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PHARMACOLOGICAL EVALUATION OF ALCOHOLIC EXTRACT OF *EUPHORBIA ANTIQUORUM* WITH SPECIAL EMPHASIS ON DIABETIC COMPLICATION

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Nephropathy, Hepatoprotective, Urination, Micro vascular

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ABSTRACT: Diabetes is a metabolic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and post prandial blood sugar levels. Diabetes is the most common cause of progressive kidney failure leading to dialysis or transplantation. Nephropathy is reported to develop in 30-40% of patients with diabetes and has become a leading cause of end-stage renal failure worldwide. The various parts of *Euphorbia antiquorum* have been associated with a number of pharmacological activities which are cytotoxic, hepato-protective and antioxidant. The juice of the plant is acrid, anti-inflammatory, purgative and is useful in rheumatism, dropsy, gout, neuropathy, deafness, cough. The aim of the study was to evaluate the effects of *Euphorbia antiquorum* on alloxan induced diabetic nephropathy in Wistar rats. Experimental diabetic nephropathy was induced by a single intraperitoneal (i.p) administration of alloxan 120 mg/kg body weight (b.w.). Wistar rats were randomly divided into normal control, diabetic control, diabetic control with standard drug and diabetic treated with ethanolic extract of *Euphorbia antiquorum* at 200 and 400 mg/kg doses respectively. The group I animals were non diabetic and served as normal control and received 0.9% normal saline. Six healthy animals with moderate hyperglycemia, *i.e.* serum glucose between the normal ranges were randomly selected. Group III & IV Diabetic rats were treated with ethanolic plant extract of EAE by gavages (200, 400 mg/kg/day b.w., p.o) in 0.9% normal saline once daily for 21 days. All animals were treated for 21 days and sacrificed on the last day. The body weight, plasma glucose and blood urea nitrogen parameters were investigated in various groups of rats. EAE at 200 and 500 mg/kg, in a dose-dependent manner, significantly improve all levels as compared to diabetic rats.

INTRODUCTION: Diabetes is the most common cause of progressive kidney failure leading to dialysis or transplantation. Nephropathy is reported to develop in 30-40% of patients with diabetes and has become a leading cause of end-stage renal failure worldwide.

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Diabetic nephropathy is characterized by structural as well as functional abnormalities. Poor glycemic control and accumulation of advanced glycation end products (AGEs) play a significant role in the development of diabetic nephropathy. Furthermore, advanced glycation end products have been implicated in tissue damage associated with diabetic nephropathy [1].

The average incidence of diabetic nephropathy is high (3% per year) during the first 10 to 20 years after diabetes onset. Typically, it takes 15 years for small blood vessels in organs like kidney, eyes and nerves to get affected.

It is estimated that more than 20 and up to 40% of diabetic patients will develop chronic kidney disease (CKD), depending upon the population, with a significant number that develop end stage kidney disease (ESKD) requiring renal replacement therapies such as kidney transplantation. Incidentally, diabetes with no clinical sign of kidney damage during the initial 20 to 25 years is significantly less likely (1% a year) to cause major renal complication later in life [2].

Diabetes mellitus (DM) is one of the leading causes of mortality and morbidity in developed and developing countries. DM is characterized by increased levels of glucose that eventually progress to frequent urination, increased thirst, and increased hunger. According to global estimates of diabetes, in 2013, 382 million people had diabetes; this number is however expected to rise to 592 million by 2035. Majority of the people with diabetes live in low- and middle-income countries and these will experience the greatest increase in cases of diabetes over the next 22 years [3].

This microvascular complication develops in approximately 30% of patients with type1 diabetes mellitus (DM1) and approximately 40% of patients with type 2 diabetes mellitus (DM2) [4].

MATERIALS AND METHODS:

Plant Material: Fresh stems of *E. antiquorum* were collected from the vicinity of Varanasi district in Uttar Pradesh, India during the month of January, 2018 and air dried. The plant material was identified and authenticated by Prof. N. K. Dubey, Taxonomist, FNASc and FNAAS, Center of Advanced Study in Botany, Institute of Science, BHU, Varanasi, India. The voucher herbarium specimen (Euphorbia.2018/4) has been deposited in the Department of Botany.

Animals: All the animals used for the study were healthy and active in their cage. Studies were carried out using male Wistar rats weighing 170-200 g. The animals were bred, reared and housed in the animal house of BNU University with CPCSEA no. (03/BNCP/IAEC/2018). The rats were group housed in polyacrylic cages (38 × 23 × 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 °C) and relative humidity 50% (±10%), with a dark and light cycle of 12 ± 1 h. They were allowed free access to standard dry pellet diet (Amrut, India) and water *ad libitum* and kept in quarantine for a week to acclimatize with animal house facility (CPCSEA guideline, 7th January, 2010).

The animals were maintained in accordance with the guidelines specified by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests (Animal Welfare Division) Govt. of India, New Delhi.

Preparation of Extracts: Coarsely powdered (200 g) stem was extracted with 95% (v/v) alcohol in a Soxhlet apparatus. The extract obtained was filtered and subjected to concentration at a temperature below 50 °C to obtain a dark brown semi solid mass. The extract was suspended in distilled water and used for further studies.

Experimental Design:

Group I: (Normal or Vehicle Control): Group I animals were non diabetic and served as normal control and received 0.9% normal saline. Six healthy animals with moderate hyperglycemia, *i.e.* serum glucose between the normal ranges were randomly selected.

Group II (Diabetic Control): Rats served as diabetic control, which were injected a single dose of 150 mg/kg b.w. of alloxan monohydrate (ip) with 0.9% normal saline.

Group III: Diabetic rats were treated with ethanolic plant extract by gavages (200 mg/kg/day b.w., p.o) in 0.9% normal saline once daily for 21 days.

Group IV: Diabetic rats were treated with ethanolic plant extract by gavages (400 mg/kg b.w., p.o) in 0.9% normal saline once daily for 21 days.

Group V: Which served as positive control, diabetic rats were treated with metformin by gavages (200 mg/kg b.w., p.o) in 0.9% normal saline once daily for 21 days.

All animals were treated for 21 days and sacrificed on the last day. Unwanted placebo effect in case of each control group was blocked by executing

appropriate vehicle treatment and same animal handling pattern. Extract and standard drug doses given orally were suspended in 0.9% normal saline. Blood samples were drawn from tail vein and retro orbital sinus using diethyl-ether anesthesia after 3 weeks. Body weight measurement and blood sampling were done on 21st day of the study. Animals (rats) were sacrificed, dissected and organs were cleaned and collected and kept in a suitable container [5].

Acute Toxicity: Acute toxicity studies were carried out following OECD guidelines 420 [6].

Following Parameters would be studied:

- ✓ Blood glucose level
- ✓ Blood urea Nitrogen
- ✓ Body weight

Blood Glucose Level: Blood sample for glucose estimation was collected from rat tail vein after applying xylene to make vein prominent. Blood glucose was estimated by glucose oxidase-peroxidase reactive strips and a glucometer.

Blood Urea Nitrogen: Analysis of serum for blood urea nitrogen (BUN) was estimated by using BUN GLDH kit (Bhat Bio-tech Pvt. Ltd., Bangalore, India) technique as per instructions of manufacturers provided in BUN kits.

Body Weights: Body eights was measured prior to euthanasia of all the animals on 21st day of the study. Last documentation of body weight was shortly before euthanasia. Body weight was determined to the nearest 0.1 g, using a precision scale [7-8].

RESULTS AND DISCUSSION: Extract of *Euphorbia antiquorum* significantly decreased the blood glucose level, blood urea nitrogen and increased in body weight with compare to diabetic control in a dose dependent manner.

Effect of *Euphorbia antiquorum* Ethanolic Extract on Alloxan Induced Diabetic Nephro-pathy:

Effect on Blood Glucose Level: The virtual results of blood glucose alteration at various doses of test samples (Group III & IV) with normal or vehicle control (NC) (Group I), diabetic control (DC) (Group II) and standard drug (Metformin) (Group V) in normal rats was tabulated in **Table 1** and **Fig. 1**. Substantiation has been gathered in the past few years supporting that diabetes was precipitated by stress. Moreover, it was also reported that hyperglycaemia itself increases stress. In this research work, the diabetes was induced with alloxan, because it was more inexpensive and easily accessible. Moreover, alloxan was reported to turn out diabetes by destructive pancreas by free radical associated mechanisms. Rat was used since it was regularly used animal model for fast showing of drugs for their hypoglycaemic/antihyper-glycaemic action. Since little amount of blood was necessary for glucose analysis, the blood samples were collected by retro orbital puncture as it was reported to be good method when minute samples of blood were required. According to the standard working protocol, 21 days daily treatment with test drug moderately reduced the prominent blood glucose in alloxan induced diabetic rats while it had no result on blood glucose of normal rats.

TABLE 1: EFFECT OF *EUPHORBIA ANTIQUORUM* ETHANOLIC EXTRACT ON BLOOD GLUCOSE LEVEL IN ALLOXAN INDUCED DIABETIC RAT

Group	Treatment	Dose (mg/kg)	Blood Glucose Level mg/dL (Mean ± SEM)
1	NC	-	105.5±3.030
2	DC	120	303.3±23.12
3	DC+EAE	200	189.2±5.839***
4	DC+EAE	400	128.0±4.517***
5	DC+Metformin	200	110.5±6.065***

Statistical significance test was done by ANOVA followed by Tukey's t test (n=6). Values are Mean ± SEM of 6 animals per group, *Difference in blood glucose level of a group when compared with diabetic control significant at p<0.05. **Difference in blood glucose level of a group when compared with diabetic control significant at p<0.01. ***Difference in blood glucose level of a group when compared with diabetic control significant at p<0.001.

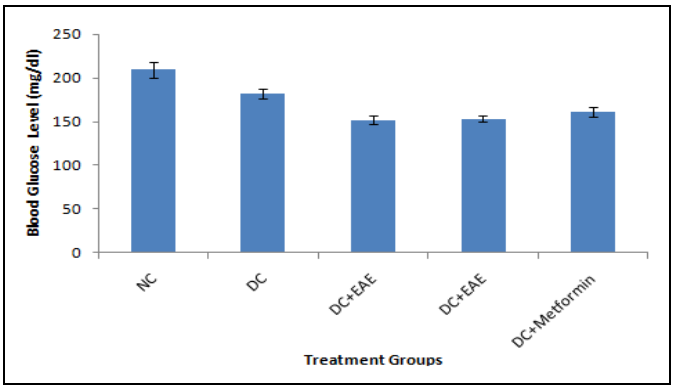

FIG. 1: EFFECT OF *EUPHORBIA ANTIQUORUM* ETHANOLIC EXTRACT ON BLOOD GLUCOSE LEVEL IN ALLOXAN INDUCED DIABETIC RAT

Effect of Different Doses of Ethanolic Extract of Stems of *Euphorbia antiquorum* (EAE) and Metformin on Blood Glucose Level of Alloxan-Induced Diabetic Rat: EAE at doses of 400 mg/kg b.w. (Group IV) significantly reduced the blood glucose of alloxan-induced diabetic rats **Table 1** & **Fig. 1**, similar to what was seen in the standard (metformin) group (Group V). The animals of non-diabetic normal or vehicle control group (Group I) had fasting blood glucose levels of 105.5 ± 3.03 mg/dl at the day of 21, whereas that of diabetic control group was 303.3 ± 23.12 mg/dl (Group II). The group treated with EAE at doses of 200 mg/kg b.w. (Group III) showed significant reduction of blood glucose levels of 189.2 ± 5.839 mg/dl (***p < 0.001 vs. diabetic control group) at the day of 21. At the day of 21, the doses of EAE at doses of 400 mg/kg b.w. (Group IV) significantly reduced blood glucose levels to 128.0 ± 4.517 mg/dl (***p < 0.001 *vs.* diabetic control group). The blood glucose levels of EAE at doses of 400 mg/kg b.w. (Group IV) demonstrated a notable significant to 128.0 ± 4.517 mg/dl (***p < 0.001 *vs.* diabetic control group) but which is not even better than metformin (200 mg/kg b.w) treated group (Group V) that reduced blood glucose levels to 110.5 ± 6.065 mg/dl (***p < 0.001 *vs.* diabetic control group) at day of 21. So, it can be concluded that, in this present study it was observed that treatment of diabetic induced rats with 400 mg/kg b.w. (Group IV) of test samples and metformin showed significant decrease in blood glucose level, compared to the dose at 200 mg/kg b.w. (Group III) of test samples of both the extracts.

TABLE 2: EFFECT OF *EUPHORBIA ANTIQUORUM* ETHANOLIC EXTRACT ON BLOOD UREA NITROGEN IN ALLOXAN INDUCED DIABETIC RAT

Group	Treatment	Dose (mg/kg)	Blood Urea Nitrogen in µmol/l (Mean ± SEM)
1	NC	-	17.33±1.109
2	DC	120	73.18±2.048
3	DC+EAE	200	42.51±1.019***
4	DC+EAE	400	33.57±1.391***
5	DC+Metformin	200	24.65±0.8327***

Statistical significance test was done by ANOVA followed by Tukey's t test (n=6). Values are Mean ± SEM of 6 animals per group, *Difference in blood urea nitrogen level of a group when compared with diabetic control significant at p<0.05. **Difference in blood urea nitrogen level of a group when compared with diabetic control significant at p<0.01. ***Difference in blood urea nitrogen level of a group when compared with diabetic control significant at p<0.001.

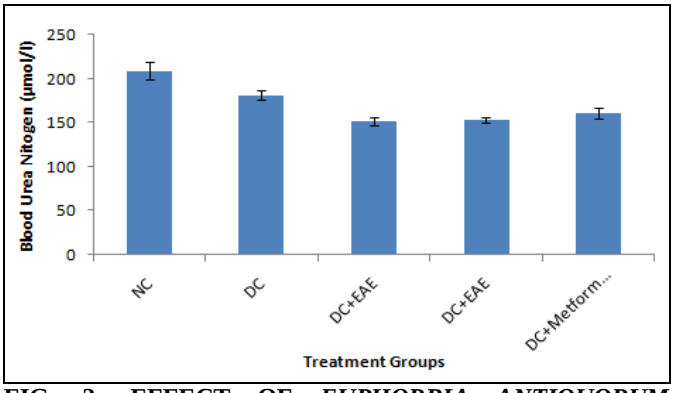

FIG. 2: EFFECT OF *EUPHORBIA ANTIQUORUM* ETHANOLIC EXTRACT ON BLOOD UREA NITROGEN IN ALLOXAN INDUCED DIABETIC RAT

Effect on Blood Urea Nitrogen (BUN) Level: The fundamental results of blood urea nitrogen modification at various doses of test samples (Group III & IV) with normal or vehicle control (NC) (Group I), diabetic control (DC) (Group II) and standard drug (Metformin) (Group V) in rats was tabulated in **Table 2** and **Fig. 2**.

TABLE 3: EFFECT OF *EUPHORBIA ANTIQUORUM* ETHANOLIC EXTRACT ON BODY WEIGHT IN ALLOXAN INDUCED DIABETIC RAT

Group	Treatment	Dose (mg/kg)	Body Weight in gm (Mean ± SEM)
1	NC	-	209.2±9.697
2	DC	120	181.7±5.869
3	DC+EAE	200	151.7±4.773*
4	DC+EAE	400	153.3±3.073*
5	DC+Metformin	200	160.8±5.833*

Statistical significance test was done by ANOVA followed by Tukey's t test (n=6). Values are Mean ± SEM of 6 animals per group, *Difference in body weight of a group when compared with diabetic control significant at p<0.05. **Difference in body weight of a group when compared with diabetic control significant at p<0.01. ***Difference in body weight of a group when compared with diabetic control significant at p<0.001.

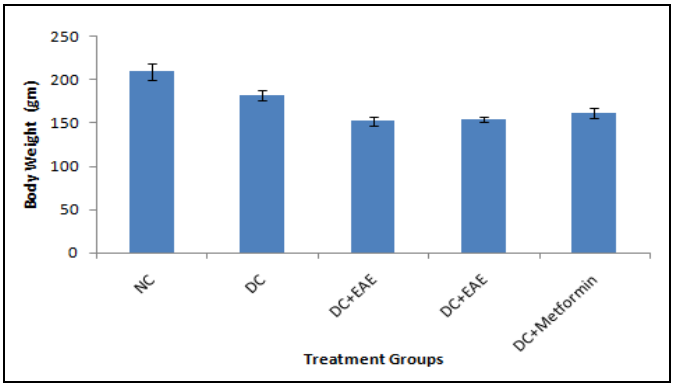

FIG. 3: EFFECT OF *EUPHORBIA ANTIQUORUM* ETHANOLIC EXTRACT ON BODY WEIGHT IN ALLOXAN INDUCED DIABETIC RAT

Effect of Different Doses of Ethanolic Extract of Stems of *Euphorbia antiquorum* (EAE) and Metformin on Blood Urea Nitrogen Level of Alloxan-Induced Diabetic Rat: EAE at doses of 200 and 400 mg/kg b.w. (Group IV) significantly reduced the blood urea nitrogen of alloxan-induced diabetic rats **Table 2** and **Fig. 2**, similar to what was seen in the standard (metformin) group (Group V). The animals of non-diabetic normal or vehicle control group (Group I) had blood urea nitrogen levels of 17.33 ± 1.109 mg/dl at day 21, whereas that of diabetic control group was 73.18 ± 2.048 mg/dl (Group II). The group treated with EAE at doses of 200 mg/kg b.w. (Group III) showed significant reduction of blood urea nitrogen levels of 42.51 ± 1.019 mg/dl (***$p < 0.001$ *vs*. diabetic control group) at day of 21. At the day 21 the doses of EAE at doses of 400 mg/kg b.w. (Group IV) significantly reduced blood urea nitrogen levels to 33.57 ± 1.391 mg/dl (***$p < 0.001$ *vs*. diabetic control group). The blood urea nitrogen levels of EAE at doses of 400 mg/kg b.w. (Group IV) demonstrated a notable significant decline to 42.51 ± 1.019 mg/dl (***$p < 0.001$ *vs*. diabetic control group) but which is not even better than metformin (200 mg/kg b.w) treated group (Group V) that reduced blood urea nitrogen levels to 24.65 ± 0.8327 mg/dl (***$p < 0.001$ *vs*. diabetic control group) at day of 21.

Effect on Body Weight: EAE at doses of 200 and 400 mg/kg (Group III & IV) significantly reduced body weight of treated animals at days of 21, compared to the initial body weights (Group I) of each and individual groups. These special effects of weight reduction were similar to that of metformin group. In the present study a decrease in body weight was observed rats after induction of diabetes using alloxan (Group II). The results of body weight conversion at various doses of test samples (Group III & IV) with normal or vehicle control (NC) (Group I), diabetic control (DC) (Group II) and standard drug (Metformin) (Group V) in rats was tabulated in **Table 3** and **Fig. 3**.

Effect of Different Doses of Ethanolic Extract of Stems of *Euphorbia antiquorum* (EAE) and Metformin on Body Weight of Alloxan-Induced Diabetic Rat: EAE at doses of 200 & 400 mg/kg b.w. (Group III & IV) significantly reduced the body weight of alloxan-induced diabetic rats **Table 3** and **Fig. 3**; similar to what was seen in the standard (metformin) group (Group V). The animals of non-diabetic normal or vehicle control group (Group I) had body weight of 209.2 ± 9.697 gm at day 21, whereas that of diabetic control group was 181.7 ± 5.869 gm (Group II). The group treated with EAE at doses of 200 mg/kg b.w. (Group III) showed significant reduction of body weight of 151.7 ± 4.773gm (*$p < 0.05$ *vs*. diabetic control group) at day of 21. At the day 21 the doses of EAE at doses of 400 mg/kg b.w. (Group IV) significantly reduced body weight to 153.3 ± 3.073 gm (*$p < 0.05$ *vs*. diabetic control group). The body weight of EAE at doses of 400 mg/kg b.w. (Group IV) demonstrated a notable significant decline to 153.3 ± 3.073 gm (*$p < 0.05$ *vs*. diabetic control group) but which is almost like metformin (200 mg/kg b.w) treated group (Group V) that reduced body weight to 160.8 ± 5.833 gm (*$p < 0.05$ *vs*. diabetic control group) at day of 21.

SUMMARY AND CONCLUSION: The presented study is an attempt to investigate the effect of alcoholic extract of *Euphorbia antiquorum* L. (EA) on Alloxan induced diabetic in Wistar rats. The no. of previous phytochemical study was screening showed the presence of tannins, carbohydrate, flavonoids and reducing sugar which is responsible for the anti-diabetic activity. The animals were induced with alloxan at a dose of 120 mg/kg intraperatoneal and the diabetic animals were treated with EAE (200, 400 mg/kg) for 21 days orally. The blood glucose level, body weight, Blood urea nitrogen were measured from the pancreas and kidney homogenate were measured which showed significant activity. The finding of the presence investigation suggests the EAE has potential for its evaluation as protective agents against toxicity induced by alloxan. Clinical assessments EA determination of underlying mechanism of the protective effects in interesting topics requiring further study.

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CONFLICTS OF INTEREST: Nil

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