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EVALUATION OF ANTI DIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF IXORA COCCINEA LEAVES

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Abstract:

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Ixora coccinea was made on the basis of its High therapeutic value. They are mostly shrubs and small trees that are part of the under-storey plant community in the tropical forest. The preliminary phytochemical investigation of powdered Ixora coccinea showed the presence of Carbohydrates, Alkaloids, Glycosides, Steroid, Terpenoids, Flavonoids, Proteins and Amino acids. The pharmacological and acute toxicity studies of ethanolic extract was performed by following, OECD-423 guidelines (Acute toxic class method). No mortality or acute toxicity was observed upto 2000mg/kg of body weight. The Biological dose of extract Ixora coccinea dose was selected 200mg/kg and 400mg/kg in this dose possessed significant antidiabetic activity. Alloxan causes a massive destruction of β-cells of the islets of Langerhans, resulting in reduced synthesis and release of insulin. The function of the insulin suppressed, which leads to high level of hyperglycemic and eventually to death, but the different extracts of Ixora coccinea showed antidiabetic effect in alloxan induced diabetic rats and reduced the mortality rate significantly.

KEY WORDS: *Ixora coccinea, Anti-diabetic activity, Leaves, Ethanolic extract*

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INTRODUCTION:

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Metabolic abnormalities in carbohydrates, lipids, and proteins result from the importance of insulin as an anabolic hormone. Low levels of insulin to achieve adequate response and/or insulin resistance of target tissues, mainly skeletal muscles, adipose tissue, and to a lesser extent, liver, at the level of insulin receptors, signal transduction system, and/or effector enzymes or genes are responsible for these metabolic abnormalities. The severity of symptoms is due to the type and duration of diabetes. Some of the diabetes patients are asymptomatic especially those with type 2 diabetes during the early years of the disease, others with marked hyperglycemia and especially in children with absolute insulin deficiency may suffer from polyuria, polydipsia, polyphagia, weight loss, and blurred vision. Uncontrolled diabetes may lead to stupor, coma and if not treated death, due to ketoacidosis or rare from nonketotic hyperosmolar syndrome [1-3]. Although classification of diabetes is important and has implications for the treatment strategies, this is not an easy task and many patients do not easily fit into a single class especially younger adults [1,4-6] and 10% of those initially classified may require revision [7]. The classical classification of diabetes as proposed by the American Diabetes Association (ADA) in 1997 as type 1, type 2, other types, and gestational diabetes mellitus (GDM) is still the most accepted classification and adopted by ADA [1]. Wilkin [8] proposed the accelerator hypothesis that argues "type 1 and type 2 diabetes are the same disorder of insulin resistance set against different genetic backgrounds" [9]. The difference between the two types relies on the tempo, the faster tempo reflecting the more susceptible genotype and earlier presentation in which obesity, and therefore, insulin resistance, is the center of the hypothesis. Other predictors of type 1 diabetes include increased height growth velocity [10,11] and impaired glucose sensitivity of β cells [12]. The implications of increased free radicals, oxidative stress, and many metabolic stressors in the development, pathogenesis and complications of diabetes mellitus [13-18] are very strong and well documented despite the inconsistency of the clinical trials using antioxidants in the treatment regimens of diabetes[19-21]. The female hormone 17-β estradiol acting through the estrogen receptor-α (ER-α) is essential for the development and preservation of pancreatic β cell function since it was clearly demonstrated that induced oxidative stress leads to β-cell destruction in ER-α knockout mouse. The ER-α receptor activity protects pancreatic islets against glucolipotoxicity and therefore prevents β-cell dysfunction [22].

The common names are West Indian Jasmine, Rangan, Kheme, Ponna, Chann tanea, Techi, Pan, Santan, Jarum-jarum, Jungle flame, Jungle geranium and many more. They are mostly shrubs and small trees that are part of the under-storey plant community in the tropical forest, but some species can become very tall. People of the region have been using Ixora's for generations, not only for ornamental purposes but more importantly because of their medicinal values. Ixora is a popular flowering plant in gardens. Red Ixora flowers are commonly used in Hindu worship, as well as in Indian folk medicine.

In recent year there has been a tremendous increase in demand for herbal drugs due to its safety, efficacy and better therapeutic results and also due to its economic pricing as compared to synthetic or allopathic drugs, which have several therapeutic complications. The selection of this plant, Ixora coccinea was made on the basis of its High therapeutic value, Easy availability, Degree of research work which is not done Very less pharmacological studies have been carried out on the leaves of Hence, I have decided to choose the Ixora coccinea project on Which detailed studies on Preliminary Phytochemical and Pharmacological activities of Oral glucose tolerance, and In-vivo Anti-diabetic studies is done.

MATERIALS AND METHODS:

Plant collection and identification

Fresh plant of Ixora coccinea was collected from the forest from chittur dist. The plant materials were identified and authenticated by Prof. Madhav Shetty, Dept. of botany, Taxonomist, SV University, Tirupati. A voucher was kept in the Department of Pharmacognosy for reference.

Preparation of plant extract

The sample was washed with distilled water to remove any adherent particles, shade dried and powdered. 25g of each sample was weighed and extracted with 300ml of ethanol by continuous hot percolation with the help of soxhlet apparatus for 10hrs of time. On completion the extract was filtered and concentrated using rotary evaporator under reduced pressure and controlled temperature of 500C – 600 C. The concentrates were stored in the refrigerator for further use.

Experimental design

Adult Male Wistar rats of weighing 180-230 gms were used for this study. The inbred animals were

procured from the animal house. They were housed five per cage under standard laboratory conditions at a room temperature at 22±2^0 C with 12 hr light/dark cycle. The animals were acclimatized to laboratory conditions one week and provided with standard pellet chow and water *ad libitum*. Ethical committee clearance was obtained from IAEC of CPCSEA.

Phytochemical Qualitative Analysis
The plant extracts were assessed for the existence of the phytochemical analysis [6]

Determination of LD50value of ethanolic extract of *Ixora coccinea*

Acute Oral Toxicity Study:
The procedure was followed by using OECD guidelines 423 (Acute toxic class method). The acute toxic class method is a step wise procedure with 3 animals of single sex per step. Depending on the mortality and / or moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test animals while allowing for acceptable data based scientific conclusion. The method uses defined doses (5, 50, 300, 2000mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemical which cause acute toxicity.

Animals:
Female albino mice weighing 20-25g were used in the present study. All rats were kept at room temperature of 22-25ºC in the animal house. All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food for one week in order to adapt to the laboratory conditions.

Procedure:
Twelve animals Albino mice, (25-30gm) were selected for studies.

The starting dose of ethanolic extracts of *Ixora coccinea* 300mg/kg, b.w, p.o, was administered.

Most of the crude extracts possess LD50, value more than 2000mg/kg of the body weight of the animal used. Dose volume was administered 0.1ml/100gm body weight to the animal by oral route. After giving the dose toxic signs were observed within 3-4 hours. Body weight of the animals before and after administration, onset of toxicity and signs of toxicity like changes in the skin and fur, eyes and mucous membrane and also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity and behavior pattern, sign of tremors, convulsion, salivation, diarrhea, lethargy and sleep and coma was also to be noted, if any, was observed. The animal toxic or death was observed upto 14 days.

Observation
Acute toxicity studies and evaluation of dates are studied as per the guideline of OECD (423).No toxicity or death was observed for these given dose levels, in selected and treated animals. So the LD50 of the ethanolic extract of leaves of Ixora coccinea wasgreater than 2000mg/kg (LD50>2000mg/kg). Hence the biological dose was fixed at three levels, 200 and 400mg/kg body weight for the extract.

ORAL GLUCOSE TOLERANCE TEST (OGTT):
The overnight fasted (18hr) normal rats were taken and divided into fourgroups consists of six animals. They were provided with drinking water only. Normal saline solution was administered to group I animals. Group II animals were received *Glibenclamide* (3mg/kg,b.w) as a standard. *Ixora coccinea* ethanol extract (200 and 400 mg/kg) was administered by oral route to group III and IV Glucose (2mg/kg) load was fed 30 minutes after the administration of extracts. Blood was withdrawn from tail vein under mild ether anesthesia initial, 30,60 and 90 minutes after glucose administration and glucose level were estimated using glucose strips and a glucometer (Standard diagnostics Ltd). Blood glucose levels were noted and reported.

EVALUATION OF ANTI-DIABETIC ACTIVITY:
Animals:
Wistar albino rats (150-200g) were selected for either sex, for studies and they were kept in a standard polypropylene cage at room temperature of 27±2ºC, relative humidity 60-70% and well ventilated. They were fed a standard rat pellet and water adlibtium. Animals were deprived of food initially for 16 hrs but had free access to water. The experimental protocol has been approved by institutional animal ethics, committee.

Chemicals:
Alloxan monohydrate (LOBA Chemie, Mumbai, India) was purchased, preserved at 25ºC and used for this study. Glibenclamide is an oral antidiabetic preparation with an efficient hypoglycemic action. Daonil (Glibenclamide) (S.K.Prasad *et.al*, 2009) manufactured by Aventis Pharma Ltd. Goa, India, was collected from market and preserved at room

temperature.

Induction of Experimental Diabetes:
Hyperglycemia / Diabetes was induced by single intraperitoneal injection of freshly prepared aqueous solution of alloxan monohydrate 150 mg/kg, to overnight fasted rats. After 48 hrs of alloxan injection, the animals which did not developed hyperglycemia i.e glucose level > 200mg/dl, were injected or replaced. Immediately after confirmation of diabetes, rats were classified into five groups of six rats each. Standard drug used for treatment, Glibenclamide, 5 mg/kg, ethanolic test extract were prepared, 200mg/kg and 400mg/kg in 2% Carboxy Methyl Cellulose (CMC) and were given orally. Taking six rats in each five groups did evaluation of antidiabetic effect.

Experimental Design:
Experimental rats were divided into 5 groups of six animals each all the group of animals were induced diabetic except control and treated for 21days as follows.

- Group I: Normal control rats fed with vehicles only. (Normal saline with 1%CMC)
- Group II: Diabetic controls rats (Alloxan monohydrate 120mg/kg body weight of rats, once i.p injection).
- Group III: Diabetic rats treated with standard drug, Glibenclamide 3mg/kg per oral body weight.
- Group IV: Diabetic rats treated with ethanolic extract of *Ixora coccinea* 200mg/kg, per oral, dissolved in 1% carboxy methyl cellulose (CMC).
- Group V: Diabetic rats treated with ethanolic extract of 400mg/kg, per oral, dissolved in 1% carboxy methyl cellulose (CMC).

Sample collection:
Fasting blood glucose (FBG) of all rats was determined before the start of the experiment. Blood sample was collected at weekly intervals from tail vein puncture till the end of study. In the continuous 21 days of drug treatment, a blood glucose level of all animals was determined at the 0, 7, 14, 21 day by using one touch glucometer (SD Check) method.

EVALUATION OF PARAMETERS:
Estimation of changes in body weight of the animals:
Body weight of all rats was measured on starting day (0 day) of the experiment and 21stday of the experiment. Both initial and final body weights were noted and reported.

Estimation of blood glucose level:
Glucose level in plasma was estimated by glucose oxidase/ peroxidase method using a commercial kit from Med source Ozone Biomedicals Pvt Ltd followed by Trinder, p.(1969)Annals.Clin. Bio chem.6,24.

Reagents:

1. Enzyme reagent
2. Buffer solution
3. Glucose standard (100 mg%)

Procedure:
10 µl of plasma was added to 1.0 ml of working enzyme reagent, mixed well and incubated at 37°C for 15 min. The colour developed was read at 505 nm against blank containing distilled water instead of the sample. A standard was also processed similarly.

The level of glucose is expressed as mg/dl.

STATISTICAL ANALYSIS
All the values of body weight and fasting blood glucose level were expressed as mean ± standard error of mean (S.E.M) and was analyzed for significance by ANOVA and groups were compared by Tukey-Kramer multiple comparison test. Differences between groups (p Value) were considered significant at $P<0.05$ level

RESULTS AND DISCUSSION:
Based on literature review the leaves of *Ixora coccinea* were selected and project work was carried on *Ixora coccinea* belonging to the family Rubiaceae was collected and authenticated. The result of the present study show that the ethanol extract ofexerts anti-diabetic *Ixora coccinea* effective against alloxan induced diabetes.

PHARMACOGNOSTICAL STUDIES
ANALYTICAL PARAMETERS
PRILIMINARY PHYTOCHEMICAL STUDIES
Table No. 1: Weight of extract of *Ixora coccinea*

Name of extract	Yield(%w/w)
Ethanol	8

The extract obtained were subjected to qualitative Phytochemical test to find out the active constituents.

Table No. 2: Qualitative Phytochemical analysis of heart wood parts extract

TEST FOR PHYTOCONSTITUENTS	RESULT
Saponins	_
Alkaloids	+
Glycosides	+
Tannins and phenolic compounds	+, _
Carbohydrates	+
Proteins and aminoacids	+
Flavonoids	+
Steroids	+

(+) - Present (-) - Absent

DISCUSSION:

The preliminary Phytochemical studies were done in the ethanolic extract of *Ixora coccinea* leaves result suggest that presence of **Alkaloids, Carbohydrate, Glycosides, Proteins and aminoacids, flavonoids, Steroids, and tannins.**

PHARMACOLOGICAL STUDIES

ACUTE ORAL TOXICITY STUDIES

The acute oral toxicity of the ethanolic extract of *Ixora coccinea*was carried out as per OECD 423-guidelines (Acute toxic class method). Acute toxicity studies revealed that LD_{50}>2000mg/kg for the extract. Hence, the biological dose was fixed at EEIC 200mg and 400mg of body weight for the extract.

EFFECT ON GLUCOSE TOLERANCE

In OGTT, the doses of EEIC 200 mg/kg and 400 mg/kg increased the tolerance for glucose suggesting increased peripheral utilization of glucose. The reduction in blood glucose level was dose dependent.

Table No. 3: Effect of ethanolic extract of *Ixora coccinea*and *Glibenclamide* on glucose tolerance of diabetic rats.

Groups	Treatment	Change in blood glucose levels(mg/dl)			
		Fasting	After 30 Minutes	After 60 minutes	After 90 minutes
1.	Glucose 2mg/kg	84.99±2.90	125.22±2.02	127.88±1.90	108.51±2.89
2.	*Glibenclamide* 3mg/kg	68.01±3.32	83.10±1.50 a	62.01±2.55 a	52.71±3.24 a
3.	EEIC 200mg/kg	67.05±1.49	113.08±6.99 b	113.76±3.02	96.42±2.98 a
4.	EEIC 400mg/kg	78.69±2.80	99.92±1.99 a	105.11±7.88 b	90.65±1.91 a

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = $p<0.001$; b= ** = $p<0.01$; c= * =$p<0.05$.EEIC. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). Normal control group I was compared with group 2(std drug) and extract treated groups III , IV.

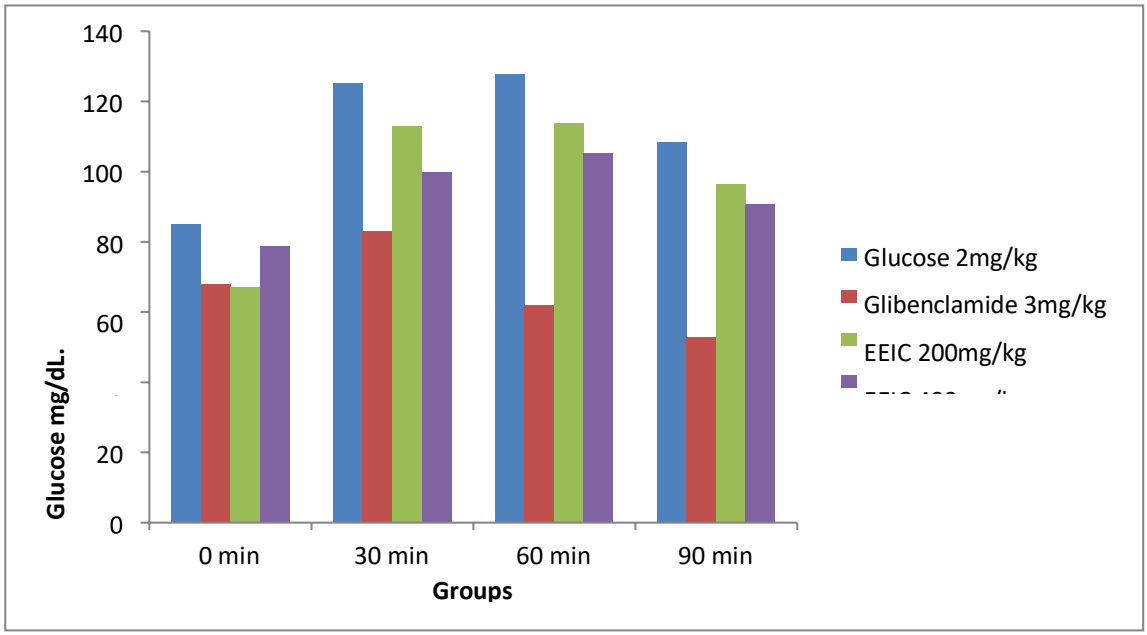

Figure No. 1: Effect of ethanolic extract of *Pouteria campechiana* and *Glibenclamide* on glucose tolerance of diabetic rats.

EVALUATION OF PARAMETERS

Changes in body weight:

Vehicles control animals were found to be stable in their body weight but significant reduction in diabetic control group during 21 days. Alloxan caused body weight reduction, which is slightly reversed by ethanolic extract of *Ixora coccinea* treated (200mg/kg and 400mg/kg) groups after 21 days.

While, significant ($p<0.01$, $p<0.001$) increase in body weight was observed in rats treated with ethanolic extract of *Ixora coccinea* The EEIC treated diabetic rats (400mg/kg) were slightly increased the body weight level.

Table No. 4: Body weight changes in ethanolic extract of*Ixora coccinea* and *Glibenclamide* on control and experimental groups of rats

Group	Treatment	Body weight changes (g)	
		Day 0	Day 21
I	Normal control rats (vehicles only)	145±7.67	204.15±11.94
II	Diabetic control rats	162.5±8.54 b	129.18±7.67 b
III	Diabetic group + Glibenclamide 5mg/kg	150±2.44 a	208.37±02.37 a
IV	Diabetic group + EEIC (200mg/kg)	154.17±7.67 b	200±6.46 b
V	Diabetic group + EEIC (400mg/kg)	162.6±04.05 a	210.6±17.98 c

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = $p<0.001$; b= ** = $p<0.01$; c=* =$p<0.05$. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiplecomparison tests). (Diabetic control group II was compared with Normal control group I, group III and extract treated groups IV, V compared with Diabetic control group II.)

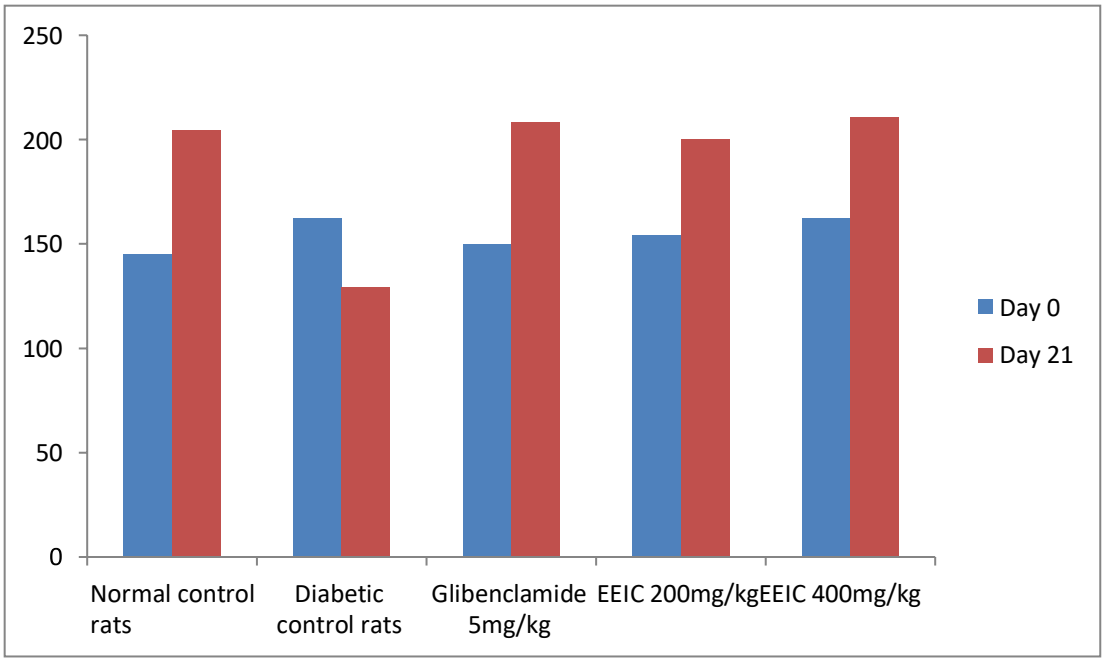

Figure No 2: Body weight changes in ethanolic extract of and *Ixora coccinea Glibenclamide* on control and experimental groups of rats

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = p<0.001; b= ** = p<0.01; c=* =p<0.05. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiplecomparison tests). (Diabetic control group II was compared with Normal control group I, group III and extract treated groups IV, V compared with Diabetic control group II.)

Changes in blood glucose:

A significant increase in the level of blood glucose, was observed in diabetic control rats when compared to control rats. Administration of EEIC and *Glibencla mide* to diabetic rats significantly decreased the elevated level of blood glucose, near tocontrol level.

Table No. 5: Effect of *Ixora coccinea* ethanolic extract of and *Glibenclamide* on blood glucose level

Group	Treatment	Blood glucose level (mg/dl)			
		Day 0	Day 7	Day 14	Day 21
I	Normal control rats (vehicles only)	70.65±1.42	80±2.34	78.83±2.36	72.33±1.82
II	Diabetic control rats	380.6±1.57 a	336.84±2.18 a	354.84±2.81 a	369.32±1.09 a
III	Diabetic group + Glibenclamide 3mg/kg	313.6±2.09 a	281.34±1.56 a	233.65±1.42 a	147.67±1.05 a
VI	Diabetic group + EEIC (200mg/kg)	334.66±8.90 c	285±03.26 a	174.82±2.91 a	161±2.08 a
V	Diabetic group + EEIC (400mg/kg)	321.84±12.16 c	286±5.08 b	157.82±1.30 a	160.5±2.74 a

Values are expressed as Mean ±SE, n = 6 by Dunnett's t test; *P <0.01 Vs Control **P > 0.001 Vs Control.

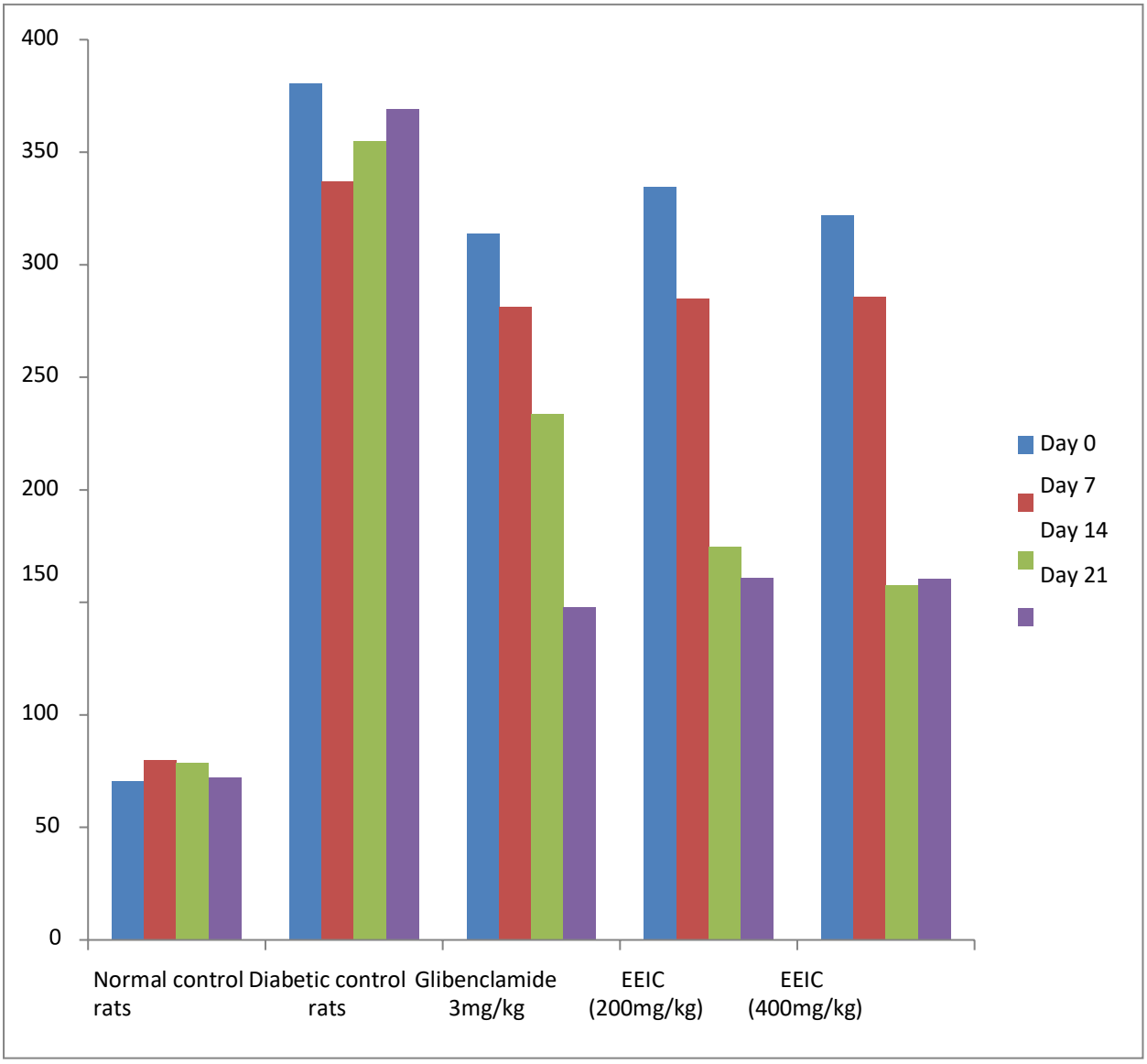

Figure No 3: Effect of ethanolic extract of *Ixora coccinea* and *Glibenclamide* on blood glucose level.

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = p<0.001; b= ** = p<0.01; c=* =p<0.05. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiplecomparison tests). (Diabetic control group II was compared with Normal control group I, group III and extract treated groups IV, V compared with Diabetic control group II.)

SUMMARY AND CONCLUSION:

The leaves of *Ixora coccinea* belonging to the family Rubiaceae has been examined to gain an insight of its phytochemical and pharmacological behavior.

The preliminary phytochemical investigation of powdered *Ixora coccinea* showed the presence of Carbohydrates, Alkaloids, Glycosides, Steroid, Terpenoids, Flavonoids, Proteins and Amino acids.

The pharmacological and acute toxicity studies of ethanolic extract was performed by following, OECD-423 guidelines (Acute toxic class method). No mortality or acute toxicity was observed upto 2000mg/kg of body weight. The Biological dose of extract *Ixora coccinea* dose was selected 200mg/kg and 400mg/kg in this dose possessed significant antidiabetic activity.

Alloxan causes a massive destruction of β-cells of the islets of Langerhans, resulting in reduced synthesis and release of insulin. The function of the insulin suppressed, which leads to high level of hyperglycemic and eventually to death, but the different extracts of *Ixora coccinea* showed antidiabetic effect in alloxan induced diabetic rats and reduced the mortality rate significantly.

Overall, it can be concluded that ethanolic extract of *Ixora coccinea* can be used as a natural source of anti-diabetic activity.

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