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Investigating the Anti-diabetic and Lipid-regulating Properties of *Eclipta alba* in Alloxan-induced Diabetes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Since the dawn of human civilization, humanity has used herbal medicine for medicinal purposes. Traditional medicine has experienced a substantial surge in popularity in recent years. This research sought to evaluate the antidiabetic effectiveness and lipid profile of *Eclipta alba*. We evaluated the antidiabetic efficacy using the alloxan-induced diabetic model. The dosages of 800 mg/kg and 1200 mg/kg in groups 5 and 6, respectively, yielded statistically significant findings ($p < 0.05$) regarding antidiabetic efficacy. The group receiving a 1200 mg/kg dosage had statistically significant outcomes for total cholesterol and LDL, with findings of 196.15 ± 8.91* and 123.77 ± 6.50*, respectively ($p < 0.05$). However, no groups exhibited statistically significant results for HDL and triglyceride levels, despite a reduction in these parameters in the blood after the administration of the extract. Group 6, with a dosage of 1200 mg/kg, exhibited statistically significant results ($p < 0.05$) for SGPT and SGOT, with values of 88.71 ± 6.23* and 92.82 ± 7.50*, respectively. In the kidney function test, groups 5 and 6 exhibited statistically significant results ($p < 0.05$) at doses of 800 and 1200 mg/kg. So ethanolic extract of this plant shows significant antidiabetic and antihyperlipidemic activity and further advanced research on this plant may add alternative medicine to the treatment system.

Keywords: Eclipta alba; HDL; LDL; diabetes; herbal medicine; triglyceride.

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic condition characterized by a persistent increase in blood glucose levels (BGL) due to reduced insulin action, secretion, or both in target tissues. It is known that all types of diabetes mellitus are linked to long-lasting microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (coronary and peripheral artery disorders and stroke) complications. These issues occasionally lead to the detection of organ injury and mortality at a late stage or without sufficient medical supervision (Yikna and Yehualashet, 2021). The liver, the body's largest glandular organ, is responsible for regulating most physiological activities. The liver receives an individual's entire blood volume numerous times throughout the day. It is essential for the metabolic functions of humans (Himi et al., 2024). Rising levels of reactive oxygen species (ROS), which include OH, H2O2, and O2, can happen when someone drinks too much, is addicted to drugs, is exposed to some dangerous chemicals, or has a virus or parasite infection (FM et al., 2023). Hepatocellular injury may result from this. The Centers for Disease Control and Prevention researched 1492 clinicians who provide ambulatory treatment in non-government facilities. The survey revealed that these physicians encounter hyperlipidemia as the second most common chronic disease, with hypertension being the only condition they encounter more frequently (Baroi et al., 2023). The results of the study indicate that the primary factor contributing to hyperlipidemia is the excessive consumption of high-fat meals (Zhang et al., 2021). The liver plays a crucial role in the metabolism of commonly prescribed anti-hyperlipidemic medications such as atorvastatin, pravastatin, fluvastatin, simvastatin, lovastatin, and rosuvastatin. As a result, the bioavailability of these medications is low (Srinivasa et al., 2011). Statins can transiently inhibit the enzyme 3-hydroxy-3-methylglutaryl-co-A reductase (HMG-CoAR). This enzyme lowers cholesterol levels. This enzyme facilitates the reduction of cholesterol synthesis within the cells. The reason for this is that statins can enter hepatocytes and inhibit HMG-CoAR, which is responsible for their pharmacological effects (Schachter, 2005). Statin-associated muscle symptoms (SAMS), also known as muscular problems, are the primary adverse effects that restrict the use of statins. The onset of diabetes mellitus (DM) and complications influencing the central nervous system are two additional potentially detrimental consequences (Thompson et al., 2016). These synthetic medicines are not only costly, but they also have significant adverse effects, which may result in financial hardships for patients who are required to continue taking them throughout the therapy (Rupak et al., 2022). Consequently, it is imperative to create antihyperlipidemic medications that are highly effective and have minimal adverse effects. The discovery and synthesis of novel therapies are contingent upon the presence of plants (Islam et al., 2022). They function as a plentiful and beneficial source of naturally occurring compounds that are suitable for therapeutic purposes. Experts in the field suggest that specific chemical constituents

extracted from medicinal plants possess therapeutic properties. Consequently, researchers are perpetually in pursuit of innovative herbal remedies and other plant-based medications that can effectively address a variety of ailments (Baroi et al., 2023). For centuries, numerous countries worldwide have employed traditional medicines as remedies derived from botanicals, dietary supplements, and alternative medical methods. Traditional medicine has experienced a substantial surge in popularity in recent years, with a significant number of individuals throughout the nation relying on it as their primary form of care (Chowdhury et al., 2024). Plants used for medical purposes contain a wide variety of chemical constituents, enabling them to produce a wide range of therapeutic and pharmacological effects. Tanning agents, glycosides, alkaloids, saponins, polysaccharides, essential oils, terpenoids, resins, and plant lipids are among the numerous constituents of these substances (Bhowmik et al., 2024; Chowdhury et al., 2024; Saxena et al., 2013). Genetically engineered plants ultimately achieve the desired therapeutic outcome by precisely regulating chemical levels. Increasing the production of secondary metabolites, which includes making alkaloids, is one of the many possible uses of reverse genetics (Lima et al., 2023). Global advancements in scientific research have facilitated the investigation of the therapeutic properties of plant species (Pracheta et al., 2011). In comparison to synthetic pharmaceuticals, plants are becoming increasingly popular due to their inherent safety, potent pharmacological properties, and cost-effectiveness.

Eclipta alba (L.) Hassk., commonly referred to as false daisy or ink plant, is an annual herb belonging to the Asteraceae family. *E. alba* is a medium-sized, branched plant characterized by small white flowers. Warm, humid climates across Asia, South America, and Africa frequently harbor this plant, with reports of its presence in French rice fields in 1991. Often considered a common weed by farmers, traditional medicine uses the plant in various native countries such as India, China, Thailand, Brazil, Korea, and Ivory Coast (Jahan et al., 2014; Feng et al., 2019). China, India, and Thailand traditionally use *E. alba* to treat skin conditions such as atopic dermatitis, which is associated with inflammatory processes, and vitiligo (Galli et al., 2008). People extensively use the plant to promote hair growth and treat hepatitis and jaundice. In the Eastern Ivory Coast, pregnant women utilize the plant to promote fetal development and facilitate childbirth (Jahan et al., 2014). It exhibits antimicrobial, anti-hyperlipidemic, and anti-diabetic properties (Mithun et al., 2011). The plant extract of *E. alba* contains a diverse array of secondary metabolites, such as saponins, sterols, flavonoids, terpenoids, phenolic acids, thiophenes, polyacetylenes, and coumestans (Chung et al., 2017).

As no vigorous scientific research were done to this valuable plant this study investigates the anti-diabetic effects and lipid profile of an ethanolic extract from *Eclipta alba.*

2. MATERIALS AND METHODS

2.1 Drugs, Chemicals and Instruments

We obtained the ethanol and alloxan from Sigma Aldrich in Germany. Healthcare Pharmaceutical Limited provided us with a complimentary sample of metformin, a commonly used medication for diabetes. The blood serum analysis kits for various biomarkers were acquired from Plasmatic Laboratory Products Ltd. in the United Kingdom. Alere Inc. glucometer is used in this study. We acquired it from Shahbag in Dhaka, Bangladesh. We assessed the biochemical parameters using the Humalyzer 3000, a semiautomated clinical chemistry analyzer.

2.2 Plant Collection and Extract Preparation

Three distinct regions in Bangladesh were utilized to collect *Eclipta alba* plants: North Bengal, a hill-track area, and a lowland area. The next step involved authentication and taxonomic identification. The National Herbarium of Bangladesh maintained the plant specimen by applicable regulations. The leaves were dried in a shaded area for a duration of seven to ten days, followed by fine grinding. The powdered leaves were agitated for 96 hours in a 70% ethanol solution. Following the soaking process, the extract underwent filtration, and the resultant liquid was collected. The extract was collected using a rotary evaporator. The dried extract was collected and stored in the refrigerator for future use.

2.3 Experimental Animal Handling

100 male Wistar rats weighing 125-150 grams were obtained from the Pharmacy Department of

Jahangirnagar University in Dhaka, Bangladesh. The rats were maintained in a controlled environment at the Institute of Nutrition and Food Science, University of Dhaka, with a 12-hour dark/light cycle and a constant temperature of 25°C. We regularly provided the subjects with a standard pellet meal and clean water. The rats were housed in the facility to acclimatise prior to the commencement of the study. The rat trials adhered to the guidelines set forth by the Institutional Animal Ethics Committee (IEAC). The ethical approval was taken from the Dhaka University, Department of Zoology with the issue no 147/pharm.science.ewu. The researchers cared for and managed the animals in accordance with the guidelines set by the Swiss Academy of Medical Sciences (SAMS) and the Swiss Academy of Sciences (SCNAT).

2.4 Experimental Guidelines

The tests were conducted in adherence to the ethical principles stated in the 2013 Helsinki Declaration. The study strictly adhered to the "3R" standards, which are fundamental principles in Swiss and global legislation regarding the use of animals in research. The prefix "R" represents the concept of "replacement," encompassing both absolute replacements (such as substituting animal models with computer-generated models) and relative replacements (such as substituting live animals with cell or tissue cultures or vertebrates with invertebrates). For the purpose of conducting thorough research, an animal model was utilised. Rats were chosen as test subjects due to their distinct pancreas and beta cells, which makes them suitable for antidiabetic research. This is in contrast to invertebrates, as mammals are vertebrates. The second "R" represents "reduction," which pertains to techniques that minimise the number of animals needed to gather sufficient data for research purposes or maximise the information obtained from each animal. We selected ten rats for this study based on the sample size estimate determined by the power analysis method. We employed this approach to ensure adherence to the recommended guidelines. Refinement, the third "R," involves reducing the pain and distress experienced by experimental animals. In order to enhance the comfort of the rats during surgery and minimise any discomfort caused by pinching, the tail tips of the rats were gently rubbed with isopropyl alcohol both before and after each blood glucose level measurement. The rats were provided with sufficient nourishment throughout the trial, and they were euthanized painlessly at the conclusion, in accordance with the 2013 amendment to the Guidelines for the Euthanasia of Animals.

2.5 Experimental Design

We divided the rats into groups based on their body weight and subsequently tested them for antihyperglycemic action (Table 1). The rodents were categorized into groups according to their body weight, with 10 rats in each group. Table 1 illustrates the alloxan control group, consisting of rats that received only alloxan therapy. N/A indicates the absence of therapeutic treatment in this group.

2.6 Biological Sample Collection

Blood glucose levels were measured by obtaining samples through puncturing the tip of the rat's tail. Blood was collected from the slaughtered animal immediately after a heart puncture and transferred to a microcentrifuge tube. The supernatant fluid was obtained by centrifuging the collected samples for 5 minutes at 5,000 rpm. The fluid was transferred to a different microcentrifuge tube for biochemical testing. The kidneys and liver were promptly extracted from the animal's body post-sacrifice and thoroughly rinsed with an ice-cold saline solution for subsequent analysis of kidney and liver function. Rats were categorized into distinct groups based on body weight, followed by tests to assess their antihyperglycemic action (Table 1). Rodents were categorized according to body weight, with each group consisting of 10 rats. The control group in Table 1 consisted exclusively of rats treated with alloxan. This group does not receive therapeutic treatment when not applicable is indicated.

2.7 Estimation of Biochemical Parameters

By using a glucometer, the blood glucose level was ascertained. The Humaluzer 3000 was one of many tests administered, along with those for the lipid profile (HDL, LDL, Cholesterol, triglyceride|), kidneys (Urea, Creatinine), and liver (SGPT and SGOT). We also tested liver and kidney samples for gluconeogenic and glycolytic enzyme activity

2.8 Statistical Analysis

All of our findings (raw data) in terms of numerical parameters were recorded and

Table 1. Anti-hyperglycemic Activity Analysis

Group number	Group Status	Treatment specimen	Dose of treatment specimen (mg/kg)	Group Abbreviation
1	Negative Control	Physiological Saline	10 mL/kg	NC
2	Alloxan control	Alloxan	150 mg/kg	AC
3	Alloxan + Metformin	Alloxan + Metformin	150 mg/kg + 100mg	A + M_{100}
4	Alloxan + *Eclipta alba*	Alloxan +*Eclipta alba* extract low dose	150 mg/kg + 400 mg/kg	A + EA_{400}
5	Alloxan + *Eclipta alba*	Alloxan + *Eclipta alba* extract medium dose	150 mg/kg + 800 mg/kg	A + EA_{800}
6	Alloxan + *Eclipta alba*	Alloxan + *Eclipta alba* extract high dose	150 mg/kg + 1200 mg/kg	A+ EA_{1200}
7	Metformin	Metformin	100 mg/kg	M
8	*Eclipta alba*	Alloxan + *Eclipta alba* extract low dose	400 mg/kg	EA_{400}
9	*Eclipta alba*	Alloxan + *Eclipta alba* extract medium dose	800 mg/kg	EA_{800}
10	*Eclipta alba*	Alloxan + *Eclipta alba* extract high dose	1200 mg/kg	EA_{1200}

analyzed on a broadsheet using the MS Excel application. The gathered data were subjected to descriptive statistics, with the findings reported as mean SEM. To evaluate statistical significance, we used the SPSS 16 software's "One-way Anova test" to interpret inter-group heterogenicity in terms of several biological factors. The occurrences are considered statistically significant since the 'p' value was less than 0.05($p<0.5$).

3. RESULTS AND DISCUSSION

Herbal medicine involves the application of medicinal plants for the prevention and treatment of illnesses, encompassing both traditional remedies prevalent in various cultures and the utilization of standardized and titrated herbal extracts. This study examined the antidiabetic effects and lipid profile of the herb *Eclipta alba* in a murine model. Diabetes represents a significant health challenge in the twenty-first century. Diabetes is a significant contributor to mortality, with its macro- and microvascular complications leading to increased disability and substantial healthcare expenditures. The dosages of 800 mg/kg and 1200 mg/kg in groups 5 and 6, respectively, yielded statistically significant findings ($p < 0.05$) regarding antidiabetic efficacy (Fig. 1). Multiple studies on plant extracts produced comparable results (Chung et al., 2017; Mandal et al., 2010). The group receiving a dose of 1200 mg/kg demonstrated statistically significant outcomes for total cholesterol and LDL, with values of 196.15 ± 8.91* and 123.77 ± 6.50*, respectively ($p < 0.05$). However, no groups exhibited statistically significant outcomes regarding HDL and triglyceride levels, despite a reduction in these parameters in the blood following the administration of the extract. Two studies on plant extracts produced comparable results (Ahmed et al., 2010; Bhowmik et al., 2024). The group 6, administered a dose of 1200 mg/kg, exhibited statistically significant results for SGPT and SGOT, with values of 88.71±6.23* and 92.82±7.50*, respectively ($p<0.05$) (Table 2). Two studies on plant extracts produced comparable results (Kumar et al., 2017; Kang et al., 2012). Groups 5 and 6 demonstrated statistically significant results ($p < 0.05$) in the kidney function test at doses of 800 mg/kg and 1200 mg/kg. Two studies on plant extract produced comparable results (El et al., 2013; Osman et al., 2010; Mishra and Pancholi, 2013).

Table 2. Lipid profile after administration of different dose of *Eclipta alba*

Groups	Total Cholesterol	HDL	LDL	Triglyceride	SGPT	SGOT	Urea	Creatinine
N	123.24± 5.25	93.50±6.18	25.77 ± 2.90	50.53 ± 5.81	42.34±3.08	41.28±4.10	35.77±3.28	0.84±0.092
A	209.50 ± 9.28	46.24±4.20	134.77±12.13	110.77±6.83	98.01±9.75	103.73±12.21	107.21±6.21	2.72±0.75
$A+M_{100}$	137.70 ± 8.21	70.50±4.28	55.42±4.87	58.162±4.97	58.72±8.24	54.28±11.10	54.39±7.12	1.41±0.83
$A+EA_{400}$	204.23 ± 7.50	48.10±5.73	130.33±9.82	107.53±9.28	96.28±6.23	100.25±9.87	103.55±7.50	2.4±0.73*
$A+ EA_{800}$	201. 19 ±8.24	49.70±4.73	127.81±8.37	104.73±7.08	94.73±7.28	97.82±8.73	100.29±6.20*	1.9±0.084*
$A+ EA_{1200}$	196.15 ± 8.91*	52.37±3.08	123.77±6.50*	98.50±6.25	88.71±6.23*	92.82±7.50*	94.90±5.29*	1.48±0.084*
EA_{400}	120.15 ± 6.25	95.08±5.73	27.10±3.10	52.28±6.24	44.28±3.29	42.29±4.10	34.08±2.05	0.77±0.052
EA_{800}	124.19 ± 7.10	91.73±4.88	24.50±2.30	49.28±5.10	44.70±4.10	39.73±3.15	37.50±3.90	0.88±0.073
EA_{1200}	128.17 ± 6.10	94.28±5.77	22.08±1.90	50.50±4.20	43.93±3.08	41.40±4.02	38.20±4.01	0.81±0.094
M_{100}	123.5 ± 5.10	93.93±4.70	22.77±2.80	54.81±5.10	40.73±3.19	44.50±3.25	37.20±3.20	0.76±0.091

*Note: The results were expressed in Mean±SEM (standard mean error) *p< 0.05 were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control.*

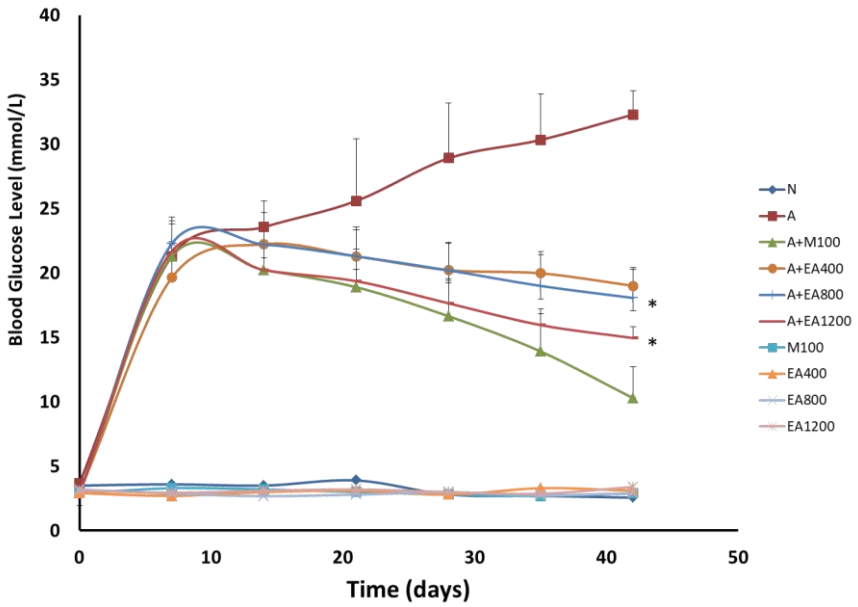

Fig. 1. Antidiabetic activity of different dose of *Eclipta alba*

4. CONCLUSION

The ethanol extract of *Eclipta alba* demonstrates significant protective effects against diabetes, hypercholesterolemia, liver damage, and impaired kidney function. The extract exhibited a notable effect on the specified outcomes, indicating its possible therapeutic significance. Further research is required to isolate and identify the specific active compounds that contribute to a better understanding of the mechanisms of action and facilitate the advancement of more effective treatments derived from this plant.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The ethical approval was taken from the Dhaka University, Department of Zoology with the issue no 147/pharm.science.ewu.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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