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Experimental Study of *Manjistha* root (*Rubia cordifolia* Linn.) w.s.r. to Anti-Diabetic Activity

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ABSTRACT

Manjistha is well known for its various activities, one of which is *Pramehagna* (Anti-Diabetic) activity. The *Manjistha* was collected from market and its authenticity was approved by its organoleptic characters and is used for present study. The sample was processed and its alcoholic and aqueous extracts were prepared. Then the drug was subjected to experimental study on albino rats. Study was carried out to assess the anti-diabetic activity. Results showed that there is significant reduction in sugar level which indicates that both aqueous and alcoholic extracts do possess significant anti-hyperglycemic activity as compared to Metformin (Standard drug), which could be due to anti-oxidants and flavonoids present in it. The variations in above said analysis make it very interesting and important to analyse the expected Anti-microbial and immune modulatory activity of *Manjistha* root.

Key words: *Manjistha*, *Rubiocordifolia*, *Experimental Study*, *Anti-Hyperglycemic Activity*.

INTRODUCTION

Ayurvedic treatments are preferred now a days due to their excellent effectiveness and least toxicity. The threatened world is looking towards Ayurveda as a treatment modality which promotes health of a person and also cures the disease. Ayurveda, a part of Atharvaveda displays description and information about drugs useful in the treatment of *Prameha* (Diabetes Mellitus). In 2013, according to International Diabetes Federation, on estimates 381 million peoples had diabetes, and by 2030, the

number is estimated to be double.^[1]

As per WHO, Diabetes mellitus is defined as a heterogeneous metabolic disorders characterized by common feature of chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism.^[2]

The objective of present study was to investigate the phyto-chemical analysis of alcoholic and aqueous extract of *Manjistha* root was investigated in alloxan monohydrate induced diabetic root.

Presently two main groups are recognized as oral anti diabetic agents. They are Sulphonemides derivatives and gunidide derivatives and insulin, but these drugs produce some hazards such as Vertigo, Gastric and Hepatic disorders, Nephrotoxicity, Cardiac disorders etc. Many Anti diabetic drugs have been explained in Ayurvedic texts like *Bimbi*, *Manjistha*, *Jambu*, *Chakramarda* etc. In *Charaka* and *Sushruta Samhita* and in *Nighantus* like *Bhavaprakash* and *Dhanvantari Nighantu* explanation is there stating that *Manjistha* possesses *Pramehagna* properties.^[3-9]

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Therefore *Manjistha* is expected to have anti diabetic activity apart from its other physiological activities such as immunomodulatory, rejuvenative etc. Hence to know its anti diabetic activity and to provide scientific data and statistical validation, Experimental Study of *Manjistha* Root (*Rubia cordifolia* Linn.) w.s.r. to Anti-Diabetic Activity was undertaken.

MATERIALS AND METHODS

Plant Material - Roots of *Rubia cordifolia* were collected from market sample.

Extraction - Roots of *Rubia cordifolia* were coarsely powdered and extracted. The extraction process was done with the help of soxhlet apparatus. Ethenol and water were used as solvents. Extracts were kept in desiccators for the removal of remaining moisture.^[10]

Animals - Either sex albino rats weighing 150-200g maintained under standard experimental condition (temperature 26±2°C, relative humidity 45-55% and 12 hours light/dark cycle) were housed in standard environmental condition.

Induction of diabetics - Hyperglycemia was induced by injecting Alloxan monohydrate at a dose 160mg/kg intraperitonially. The animal were kept under observation and after 24hrs were tested for hyperglycemia using glucometer.

Preparation of extracts

The dried roots of *Rubia cordifolia* were purchased from local market. Then the roots were coarsely powdered by using grinder, then packed into Soxhlet column and subjected to successive extraction using ethanol and distilled water. Excess of solvents from the extracts were recovered using rotary flash evaporator resulted to obtain semisolid crude extracts. The obtained crude extracts were stored in airtight container in refrigerator below 10°C for further investigation.

The stock solution of ethanolic extract of *Rubia cordifolia* roots (EERCR) and aqueous extract of *Rubia cordifolia* roots (AERCR) were prepared using distilled water and then subjected to the following studies.

METHODOLOGY

The stock solutions of the both extracts were administered 0.5 ml/100g of body weight of Wistar rats orally.

- **Group 1 (Control)** - received Alloxan Monohydrate (160mg/kg) and distilled water (1mg/100g b.w.)
- **Group 2 (Standard)** - received Alloxan Monohydrate + Metformin (150mg/kg.p.o.)
- **Group 3** - Alloxan Monohydrate + EERCR (100mg/kg.p.o.)
- **Group 4** - Alloxan Monohydrate + EERCR (200mg/kg.p.o.)

Oral Glucose Tolerance Test in normal rats (OGTT):

Rats were divided into four groups and were administered normal saline and dose of 100mg/kg oral of alcohol extract and 200mg/kg of aqueous extract. Glucose solution 2g/kg was administered 30min after the administration of the extract. Blood samples were withdrawn from retro-orbital at intervals of 1,2,3,4,and 6th hourly of glucose administration and the level of blood glucose was measured.

Evaluation of anti-diabetic activity

Fasting blood glucose of rats was measured after depriving food for 16hrs with free access of drinking water. Hyperglycemia was induced by a single i.p. injection of 160 mg/kg of alloxan monohydrate in sterile saline. After 3 days (i.e. 72 hr.) of Alloxan injection, the hyperglycemic rats (glucose level between 200 - 250 mg/dl) were separated and divided into four different groups comprising of 6 rats each for the anti-diabetic study. Then the animals were given orally both extracts i.e. EERCR and AERCR except normal control and diabetic control groups. After the administration of test extracts the measurement of blood glucose level was performed by obtaining blood through retro orbital plexes of rats at an interval of 1, 2, 3, 4 and 6th hours using glucometer.

STATISTICAL ANALYSIS

The data obtained from the above findings subjected to statistical analysis using one way ANOVA followed by Tukey Kramer Multiple Comparison Test to assess the statistical significance of the results through effect of EERCER and AERCER on fasting blood glucose level in alloxan intoxicated diabetic rats.

OBSERVATIONS AND RESULTS

EERCER and AERCER were evaluated for anti-diabetic activity in rats where alloxan monohydrate (160 mg/kg b.w.,i.p.) used as the diabetogenic agent. A marked rise in fasting blood glucose level was observed in diabetic control compare to normal control rats. Both the EERCER and AERCER decreased the elevated serum blood glucose level at 1st and 2nd hour in a considerable manner. However, antidiabetic effect of both test extract was found to be more effective at 3rd, 4th and 6th hour of interval. Blood glucose lowering effect of both the extracts at doses of 100 and 200 mg/kg b.w. was found to be statistical significant at interval of 1 - 6 hours except EERCER 100 mg/kg at 1 hr interval. The reference drug, Metformin exhibited a significant reduction in blood glucose as compared to diabetic control rats. However, the effect of test extracts on blood glucose level was found to be less potent than the reference standard drug, Metformin. Among the tested extracts the EECRE demonstrated more potent antidiabetic activity in a dose dependent manner compared to AECRE. The results are shown in the Table no. 1 and graphically represented in Figure no. 1 - 5.

Table 1: Effect of EERCER and AERCER on fasting blood glucose level in alloxan induced diabetic rats

| Group | Treatment | Basal value | 1 hr | 2 hr | 3 hr | 4 hr | 6 hr |
|-------|--------------------------|---------------|---------------|---------------|---------------|---------------|---------------|
| 1 | Normal Control | 110.78 ± 2.89 | 109.18 ± 1.98 | 110.45 ± 1.47 | 112.01 ± 2.12 | 111.20 ± 2.45 | 108.98 ± 2.14 |
| 2 | Diabetic Control Alloxan | 248.56 ± 2.24 | 247.46 ± 2.21 | 250.42 ± 2.12 | 249.47 ± 3.01 | 246.56 ± 3.21 | 245.41 ± 3.54 |

| | (160 mg/kg) + Vehicle | | | | | | |
|---|--------------------------------|---------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 3 | Alloxan + Metformin 150 mg/kg) | 238.44 ± 3.65 | 232.41 ± 1.65** | 200.46 ± 2.01** | 180.25 ± 1.65** | 161.01 ± 2.10** | 130.14 ± 2.85** |
| 4 | Alloxan + EERCER (100 mg/kg) | 245.14 ± 2.01 | 240.17 ± 2.98ns | 238.56 ± 2.11* | 180.84 ± 2.13** | 171.71 ± 2.01** | 156.35 ± 3.14** |
| 5 | Alloxan + AERCER (200 mg/kg) | 241.43 ± 2.48 | 235.41 ± 3.56* | 227.12 ± 2.98** | 175.45 ± 2.21** | 170.01 ± 2.98** | 146.11 ± 3.10** |

Values are Mean ± S.E.M, n=6, ***P < 0.001 vs Diabetic Control @ p < 0.001 vs Normal Control

Figure 1: Effect of EERCER and AERCER on blood glucose level (mg/dl) at 1st hr. interval

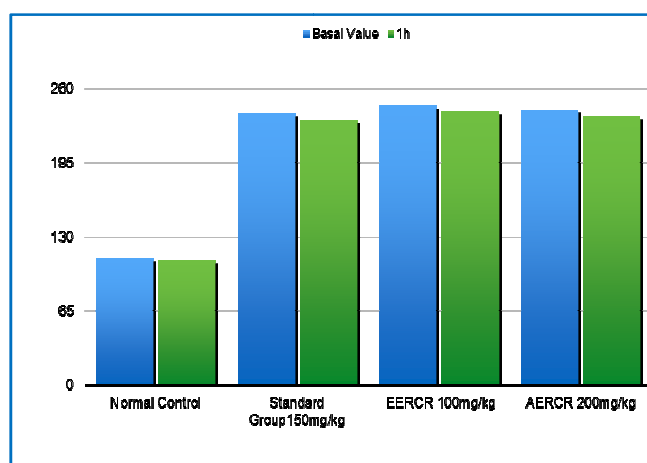


Figure 2: Effect of EERCER and AERCER on blood glucose level (mg/dl) at 2 hr. interval

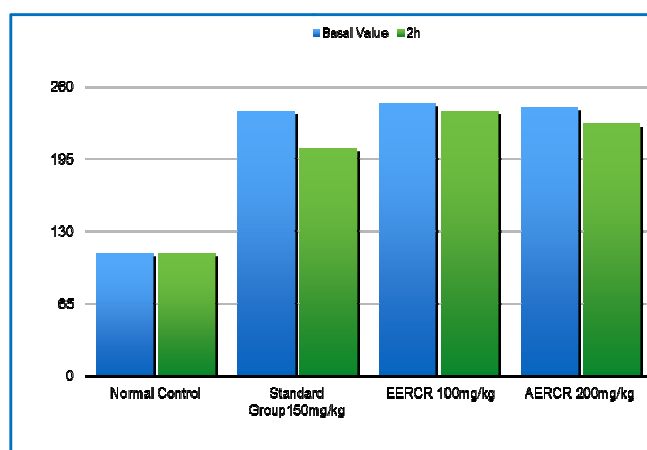


Figure 3: Effect of EERCRCR and AERCRCR on blood glucose level (mg/dl) at 3 hr. interval

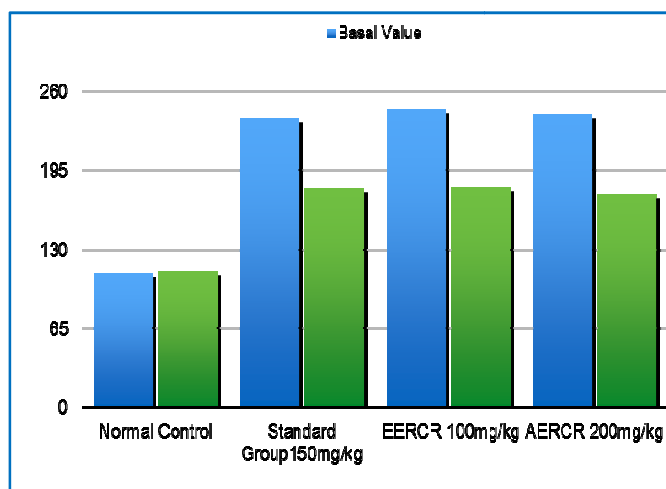


Figure 4: Effect of EERCRCR and AERCRCR on blood glucose level (mg/dl) at 4 hr. interval

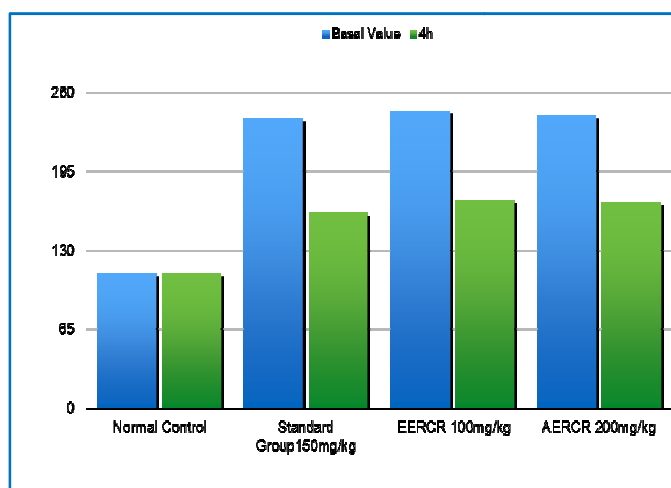
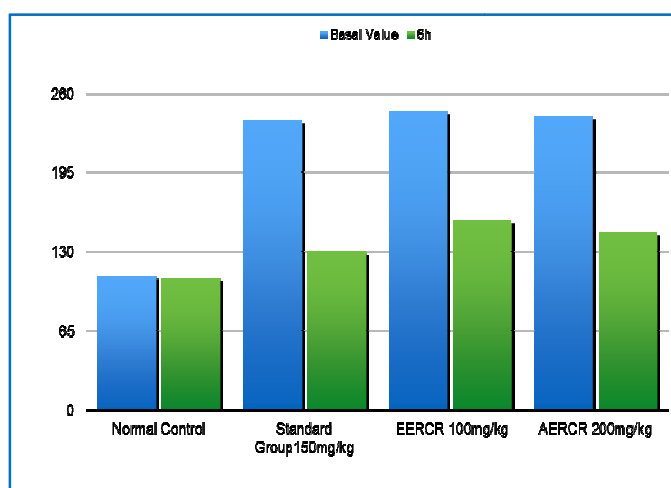


Figure 5: Effect of EERCRCR and AERCRCR on blood glucose level (mg/dl) at 6 hr. interval



DISCUSSION

Diabetes mellitus is an endocrinal disorder characterized by high blood glucose levels due to absolute or relative lack of circulating insulin levels. Though different types of oral anti diabetics are available along with insulin for the treatment of diabetes mellitus, there is a growing interest in natural remedies, due to the side effects associated with these allopathic medications. Herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their minimal side effects and relatively low costs.^[11]

Alloxan causes massive reduction in insulin release by the destruction of β cells of islets of Langerhans there by inducing hyperglycemia. Insulin deficiency leads to various metabolic alteration in animal viz. increase blood glucose, triglycerides, cholesterol and urea.^{[12],[13]}

In the present investigation, treatment with EECRE and AECRE in alloxan intoxicated diabetic rats exhibited significant hypoglycemic effect. The observed hypoglycemic effect of the test extracts may be due to due to regeneration of pancreatic β cells that were partially destroyed by alloxan.

Natural antioxidants strengthen the endogenous antioxidant defense mechanism against reactive oxygen species (ROS) and restore the optimal balance by neutralizing the reactive species.^[14] In the present study, in vivo antioxidant property of the title plant reported in the literature could be the one of the reason for the observed antidiabetic activity of the root extract of *Rubia cordfolia*.^[15]

CONCLUSION

The Experimental study on Albino rats to assess the Anti diabetic activity using alcoholic and aqueous extract of *Manjistha* root showed that there is significant reduction in sugar level which indicates that the extracts aqueous and alcoholic do possess significant anti-hyperglycemic activity as compared to Metformin (Standard drug). Natural antioxidants strengthen the endogenous antioxidant defense mechanism against reactive oxygen species (ROS) and

restore the optimal balance by neutralizing the reactive species in vivo antioxidant property of the *Manjistha* could be the one of the reason for the observed antidiabetic activity of the root extract. Presence of flavonoids in the ethanolic and aqueous extracts which was evident by preliminary phytochemical investigation may be attributed for the observed antidiabetic activity.

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