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Effect of Co administration of extract of *Bougainvillea spectabilis* and *Catharanthus roseus* on acid phosphatases and alkaline phosphatases on alloxan induced diabetic albino rats

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Abstract
Background and purpose of study: *Bougainvillea spectabilis* and *Catharanthus roseus* extracts are part of various herbal formulations for the treatment of diabetes. The present study aims to determine the biochemical alterations due to Co-administration of *Bougainvillea spectabilis* and *Catharanthus roseus* leaf extracts on female diabetic albino rats.
Materials and Methods: *Bougainvillea spectabilis* and *Catharanthus roseus* leaves extracted with methanol and administered to both normal and alloxan induced diabetic rats. The acid phosphatase and alkaline phosphates levels were measured at 7, 14 and 21 days at a dose level of 300 mg/kg b.wt.
Results and Discussion: In the present study, effect of Methanolic extract of combination of *Bougainvillea spectabilis* and *Catharanthus roseus* in various organs of diabetic albino rats significantly decreased the acid phosphatse as well as alkaline phosphatase content at 300mg/kg b.wt for intervals of 7, 14 and 21 days. It may be due to the presence of certain phytoconstituents of plants such as flavonoids, Saponins etc.

Keywords: *Bougainvillea spectabilis*, *Catharanthus roseus*, alloxan, acid phosphatase, alkaline phosphatase

1. Introduction
Plants have always been part of the medicinal science from the beginning of human civilisation to the present modern world of synthetic medicines. Medicinal plants are widely used in the management of diseases all over the world [1]. Several species of medicinal plants used in traditional treatment and management of diabetes worldwide have been evaluated [2, 3].
Bougainvillea spectabilis is a familiar ornamental plant belongs to the family Nyctaginaceae. The *Bougainvillea* leaves are reported to have medicinal properties *viz.* antiviral, antibacterial etc [4, 5]. It is also reported that ethanolic extract of *Bougainvillea spectabilis* leaves have beneficial effect on serum cholesterol concentration reduction [6]. This traditional plant has also antidiabetic potential. The alcoholic extract of the leaf has been reported to possess hypoglycemic effect and has been used for the management of diabetes mellitus. The hypoglycemic principle of the leaf extract has been isolated and named as pinitol [7].
Catharanthus Roseus is known with various names (Madagascar periwinkle; *Vinca rosea; Lochnera rosea*) in India and all over world. *Catharanthus roseus* contains more than 400 known alkaloids, some of which are approved as antineoplastic agents to treat leukemia, Hodgkin's disease, malignant lymphomas, neuroblastoma, rhabdomyosarcoma, Wilmer's tumor and other cancers. The flower extract of *Catharanthus roseus* is reported to have wound healing activity in Sprague Dawley rats [8].

2. Materials and Methods
2.1Plant Material
Bougainvillea spectabilis and *Catharanthus roseus* leaves were collected from the Bundelkhand University Campus Jhansi (U.P) and adjacent area. The leaves were removed from the stalk and air dried at room temperature (25 - 30 ^{0}C) after which it was ground and sieved to fine powder and made into extract with methanol used for the experiment.

2.2 Animal used
Albino rats of Wister strain weighing about 300±10 gms.

The study was carried out at Department of Zoology, Institute of Basic science, Bundelkhand University campus, Jhansi. The study was conducted in sexually mature albino rats (300±10gm/kg b.wt.), purchased from DRDE (Defence Research Development Establishment) Gwalior. Prior to study, the ethical clearance was obtained from the animal ethical committee, (CPCSEA, MOEF, and Government of India). Proposal No. BU/Pharm/IAEC/12/035.

2.3 Preparation of dose

Doses were prepared with Gum acacia in saline (0.9%). The dose was prepared according to 300mg/kg b.wt. Concentration and then it is given orally to rats for different duration and their effect was studied after 7, 14 and 21 days of daily administration.

2.4 Route of administration

The dosages were given to the animals via oral route by gastric feeding needles. The entry normally obtained without anaesthesia. Feeding needle with a ball tip was used to prevent introduction of the needle into the trachea and prevent trauma to the oral cavity.

2.5 Experimental design

Present study has been planned to study the effect of the methanolic extract of *Bougainvillea spectabilis* and *Catharanthus roseus*. The diabetic induced animals were divided into four groups. One group served as experimental, which received dose of plant extract at a level of 300mg/kg b.wt., the second group received the dose of Standard drug (Glibenclamide) at 5mg/kg b.wt, third group served as diabetic experimental (Alloxan) and simultaneously fourth group served as normal control which received vehicle only. The animals were kept in same environment conditions after daily 7, 14, and 21 days of treatment. The biochemical parameters viz. protein, glycogen were studied by standardized techniques or methods.

Alloxan was injected through the penial vein at a dose of 5 mg alloxan/ kg b.wt after a 24-hour fast, and confirmation of an elevated blood sugar was done 3 days later.

2.6 Acid and alkaline phosphatase

Method outlined by Fiske and Subba Row (1925) to determine the inorganic phosphorus and then the activity of enzyme was assessed using the method of Hawk *et al*, (1954).

Reagents required

a) Acid Phosphate Buffer: 0.5 g of Sodium β-glycerophosphate and 0.424g of Sodium diethyl barbiturate were dissolved in a small amount of water, 5ml of 1N acetic acid was added and the volume was made up to 100ml by adding distilled water. The pH was adjusted to 5.00.
b) Alkaline Phosphate buffer: 0.5g of Sodium β-glycerophosphate and 0.424g of Sodium diethyl barbiturate were dissolved in a small amount of water and the final volume was made up to 100ml by adding distilled water. Finally the pH was adjusted to 9.20.
c) Triton X: 1% solution (v/v) in distilled water.
d) Trichloroacetic acid (TCA): 30% (v/v).
e) 2.5% ammonium molybdate solution: 5.0g of ammonium molybdate was dissolved in small quantity of water and to it 60 ml of 10N H_2SO_4 was added and final volume of the solution was made to 200ml by adding distilled water.
f) Amino Napthol Sulphonic Acid (ANSA) Solution: The solution was prepared by mixing 195ml of Sodium metabisulphite (15%), 0.5g of 1, 2, 4 Amino Napthol Sulphonic Acid and 5ml of Sodium Sulphite solution (20%). All the three were mixed thoroughly by shaking in 200ml glass stoppered bottle, filtered and kept in a dark brown bottle to avoid any light reaction.

Estimation procedure

0.5 ml of tissue homogenate was taken in test tubes previously containing 0.1ml of Triton-X. To this 4ml of acid buffer (for acid phosphate) and 4ml of alkaline buffer (for alkaline phosphate) was taken. Simultaneously a control tube for each set was also taken which contained 0.5ml of 30% TCA. All these tubes were incubated for 60 min at 37°C. After that 0.5ml of 30% TCA was added to the sample tubes to stop the reaction and all tubes were centrifuged at 2000 rpm for 15 minutes.

From the centrifuged tubes 2ml of supernatant was taken and to it 6.6ml of distilled water and 1ml of Ammonium molybdate (2.5%) solution and ANSA (0.4ml) were added. Similarly blank and standard solutions were prepared from distilled water (2ml) and mono potassium phosphate (2ml of 5µg/ml) respectively in place of supernatant.

After 10 minutes the optical density was read at 620 nm on spectrophotometer. Inorganic phosphorus and enzyme activity liberated during the reaction was calculated by the following formula and was expressed as mg p/100g/hr.

The amount of Inorganic phosphorus was calculated as follows

$$= \frac{\text{O.D. of Unknown}}{\text{O.D of Known}} \times 0.01 \times 1000 \times \frac{5}{12.5} \times 100$$

Where O.D is optical density.

2.7 Statistical analysis

The results were expressed as Mean±S.E. Significance of differences as compared to the control was the significance determined using student's t-test.

3. Results and Discussion

Acid phosphatase being a lysosomal enzyme is present in almost every cell and performs different functions. These are involved in catalysis and phagocytosis whenever any foreign body or chemical tends to damage the tissue. The activity of alkaline phosphatase is associated with the transport of nutrients, in the process of endocytosis and exocytosis and being a membrane bound enzyme it also alters the cell permeability.

When diabetes was induced by alloxan, the acid phosphatase level in liver, kidney, uterus and pancrease increased, but during daily administration of the dose the acid phosphatses in liver, kidney, uterus and pancrease decreased significantly.

Whereas as same trend was followed in alkaline phosphatase levels. Alloxan increased alkaline phosphatase levels whereas when plant extract was administrated daily the alkaline phosphatase levels in liver, kidney, uterus and pancrease decreased significantly.

Due to the administration of various plant extracts (crude or their chemical constituents); it leads to change in enzyme system.

Decreased enzymatic activity of glucokinase, hexokinase and phosphofructokinase has been reported in diabetic animals

resulting in depletion of liver and muscle glycogen [9, 10] have reported a decrease in alkaline phosphatase when gangetin (isolated from *Desmodiumgangetin*) is administered to female rats. Similarly, *Embelia ribes burm* and *Artabotrys odoratissimus* alter the activity of acid phosphatase in reproductive organs of adult female rats [11, 12] studied that systemic application of defined extract from *Withania somnifera* led to improvement in cholinergic activity in cortical and basal forebrain [13]. Observed an increase in the acid phosphatase after the administration of chloroquinine [14]. reported that Aristolic acid isolated from *Aristolochia indica* caused elevation in activity of alkaline phosphatase

Therefore on the basis of present study it was found that acid phosphatases as well as alkaline phosphatases decreased significantly due to the treatment of combination of *Bougainvillea spectabilis* and *Cathranthus roseus* at a dose level of 300 mg/kg.b.wt. Further studies are still needed to understand the mechanism of action. Also some other biochemical as well as histopathological studies will also help to study the exact procedure and action of the plant extract. By phytochemical isolation, it could also be studied to identify any active principle found in the crude extract. The results discussed above are presented in the tables below:

Table 1: Showing effect of daily administration of Methanolic extract of combination of *Bougainvillea spectabilis* and *Catharanthus roseus* on female diabetic albino rats indicating the content of acid phosphatases (mgP/100mg). Values are given as Mean ± SE of 6 animals in each group.

S. No.	Tissues	Control	Diabetic Control (D.C)	7 Days		14 Days		21 Days	
				D.C+P.E	D.C+STD	D.C+P.E	D.C+STD	D.C+P.E	D.C+STD
1.	Liver	295±2.5	330.5±0.68*	321.8±0.81	316.9±0.8	313.6±0.76	307.8±0.76*	304.7±0.76*	298.7±0.92*
2.	Kidney	301.4±1.5	340.5±1.09*	332.5±1.05	327.9±0.83	323.4±0.77	318.7±0.61*	312.5±0.65*	306.8±1.02*
3.	Uterus	134.7±2.7	170.5±0.62*	161.5±0.67	157.9±1.75	152.9±0.81	148.9±0.76*	147.5±0.65*	137.6±0.62*
4.	Pancrease	315.2±3.5	352.4±0.75	343.7±0.57	339.6±0.33	332.6±0.81	328.7±0.81*	322.4±0.85*	317.9±1.4*

*Values are statistically significant at $p<0.05$

Where, D.C= Diabetic Control, P.E= Plant Extract, STD= Standard Drug (Glibenclamide), and the same scheme is followed in Table II.

Table 2: Showing effect of daily administration of Methanolic extract of combination of *Bougainvillea spectabilis* and *Catharanthus roseus* on female diabetic albino rats indicating the content of alkaline phosphatases (mgP/100mg). Values are given as Mean ± SE of 6 animals in each group.

S. No.	Tissues	Control	Diabetic Control (D.C)	7 Days		14 Days		21 Days	
				D.C+P.E	D.C+STD	D.C+P.E	D.C+STD	D.C+P.E	D.C+STD
1.	Liver	295±2.5	330.5±0.68*	321.8±0.81	316.9±0.8	313.6±0.76	307.8±0.76*	304.7±0.76*	298.7±0.92*
2.	Kidney	301.4±1.5	340.5±1.09*	332.5±1.05	327.9±0.83	323.4±0.77	318.7±0.61*	312.5±0.65*	306.8±1.02*
3.	Uterus	134.7±2.7	170.5±0.62*	161.5±0.67	157.9±1.75	152.9±0.81	148.9±0.76*	147.5±0.65*	137.6±0.62*
4.	Pancrease	315.2±3.5	352.4±0.75	343.7±0.57	339.6±0.33	332.6±0.81	328.7±0.81*	322.4±0.85*	317.9±1.4*

*Values are statistically significant at p<0.05

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