park, Moulana Bhashani Road, Dhaka, Bangladesh. The plant (accession number: DACB 88046) was accurately identified by experts at the Bangladesh National Herbarium in Mirpur, Dhaka. Meanwhile, the plant's leaves were preserved, dried in shade, and then ground into powder.

2.2 Preparation of Plant Extract

The plant extraction was achieved by the cold maceration technique [10].Approximately 80g of *Polysciasscutellaria* leaf powder was immersed in 600mL of methanol for 10 days in a round bottom flask that was sealed with a stopper and covered. The mixture was then filtered and left to air dry for a further 7 days. Upon drying, a total weight of 16.15g of leaf extract was obtained.

2.3 Phytochemical Screening Test

The medicinal properties of plants are derived from their chemical components. The freshly generated MEPSL was qualitatively evaluated for the presence of phytochemicals such as alkaloids, carbohydrates, saponins, glycosides, reducing sugar, flavonoids, tannins, and steroids[11].

2.4 Anthelmintic Test

The anthelmintic activity of MEPSL was evaluated in vitro against adult *H. contortus* using the worm motility inhibition test. The worms were cleaned and then placed in phosphate-buffered saline (PBS) before being sent to the laboratory. MEPSL was dissolved in 0.5% dimethyl sulphoxide (DMSO) and evaluated at a concentration of 25 mg/ml. Albendazole, a popular anthelmintic, was utilized as the positive control at a concentration of 0.55 mg/ml dissolved in DMSO (0.5%). The negative control was DMSO at a concentration of 0.5%. Twenty worms were subjected to each treatment at a regulated temperature of 35±1°C. Each treatment was replicated three times. Reducing worm movement was the reason for the effectiveness of the anthelmintic treatment. The time taken for paralysis, full inaction, and death was measured at 0, 1, 2, and 4-hour intervals. After 4 hours, the extracts and albendazole were removed, and parasites were resuspended in lukewarm PBS for 30 minutes to assess the recovery of worm motility[12].

3. In-vivo Experiments

3.1 Experimental animals

As investigators, we obtained young, healthy *Swiss albino* mice weighing 20-26 g. We bought our mice from Jahangirnagar University at their Saver facility in Dhaka, Bangladesh. Maintaining the current state was crucial. The typical atmospheric conditions consist of a temperature of 77°F, a relative humidity ranging from 55 to 65%, and a 24-hour light/dark cycle each day. The circumstances remain constant for 8 days following collection. We provided mice with a diet of sufficient food and clean water to help them recover from water and food deprivation suffered during the trip and adapt to the laboratory setting, following Jahangirnagar University's recommendations. Following 5 days of recovery, the mice were prepared for the experiment.

3.2 Antidiabetic Test

3.2.1Induction of hyperglycemia

186.9 mg/kg of a 10% alloxan monohydrate solution was administered intraperitoneally to the subjects. The alloxan monohydrate was procured from Sigma in Switzerland.

After 48 hours of alloxan administration, blood glucose levels were assessed using a glucometer. Mice with blood glucose levels over 200 mg/mL were classified as diabetic and included in the research. Before starting the experiment, the animals were fasted for 8-12 hours but were permitted to drink water during the trial[13].

3.2.2 Experimental design

The experimental mice were randomly split into eight groups of five animals each for medication delivery via either intraperitoneal or oral route. Group, I consisted of normal mice either intraperitoneally or orally administered with 0.1 ml physiological saline; Group II consisted of alloxan-induced diabetic mice either intraperitoneally or orally administered with 0.1 ml physiological saline; Group It consisted of alloxan-induced diabetic mice intraperitoneally administered with 0.025 insulin units (0.25 insulin units in 1 ml) (1 IU/ kg body weight) in 0.1 ml physiological saline; Group Ib consisted of alloxan-induced diabetic mice orally administered with 0.075 mg glibenclamide (0.75 mg in 1 ml) (3 mg/kg body weight) in 0.1 ml physiological saline; Group IV consisted of alloxan-induced diabetic mice either intraperitoneally or orally administered with 25 mg/kg body weight in 0.1 ml physiological saline; Group V consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 48.4 mg/kg body weight in 0.1 ml physiological saline; Group VI consisted of alloxan-induced diabetic mice either intraperitoneally or orally administered with 93.5 mg/kg body weight in 0.1 ml physiological saline; Group VII consisted of alloxan-induced diabetic mice either intraperitoneally or orally administered with 180.9 mg/kg body weight in 1 ml physiological saline. Group VIII included diabetic mice induced by alloxan, which were given either insulin, glibenclamide, or a plant extract solution at a dose of 350 mg/kg body weight in 1 ml physiological saline intraperitoneally or orally.

3.2.3 Blood glucose determination

The blood sample included sterilizing the tail with 10% alcohol and then pricking it at the beginning of the experiment, and repeating this process after 1, 2, 3, 4, 6, and 24 hours. Enhanced bleeding by slowly expressing blood from the tail towards the tip. Following the surgery, the tail tips were sterilized by swabbing them with 70% ethanol. The blood glucose levels were measured using a glucose analyzer model (Hypoguard, Woodbridge, England).

3.3 Acute Toxicity Test

Each group of mice consisted of 5 individuals who were orally administered doses of either 1000 mg/kg, 2000 mg/kg, or 3000 mg/kg of MEPSL and Cinnamon oil, with water used as a control. Death rates for both groups were recorded after 24 hours of monitoring[14].

4. Statical Analysis

The experimental data was replicated three times, and the mean and standard deviation were utilized to represent the results. Excel is also used for statistical studies.

5. RESULTS

5.1 Phytochemical screening

Carbohydrates,alkaloids, glycosides, saponins, flavonoids, and reducing sugars, were encountered in MEPSL, but steroidsand tannins were not spoted in MEPSL according to the screening test results (Table 1).

Table 01. Qualitative phytochemical detection of MEPSL.

Phytochemical constituent	MEPSL
Carbohydrate	+
Alkaloid	+
Glycoside	+
Saponin	+
Flavonoid	+
Reducing Sugar	+
Steroid	-
Tannin	-

Here, (+) indicates presence, and (–) indicates absence.

5.2 Anthelmintic Activity

Earthworms were exposed to several concentrations of fresh leaf juice (5mg/mL, 10mg/mL, 20mg/mL, 50mg/mL, and 100mg/mL) to assess their anthelmintic efficacy. The leaf extract showed significant anthelmintic activity similar to the standard drug albendazole (Table 2).

Table 02: In Vitro Anthelmintic Activity of MEPSL

Test samples	Conc. (mg/mL)	Time taken for paralysis	Time taken for death
Leaf extract	5	65 min 31 sec	71 min 19 sec
	10	58 min 22 sec	63 min 13 sec
	20	55 min 18 sec	59 min 03 sec
	50	35 min 53 sec	38 min 44 sec
	100	26 min 12 sec	30 min 24 sec
Albendazole	5	76 min09 sec	82 min 19 sec
	10	62 min 47 sec	72 min 32 sec
	20	57 min 52 sec	69 min 37 sec
	50	41 min 03 sec	47 min 12 sec
	100	30 min 43 sec	38 min 18 sec

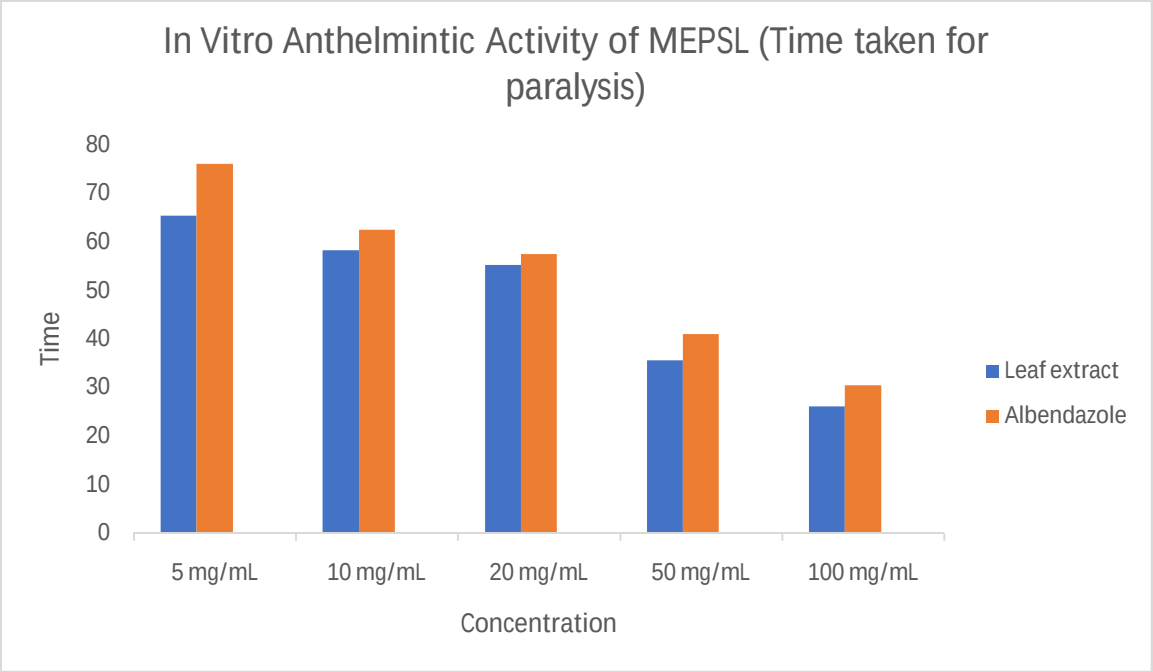

Figure 01: In Vitro Anthelmintic Activity of MEPSL (Time taken for paralysis)

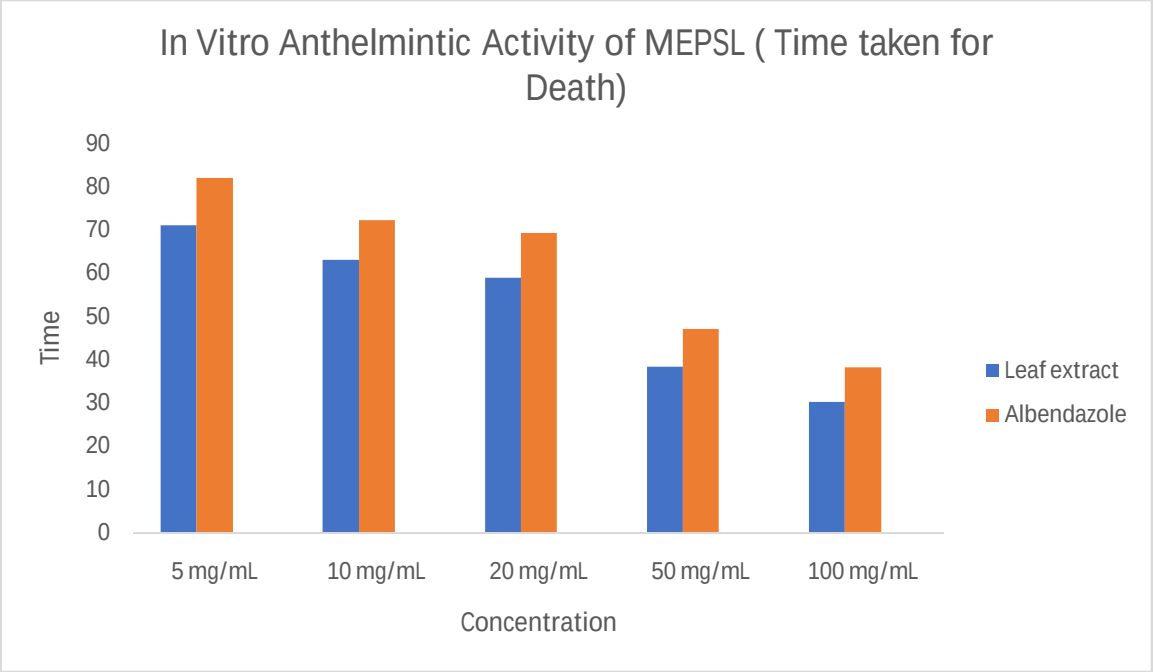

Figure 02: In Vitro Anthelmintic Activity of MEPSL (Time taken for Death)

5.3 Antidiabetic Activity

When administered intraperitoneally, MEPSL reduced blood glucose levels at all five dosages of 150 and 300 mg/kg body weight (Table 3). The event unfolded in three

stages. Initially, the extract led to a sharp decrease in blood glucose levels during the first hour, followed by a consistent reduction within the same period. There was a modest rise noted from the second to the third hour. Nevertheless, the sugar levels did not decrease according to the dosage. During the first hour, the extracts reduced blood glucose levels to 82.1%, 90.3%, 55.3%, 75.4%, and 71.2% for dosages of 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight, respectively. In comparison, insulin-treated diabetic mice had a decrease to 50.2% during the same timeframe. After three hours, each of the five treatments reduced blood sugar levels by 44.1%, 46.4%, 41.5%, 49.7%, and 44.0% correspondingly. In comparison, insulin-treated diabetic mice saw a 31.1% decrease in blood sugar levels within the same hour (Figure 3).

When taken by mouth, MEPSL reduced blood glucose levels at doses of 150 and 300 mg/kg body weight starting from the first hour in a way not dependent on the dosage. An incremental rise occurred during the second and third hours. By the second hour, the extract reduced blood glucose levels to 71.7%, 68.0%, 58.9%, 58.3%, and 69.8% for both dosages, compared to 50.2% for the usual oral medicine glibenclamide.This reaction is not dependent on the dosage. The decrease in blood glucose levels compared to the negative control was statistically significant.

Table 03: Effects of intraperitoneally administered MEPSL on blood glucose levels in alloxan induced diabetic mice

Test Samples	Blood Glucose Levelsat Varying Times (mmoles/L)			
	½ hour	1 hour	2 hours	3 hours
Control	5.21±0.05	5.31±0.11	5.33±0.03	5.28±0.07
Diabetic Saline	14.53±0.11	16.02±0.15	17.51±0.06	18.52±0.03
Standard Glibenclamide	17.43±1.30	8.51±0.07	6.21±0.42	4.96±0.11
MEPSL 150 mg/kg	12.55±0.23	9.37±1.02	7.02±0.33	5.33±1.46
MEPSL 300mg/kg	13.46±0.33	8.56±0.19	6.38±1.02	4.13±0.56

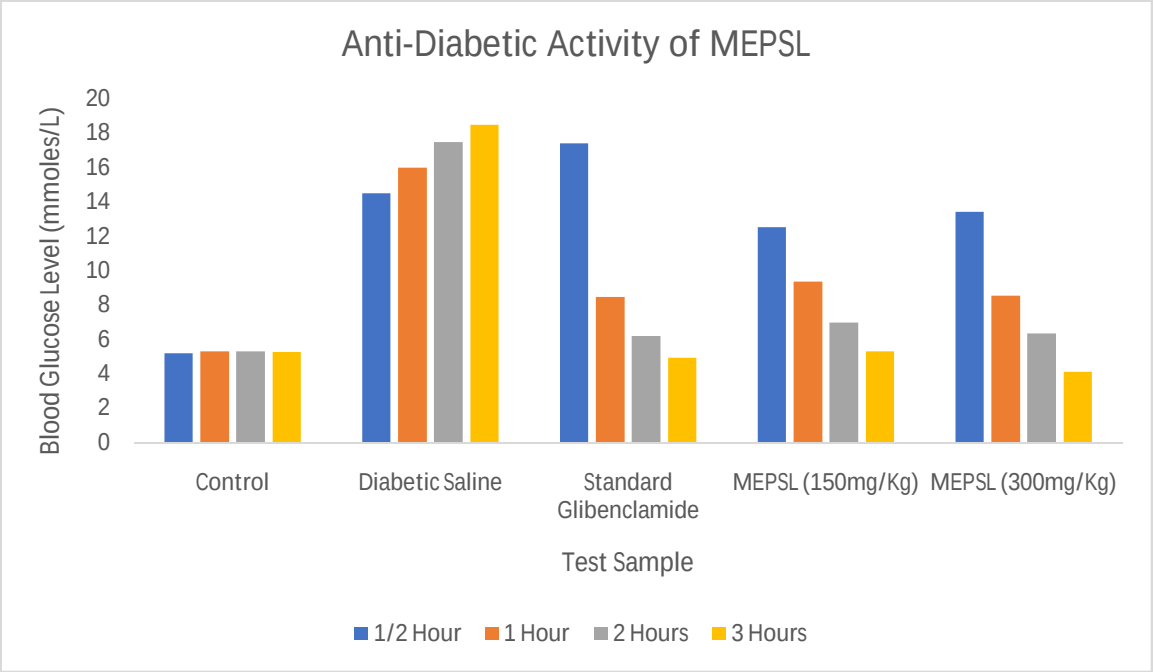

Figure 03: Anti-Diabetic Activity of MEPSL

5.4 Acute toxicological activity

During the acute toxicity test, the methanolic extract was given at doses of 1000, 2000, and 3000 mg/kg, while cinnamon oil was given at a level of 20 mg/kg. This delayed the onset of seizures compared to the negative control group. The mortality protection rates for convulsion survivors were 0/5 at 1000 mg/kg, 2/5 at 2000 mg/kg, and 3/5 at 3000 mg/kg of methanolic extract. Cinnamon oil was more effective with a rate of 5/5 at 20 mg/kg.

Table 4.Result for acute toxicological activity

Sample	**Onset time of seizure (s)**	**Mortality protection after 30min**	**Mortality protection after 24h**
Control (Normal saline)	27±2.91	0/5	0/5
Cinnamon oil (20mg/kg)	342±3.72	5/5	5/5
MEPSL (1000mg/kg)	18±1.29	0/5	0/5
MEPSL (3000mg/kg)	73±1.04	1/5	2/5
MEPSL (5000mg/kg)	114±1.82	1/5	3/5

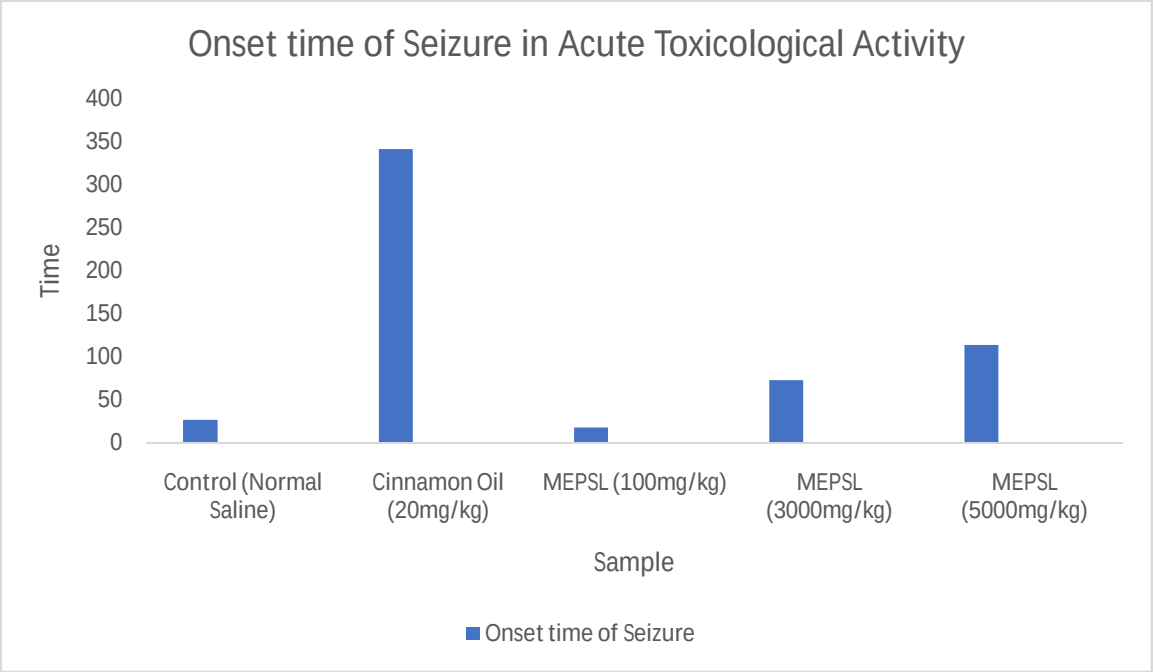

Figure 04: Onset time of Seizure in Acute Toxicological Activity

6. DISCUSSIONS

Core objective of qualitative screening of plant to invent a new drug entity which can be further analyze to determine specific chemical structure to be utilized in further aspect [15][16].Tannins and steroids are not indentified while carbohydrate, alkaloids, saponins, reducing sugar and flavonoids are recognized in rigorous screening process claiming in **Table 01** .

In Pharmacological study, the mechanism of action of anthelmintic drug is too absolute. Only a little amount of studies can precisely determinethe anthelmintic's primary mechanism of action[17]. Here, *Haemonchuscontortus*, is experimented with MEPSL to explore the abating motility and death rate [18]. From all the listed data from **Table 02** and **Figure 01 & 02**, MEPSL demonstrate significant activity at the concentration of 100 mg/mL when compared with Albendazole. Thus, MEPSL can be identified as an optimistic drug for anthelmintic property determination.

Our investigation suggests that MEPSL possess promising antidiabetic properties which shows excellency in MEPSL (300 mg/Kg) compared to standard Glibenclamide (**Table 03 and Figure 03**) . MEPSL exhibited significant anti-hyperglycemic effects in alloxan-induced hyperglycemic rats without any substantial alterations in body weight. Additionally, these extracts demonstrated the potential to improve diabetic state (DB) as evidenced by parameters such as lipid profile, body weight, serum creatinine, serum alkaline phosphatase and serum urea. It is well-established that the number of functionally intact beta-cells within the pancreatic islets is critically important for the progression and outcome of DB. Research on beta-cell regeneration in various animal models of diabetes suggests that total beta-cell mass reflects the balance between

beta-cell renewal and loss. Furthermore, previous studies have proposed that the restoration of islet beta-cells following alloxan-induced destruction might be the primary mechanism underlying the recovery observed in alloxan-injected mice[19][20].

Investigations into acute and chronic toxicity are essential for establishing the safety profile of drugs and botanical products intended for human consumption. In our experiment, rats administered MEPSL at dosages of up to 5000 mg/kg and 1000 mg/kg body weight exhibited no discernible behavioral abnormalities of clinical relevance but discovered mortality of 3 rats in total of 5 rats in the dosage of 5000 mg/kg shown in **Table 04 and Figure 04**. These findings are consistent with prior acute toxicity studies on MEPSL which reported 60% mortalities of tested animalon the experimental doses during the observation period of 24hour[21].

7. CONCLUSION

This investigation examined the chemical composition, anthelmintic activitry, antidiabetic activity and acute oral toxicity of an extract derived from *Polysciasscutellaria*.

10. ETHICAL APPROVAL

This study adhered to the regulations established by the US Food and Drug Administration, the Declaration of Helsinki, and the International Conference on Harmonisation. Stamford University Bangladesh's Faculty of Science reviewed and approved the study protocol together with the signed permission form (reference number: SUB/ERC/202302). All participants in the research were required to provide a written permission form and had the option to withdraw at any time.

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