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ANTI-DIABETIC EFFECTS OF *ECLIPTA ALBA* ON ALLOXAN-INDUCED DIABETIC MICE

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ABSTRACT: The aim of the present study was to investigate the effects of *Eclipta alba* leaves extract on the biochemical parameters in alloxan induced diabetic Swiss albino mice. Diabetes was induced in mice by injecting intraperitoneally alloxan monohydrate at dose of 135 mg/kg body weight. Ethanolic extracts of *E. alba* leaves at dose of 200 mg/kg body weight were given orally in diabetic mice daily for four weeks after established LD_{50} value. Our studies was extended to include the effect of the tested doses on different biochemical parameters including serum glucose concentration, transaminases and phasphatse activities, total protein, albumin, urea, uric acid, and creatinine levels in serum. In diabetic mice, serum glucose concentration, transaminases and phasphatse activities, urea, uric acid and creatinine levels were significantly increased but level of serum total protein and albumin were decreased in comparison with the control group. Diabetic mice group, treated with ethanolic extract of *E. alba* leaves (200 mg/kg b. wt.), on comparison with diabetic group showed a significant decrease in transaminases and phasphatse activities, urea, uric acid and creatinine level whereas, the serum total protein and albumin levels got increased as compared to diabetic mice. The results suggested that ethanolic extract of *E. alba* leaves possesses protective effect against alloxan induced diabetic mice.

INTRODUCTION: Diabetes is a major degenerative disease, characterized by hyperglycemia, lipoprotein abnormalities, defect in reactive oxygen species scavenging enzymes and altered intermediary metabolism[1]. It is a global epidemic with an estimated worldwide prevalence and according to 2006 3rd edition of the Diabetes Atlas the estimates were of 246 million people worldwide with diabetes for 2007, and an anticipated 380 million for 2025[2]. India has also the highest number of diabetic patients, and India is being called the diabetic capital of the world[3]. Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency in production of insulin by the pancreas or by the in effectiveness of the insulin produced or by a resistance to the action of insulin at the cellular level [4].

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It is associated with alteration in carbohydrate, lipid and protein metabolism causing cardiovascular, nephropathic and neurological complications in humans[5]. Diabetic mellitus is the commonest cause of liver failure and haepatomegaly because liver, an insulin dependent tissue that plays a pivotal role in glucose and lipid homeostasis and it is severely affected during diabetes[6]. Diabetic mellitus also causes renal damage due to abnormal glucose regulation, including elevated glucose and glycosylated protein tissue levels, haemodynamic changes within the kidney tissue, and increased oxidative stress[7].

Experimental diabetes, induced by chemical agents like alloxan, destroys β-cells of pancreas by generating excess reactive oxygen species and produces kidney lesions that are similar to human diabetic nephropathy[8].

Many oral antihyperglycemic agents are available along with insulin for the treatment of diabetes, but these agents are either too expensive or have significant side effects, and some are ineffective in chronic diabetes patients[9]. Thus, there is an

increasing need of new natural antihyperglycemic products with fewer side effects, safe and high antihyperglycemic potential. Many traditional medicines and extracts from medicinal plants have been extensively used as alternative medicine for better control and management of diabetes mellitus[10]. New hypoglycemic agents derived from plants have shown both hypoglycemic action and the ability to improve some of the secondary complications of diabetes such as kidney damage, fatty liver and oxidative stress. The attributed antihyperglycemic effects of these plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes[11].

Eclipta alba (Family: Asteraceae), is an annual herbaceous plant commonly known as Bringhraj in hindi, or false daisy. *E. alba* has been used in various parts of tropical and sub-tropical regions like South America, Asia and Africa. It is extensively used for the treatment of many ailments, including liver cirrhosis, jaundice, gallbladder problems and hepatitis [12, 13]. It is also reported to possess antiseptic, analgesic, antipyretic, antispasmodic, antimicrobial and antiviral properties[14]. This plant is also found active in declining high blood sugar level[15].

In view of this, the current study was designed to evaluate the antidiabetic effect of *E. alba* ethanolic leaf extract in alloxan induced diabetic mice.

MATERIALS AND METHODS:

Animals: Swiss albino mice weighing 30-32 g (obtained from the Pasteur Institute central animal house, Tehran, Iran) were housed in an air conditioned room under a 12:12 h light-dark cycle. The animals were allowed free access to tap water and standard laboratory mice food. All experimental procedures involving animals were approved by the ethics committee (Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur, India).

Induction of experimental diabetes:
Diabetes was induced in albino mice of either sex by a single intraperitoneal injection of aqueous alloxan monohydrate (135 mg/kg body weight)[16]. Blood samples were collected before and after the administration of alloxan to know the status of diabetes. After two days, diabetes was confirmed by testing blood glucose level using glucometer and they were further maintained for four days for well establishment of diabetes. The animals with blood glucose level more than 200 mg/dl (moderate diabetes) were selected for the experiment.

Plant material:
The plant material (*Eclipta alba*) which is used for study was collected and was identified by the taxonomist of Botany Department of University of Rajasthan, Jaipur. The voucher number was RUBL 20252.

Preparation of ethanolic leaves extract:
Leaves were dried in the shade after washing with cold water and then were powdered using dry grinder and passed through sieve. The coarse powder material was subjected to soxhlet extraction with ethanol. The resulting mixture was filtered and the filtrate was concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50^{0}C). The resultant residue was stored in a refrigerator in air tight containers for further uses in experiments.

Acute toxicity study: Acute toxicity study of ethanolic extract of leaves of *E. alba* was determined as per the Organization for Economic Co-operation and Development guidelines 425 (OECD)[17]. After an overnight fast of 18 h, *E. alba* was administered orally in doses of 100, 200, 500, 1000 and maximum dose of 2000 mg/Kg body weight to groups of mice (n = 6).

The behavioral changes and percentage mortality were noted beginning with 24 h up to a period of 14 days. The parameters were observed are gross behavioral changes, grooming, alertness, sedation, loss of righting reflex, tremors convulsions.

Experimental design:
All animals were randomly divided into four groups with six animals in each group.

- Control treated with normal saline (10 ml/ kg). Alloxan induced diabetic mice received normal saline (10 ml/kg).

- Positive controls treated with ethanolic leaf extract of *E. alba* (200 mg/kg body weight).
- Diabetic mice treated with ethanolic leaf extract of *E. alba* (200 mg/kg body weight).

Mice were treated daily by gavage for 6 consecutive weeks. At the end of the study, the body weight in experimental animals was determined after the study by a digital balance.

Determination of the blood glucose levels: Blood glucose level (mg/100 ml) was determined using a Glucometer (Bayer), based on the Glucose oxidase method. Blood samples were collected from the tip of tail at the defined time patterns.

Determination of Biochemical Parameters: Each mouse of different groups was weighed, blood samples were collected from the retro-orbital plexus in clean and dry test tubes, left for 10 minutes to clot and then centrifuged at 3000 r.p.m for serum separation. The centrifuged serum (15 min, 2500 rpm) was analyzed for biochemical parameters. The activities of serum AST (Aspartate aminotransferase) and ALT (Alanine aminotransferase) were assayed by the method of Reitman and Frankel (1957)[18]. ALP (*Alkaline phosphatase*) was assayed by the method of King (1959) [19].

Serum protein and serum albumins was determined by quantitative colorimetrically method by using bromocresol green[20]. Uric acid concentration in the serum was estimated by the method of Henry et al, (1964)[21], serum urea by urea berthelot method[22] and serum creatinine by alkaline picrate method[23].

Statistical analysis: All Biochemical data are expressed as mean±SD. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple tests using SPSS (version 18) of computer software. In all cases, a p-value of equal and less than 0.05 was considered to be significant.

RESULTS: It was observed that test extract was not lethal to the mice even at 2000 mg/kg dose. Hence, 10% (200 mg/kg) of this dose were selected for further experimentation.

Effect on blood glucose levels:

As shown in **Table-1**, alloxan caused significant increase (P<0.001) in blood glucose concentration which confirmed the diabetes in animals (Group-II). Normal mice treated with 200 mg/kg b. wt. of ethanolic extract of *E. alba* (Group-III) showed no significant change (P>0.05) in glucose levels when compared to the control mice (Group-I). Treatment with the dose of ethanolic extract of *E. alba* (200 mg/kg b. wt), in alloxan-induced diabetic mice (Group-IV) showed a significant (P<0.01) decrease in the elevated blood glucose level as compared with the diabetes control (Group-II) **(Table-1)**.

TABLE 1: EFFECT OF ETHANOLIC EXTRACT OF *E. ALBA* LEAVES ON BLOOD GLUCOSE LEVEL OF ALLOXAN INDUCED DIABETIC MICE AND CONTROL MICE.

Groups	Blood glucose (mg/dl)
Normal Control (Group-I)	80.2±0.78
Diabetic Control (Group-II)	290.0± 1.56***
Normal + *E. alba* (Group-III)	81.6±023
Diabetic + *E. alba* (Group-IV)	98.0± 0.59**

Values are given as mean ± SD for groups of six animals each. Values are statistically significant at ***P<0.001, **P <0.01, *P <0.05; Diabetic control mice (Group-II) and Group-III were compared with normal control mice (Group-I). Experimental group (Group-IV) was compared with diabetic control (Group-II).

Effects on body weight:

There was a statistically significant (p< 0.01) reduction in body weight in diabetic mice (Group-II) with respect to normal mice (Group-I) whereas post treatment with ethanolic extract of *E. alba* caused statistically significant (p< 0.01) improvement (p<0.01) in body weight of diabetic mice **(Table- 2)**. In contrast, normal mice showed statistically significant (p< 0.05) increase in body weight after ethanolic extract of *E. alba* treatment (Group-III) with respect to normal mice (Group-I) **(Table-2)**.

TABLE 2: EFFECTS OF ETHANOLIC EXTRACT OF *E. ALBA* LEAVES ON BODY WEIGHT OF ALLOXAN INDUCED DIABETIC AND CONTROL MICE.

Groups	Body weight (gm)
Normal Control (Group-I)	30.0±1.18
Diabetic Control (Group-II)	25.0± 1.24**
Normal + *E. alba* (Group-III)	31.2±023*
Diabetic + *E. alba* (Group-IV)	29.0± 0.59**

Values are given as mean ± SD for groups of six animals each. Values are statistically significant at ***P<0.001, **P <0.01, *P <0.05; Diabetic control mice (Group-II) and Group-III were compared with normal control mice (Group-I). Experimental group (Group-IV) was compared with diabetic control (Group-II).

Effects on serum transaminases and phosphatases activities:

The activities of serum AST, ALT and ALP of control and experimental mice are given in **Table-3**. AST, ALT and ALP activities were significantly elevated (p<0.001) in alloxan- induced diabetic mice (Group-II) as compared with normal mice (Group-I). Treatment with ethanolic leaf extract of *E. alba* for 30 days (Group-IV) showed significant reduction (p<0.001) in the activities of AST, ALT and ALP in diabetic mice as compared with untreated diabetic mice (Group-II). Treatment of normal mice with ethanolic leaf extract of *E. alba* (Group-III) showed non significant changes (p>0.05) in the activity of ALT, AST and ALP as compared to normal mice (Group-I).

TABLE 3: EFFECTS OF ETHANOLIC EXTRACT OF *E. ALBA* LEAVES ON SERUM TRANSAMINASES AND PHOSPHATASES ACTIVITIES OF ALLOXAN INDUCED DIABETIC AND CONTROL MICE.

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Normal Control (Group-I)	30.2± 0.94	38.9± 0.94	110.32±1.31
Diabetic Control (Group-II)	80.74± 0.81***	83.74± 0.81***	186.3± 1.32***
Normal + *E. alba* (Group-III)	28.23±0.99	38.16±0.57	98.56±0.36
Diabetic + *E. alba* (Group-IV)	29.5±1.12***	31.3±1.33***	104.23 ±1.56***

Values are given as mean ± SD for groups of six animals each. Values are statistically significant at ***P<0.001, **P <0.01, *P <0.05; Diabetic control mice (Group-II) and Group-III were compared with normal control mice (Group-I). Experimental group (Group-IV) was compared with diabetic control (Group-II).

Effects on serum total protein and albumin levels:

As shown in **Table-4**, there was a significant decrease (p<0.01) in the serum total protein and albumin levels in alloxan induced diabetic mice (Group-II) when compared with control mice (Group-I), while mice treated with *E. alba* showed significant increase (P<0.01) in serum total protein and albumin levels when compared with diabetic control (Group-II). Treatment of normal mice with ethanolic leaf extract of *E. alba* (Group-III) showed non significant changes (p>0.05) in the levels of serum protein and albumin when compared to normal mice (Group-I) **(Table-4).**

TABLE 4: EFFECTS OF ETHANOLIC EXTRACT OF *E. ALBA* LEAVES ON TOTAL PROTEIN AND ALBUMIN LEVELS OF ALLOXAN INDUCED DIABETIC AND CONTROL MICE.

Groups	Total protein (gm/dl)	Albumin (gm/dl)
Normal Control (Group-I)	6.9±0.89	4.1±0.63
Diabetic Control (Group-II)	5.10±0.52**	3.0±0.74**
Normal + *E. alba* (Group-III)	6.98±0.75	3.98 ±0.17
Diabetic + *E. alba* (Group-IV)	6.39±0.76**	3.84±0.69**

Values are given as mean ± SD for groups of six animals each. Values are statistically significant at ***P<0.001, **P <0.01, *P <0.05; Diabetic control mice (Group-II) and Group-III were compared with normal control mice (Group-I). Experimental group (Group-IV) was compared with diabetic control (Group-II).

Effects on serum urea, uric acid, and creatinine levels: The effects of *E. alba* in diabetic mice on the levels of urea, uric acid and creatinine were shown in **Table-5**. In diabetic mice (Group-II), statistically significant increase (P<0.01) were found in levels of urea, uric acid, and creatinine when compared with normal mice (Group-I).

Normal mice treated with 200 mg/kg b. wt. of ethanolic extract of *E. alba* (Group-III) showed no significant change (P>0.05) in urea, uric acid and creatinine levels when compared to the control mice (Group-I) while, after treatment of alloxan-diabetic mice with *E. alb*a, the levels of urea, uric acid and creatinine levels significantly decreased (P<0.05) as compared to diabetic group (Group-II).

TABLE 5: EFFECTS OF ETHANOLIC EXTRACT OF *E. ALBA* LEAVES ON UREA, URIC ACID AND CREATNINE LEVELS OF ALLOXAN INDUCED DIABETIC AND CONTROL MICE.

Groups	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Normal Control (Group-I)	30.89±0.67	3.81±0.32	0.43±0.23
Diabetic Control (Group-II)	80.25± 0.85**	15.42±0.34**	1.97±0.31**
Normal + *E. alba* (Group-III)	28.76±0.76	3.49± 0.13	0.67± 0.44
Diabetic + *E. alba* (Group-IV)	44.25±0.99*	6.98 ±0.45*	0.82±0.23*

Values are given as mean ± SD for groups of six animals each. Values are statistically significant at ***P<0.001, **P <0.01, *P <0.05; Diabetic control mice (Group-II) and Group-III were compared with normal control mice (Group-I). Experimental group (Group-IV) was compared with diabetic control (Group-II).

DISCUSSION: Diabetes mellitus is a syndrome, initially characterized by loss of glucose

homeostasis resulting from defects in insulin secretion, insulin action both resulting impaired metabolism of glucose and other energy-yielding fuels such as lipids and proteins[24]. In recent years, scientific investigation of traditional herbal remedies for diabetes in the experimental animals may provide valuable source for the development of alternative drugs thus in the present study some aspects of liver and kidney function parameters were studied in the normal diabetic mice treated with ethanolic extract of *E. alba* leaves.

Alloxan, a beta cytotoxin induces diabetes by free radical generation, which causes a massive reduction of the insulin secreting β-cells of the islets of langerhans, resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissue[25]. Our results showed that the intraperitoneal administration of alloxan to mice significantly increased glucose blood levels whereas the continuous treatment of the extracts of *E. alba* for a period of 30 days produced a significant reduction in the blood glucose level of diabetic mice and did not produce any change in the blood glucose levels of normal mice.

The possible mechanism by which plant extract brings about a decrease in blood sugar level may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from β cells of the islets of langerhans or its release from the bound form[26]. Ananthi *et al*. (2003)[15] also reported that *E. alba* possesses a hypoglycemic effect may primarily by modulating and regulating the activities of glucose-6-phosphatase and fructose 1,6 bisphosphatase enzymes either through regulation of cAMP or inhibition of gluconeogenesis.

Prajapati and Patel (2012)[27] also reported that *E. alba* plants significantly decreased blood glucose level and showed anti-diabetic effect by suppressing carbohydrate absorption from the intestine.

In alloxan induced diabetic mice, there was a decline in body weight were observed as compared to normal mice. Previously many researchers also reported that alloxan-induced diabetes is characterized by severe loss in body weights[28, 29]. Normal body weight gain is indicator of efficient glucose homeostasis; but in diabetics, glucose is not available therefore the cells use alternatively proteins for energy; consequently due to excessive breakdown of tissue protein (muscle wasting) a loss in body weight occurs[30]. Treatment with *E. alba* induced an increase in the body weight in diabetic mice and its capability to protect the body from weight loss seems to be a result of its ability to reduce hyperglycemia.

The activities of AST, ALT and ALP are the most sensitive tests employed in the diagnosis of organs damages. We have observed increased activities of serum AST, ALT and ALP in alloxan-induced diabetic mice. Our results are consistent with earlier report of Udayakumar *et al*., (2009)[31]. The elevation of serum biomarker enzymes such as ALT, AST and ALP has been observed in diabetic mice indicating impaired liver and kidney function that may be due to hepatic and renal damage induced by hyperglycemia[32].

It is also reported that the liver and kidney exhibits numerous morphological and functional alterations during diabetes[33]. Administration of *E. alba* to diabetic mice showed decreased activities of serum AST, ALT and ALP. This proves that *E. alba*, to an extent preserves the structural integrity of the liver and kidney from the adverse effects of alloxan-induced diabetes.

In this study in the alloxan induced diabetic mice levels of total protein and albumin decreases and a decline in total serum protein level in diabetics have been attributed to inhibition of oxidative phosphorylation which leads to decrease in protein synthesis, increase in catabolic processes and reduction in protein absorption[34]. Murray *et al*., (2000)[35] also reported that hyperglycemia increases gluconeogenesis and as such leads to excess protein breakdown as well as excess loss of nitrogen resulting in negative nitrogen balance.

Present study also demonstrated that the treatment of diabetic mice with the aqueous extract of *E. alba* caused a noticeable elevation in the plasma total protein and albumin levels and it may be due to *E.*

alba has been produce anabolic effects, enhancing the synthesis of certain modulator proteins in liver. On the other hand levels of urea, uric acid and creatinine increases in diabetic mice and these results also indicated that diabetes could be lead to renal dysfunction. Urea, uric acid and creatinine are waste products of protein metabolism that need to be excreted by the kidney, therefore marked increase in serum urea and creatinine, as noticed in this study, confirms an indication of functional damage to the kidney[36]. Negative nitrogen balance with enhanced tissue proteolysis and decreased protein synthesis can contribute to increased serum urea and creatinine levels, indicating impaired renal functions in diabetic animals[37].

After treatment with *E. alba* extract significantly normalized the elevated values of urea uric acid and creatinine as compared to diabetic mice. Thus, it would appear that the *E. alba* administration lowered the plasma urea, uric acid and creatinine levels by enhancing the renal function that is generally impaired in diabetic mice. Hemalakshmi et al. (2012)[38] also observed that the elevated serum urea and creatinine levels in alloxan induced diabetic rats reduced after administration of *E. alba* and concluded that the ethanolic extracts of *E. alba* has a potential therapeutic efficacy in controlling diabetes and post diabetic complications by possessing both hypoglycemic as well as antioxidant properties.

It is reported that presence of flavonoids, alkaloids, glycosides, phenolics, and tannins in the phytochemical screening of the plants are likely to be responsible for the antidiabetic effects[39]. Various previous reports and phytochemically investigation of ethanolic extracts *E. alba* in this work was in parallel, revealing the presence of carbohydrates, terpanoids, lactones, glycosides, flavonoids, esters, steroids, tannins. Thus administration of ethanolic leaves of extracts *E. alba* plants showed a potential effect may be due to the bioactive secondary compounds present in this plants.

CONCLUSION: From the overall results, it could be inferred that ethanol extracts of *E. alba* showed the beneficial effects against abnormalities in alloxan induced diabetic mice. It has antihyperglycemic effects as well as has significant capacity to reduce the altered liver and kidney biochemical parameters in diabetic mice. However, further research is needed to gain a better understanding of its potential therapeutic action, the implicated phytochemical constituents and the exact mechanism of action.

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