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INVESTIGATION OF *CLERODENDRUM SERRATUM* FOR ITS ANTIOXIDANT AND ANTI STEROIDOGENIC PROPERTIES IN MALE RATS

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ABSTRACT

The objective of the present study is to explore anti-steroidogenic and antioxidant properties of Methanolic extract of *Clerodendrum serratum* aerial plant. The different dose of methanolic extracts were given by gavage to rats in the In Vivo test at a dose of 100, 300, 500mg/kg of body weight to rats in group II, II & III respectively, along with control dose. At the end of study various parameter such as lipid profile, and anti-oxidant level in the testicular tissue were analyzed. Finding of this study explored a significantly increase ($p<0.05$) the total cholesterol, VLDL, LDL, and triglycerides levels in methanolic extract of *Clerodendrum serratum* treated rats when compared to control. But HDL cholesterol and anti-oxidant levels were significantly decreased in methanolic extract of *Clerodendrum serratum* treated rats in dose dependent manner and also we observed the role of *Clerodendrum serratum* on antioxidant defense mechanism in rat testis. The present study, it concluded that methanolic extract of *Clerodendrum serratum* capable to suppress the fertility in male rats without altering general metabolism.

Key Words:- Anti steroidogenic, Anti-oxidant, *Clerodendrum serratum*.

INTRODUCTION

The rapid increase of population has got an adverse effect on the international economy and as the increase is only limited to the developing countries, the problem becomes an acute on the fruits of improvement in the different sectors, which are being eroded by the growing population. Moreover, increasing number of births has got a deleterious effect on the health of mother and child and hinders social and economic progress. The regulation of human fertility has global consequences in terms of resources depletion, population and poverty (Ashu Chaudhary *et al*., 2008). Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources (Cragg GM & Newman DJ, 2001).

Male pills containing a combination of testosterone, estrogens and progesterone have also been tried to prevent spermatogenesis. Biomedical research supported by WHO has investigated the potential of developing new male contraceptive methods that would function at any step in male reproductive process, from sperm production in the testes through to sperm-egg interactions in female genital tract (Rajalakshmi M, 1994).

Clerodendrum serratum (Verbenaceae), shrub distributed though out country, especially common in Assam and Bengal. It is commonly known as Blue-

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flowered glory, Beetle Killer (English), cherutekku (Tamil). Bharngi is bitter in taste and pungent, the pungent taste is used for the post digestive effect. It improves tridosas, vata, pita and kapha. It is used for treatment of conditions as asthma, cough, fever, etc (Khare CP, 2007).

The major groups of chemical constituents present in the *Clerodendrum serratum* are carbohydrates, serratagenic acid, acteoside, indolizino and verbascoside, leucoanthocyanidins, flavanones, flavanonols, betulin, oleanolic acid, clerodermic acid β-sitosterol, γ-sitosterol and compesterol (Mukesh Kr. Singh *et al*., 2012).

MATERIALS AND METHODS

Plant material

The aerial parts of the *Clerodendrum serratum* were collected from tirumala hills belong to Tirupati, Andhra Pradesh, India. Taxonomical identification was made from botanical survey of medicinal plant unit, Sri Venkateswara University Tirupati, Andhra Pradesh. The aerial part of the plant was dried at room temperature, powdered by the mechanical grinder, sieved stored for future used. Exactly 2.5 kg of the fresh air-dried, powered crude drug of *Clerodendrum serratum* was extracted with methanol by adopting soxhlet extraction procedure at 60°C for 7 days in a conical flask with occasional shaking and stirring.

Animals

Adult Wistar strain male albino rats were used in the current study. Animals were housed in group five per cage made of polypropylene (8" ×12" ×8") with metal grill tops and maintained under 12 h light/12 h dark cycle with controlled conditions (21 ± 2^{0}C, 51 ± 7% humidity) and were fed by standard food (Sai Durga feeds, Bengaluru, India)and allowed water *ad libitum*. Food pellets was with-held overnight prior to dosing. All rats were handled and maintained strictly as per guidelines of Guide for the care and Use of Laboratory animals.

Design of experiment

Twenty healthy male albino rat were selected and divided in to four group containing 5 rats each and treated as follows:

Group-I: Received distilled water as normal vehicle (DW)
Group-II: Received as MECS (100mg/kg body weight)
Group-III: Received as MECS (300mg/kg body weight)
Group-IV: Received as MECS (500mg/kg body weight)

Distill water (DW) MECS extracts, 100,300,500mg/kg body weight, was administered intra gastric (i.g) route on consecutive 30 days. At the end of the treatment, animals were sacrificed by cervical dislocation and testis were collected and stored at -20°C for lipid profile and antioxidant estimation.

Determination of lipid profiles

Estimation of Cholesterol (Chod-Pap Methodology)

Principle

The cholesterol esters are hydrolyzed by enzyme cholesterol esterase to give free cholesterol and fatty acid molecules. This free cholesterol gets oxidized in presence of cholesterol oxidase to liberate cholesterol 4 en – 3 one and H_2O_2. Liberated H_2O_2 by this reaction combines with phenol and 4- amino antipyrine in presence of proxidase to from red colored quinonimine complex, the intensity of which is measured at 505nm. (490-530nm). It is directly proportional to cholesterol conc. Present in sample.

$$\text{Cholesterol Ester} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholesterol 4 – en – 3 – one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4 amino antipyrine} \xrightarrow{\text{Peroxidase}} \text{quinonmine} + 4H_2O$$

Procedure:

All the reagents were brought to the room temperature (37°C). The assay was performed with the following working assay table using Un-hemolysed Serum (Friedewald WT *et al*., 1972).

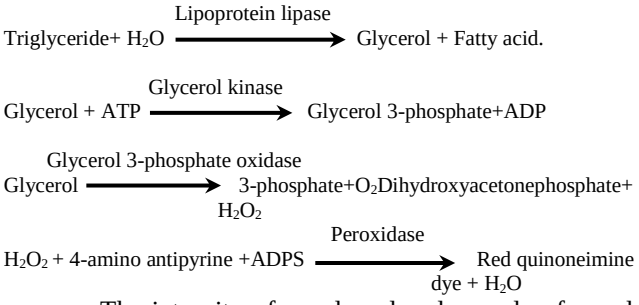
Addition Sequence	Blank (ml)	Standard (ml)	Test (ml)
Enzyme Chromogen	1	1	1
Cholesterol Standard	-	10 µl	-
Serum Sample	-	-	10 µl

The sample and reagent mixture were mixed well and incubated for 5 minutes at 37^{0}C, measured the absorbance of standard and the test sample against the reagent blank at 480nm (490nm).

Estimation of Triglycerides by Enzymatic Method

Principle:

Triglycerides in the samples originates, by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometry (*Bucclo* G & David H, 1973)



The intensity of purple colored complex formed during the reaction is directly proportional to the triglyceride concentration in the sample and is measured at 546nm.

- Reagents
- Enzyme Chromogen
- Buffer
- Standard (Triglyceride 200 mg/dl)

Procedure

Addition Sequence	Blank (ml)	Standard (ml)	Test (ml)
Enzyme Chromogen	1	1	1
Cholesterol Standard	-	10 µl	-
Serum Sample	-	-	10 µl

The Reaction mixture was incubated for 5 minutes at 37°C and mixed well. The color formed was read at 546 nm (520-570nm). The final color is stable for 30 minutes.

Calculation

$$\text{HDL in mg \%} = \frac{\text{Absorbance of HDL Test (TH)} \times 100 \times 1.1}{\text{Absorbance of Standard}}$$

From the above estimated values of TC and HDL the values of LDL and VLDL can be obtained using the following arithmetic calculations

LDL in mg % = Concentration of Triglycerides/5

VLDL Cholesterol in mg % = Concentration of Total cholesterol - Concentration of HDL cholesterol + Concentration of LDL cholesterol

Determination of Anti-oxidant enzymes in Testicular tissues

The testis was removed, cleared of excess fat and minced with anatomical scissors. The testicular tissue homogenate (10% w/v) was prepared in 0.1 M phosphate buffer (pH 7.4), centrifuged for 15 minutes at 500 x *g*. The supernatant obtained thereafter was used for various biochemical assays.

Determination of Catalase

The Catalase activity was assayed by, the incubation mixture contained in a final volume of 2.0ml, 0.1ml of diluted homogenate, 1.5ml of phosphate buffer and 0.4ml of distilled water to which 0.5ml of H_2O_2 solution was added to initiate the reaction, while the H_2O_2 solution was left out in control tubes. After incubating for 1 min at 37 °C the reaction was stopped by addition of 2 ml of potassium dichromateacetic acid reagent. The samples were kept in boiling water bath for 10 minutes, finally cooled and the absorbance measured at 570 nm against control (Sinha AK, 1972).

$$\text{Activity} = \frac{\Delta \text{ OD x Std conc. (µ mol)}}{\text{Enzyme (ml) x Std. OD x Protein (mg/ml)}}$$

Unit=µmoles/min/mg/protein.

Determination of Superoxide Dismutase (SOD)

In this test, the degree of inhibition of pyrogallol autoxidation by supernatant of the lenticular homogenate was measured. The change in absorbance was read at 470 nm against blank every minute for 3 min on a spectrophotometer. The enzyme activity was expressed as units per milligram protein (Marklund S & Marklund G, 1974).

Determination of Glutathione Peroxidase

The principle of this method is that the rate of glutathione oxidation by H_20, as catalyzed by the GPx present in the supernatant, is determined; the color that develops is read against a reagent blank at 412 nm on a spectrophotometer. In the test, the enzyme activity was expressed as units per milligram protein (one unit was the amount of enzyme that converted 1 µmol of reduced glutathione to the oxidized form of glutathione in the presence of H_20_2/min) (Rotruck JT *et al*., 1973).

Determination of Reduced Glutathione

Each testis was homogenized in 1 ml of 0.1 M phosphate buffer and was centrifuged at 5,000 rpm for 15 min at 4°C. To the supernatant of the lenticular homogenate, 0.5 ml of 10% trichloroacetic acid was added and recentrifuged. The protein-free supernatant thus obtained was reacted with 4 ml of 0.3 M Na_2HPO (pH 8.0) and 0.5 ml of 0.04% (w/v) 5, 5dithiobis-2-nitrobenzoicacid. The intensity of the resulting yellow

color was read spectrophotometrically at 412 nm. A parallel standard was also maintained. The results were expressed in micromoles per gram of tissue (Moron MS *et al*., 1979).

Statistical analysis

The data expressed as mean ±SEM and analyzed by One-way analysis of variance. P<0.05 was considered as the criterion for statistical significance.

RESULTS

Effects of *Clerodendrum serratum* extracts on lipid profile

Exposure to *Clerodendrum serratum* resulted in an increase of absolute and relative Lipid profile compared to the control in a dose-dependent manner. Total Cholesterol, VLDL, LDL cholesterol, and triglyceride levels were significantly higher in *Clerodendrum serratum* 500mg/kg bw group. Whereas the total: HDL cholesterol ratio was significantly decreased in *Clerodendrum serratum* 300mg/kg and 500mg/Kg/bw. Treated groups (Table 1). The concentration of lipid profile on 100 and 300mg of *Clerodendrum serratum* treated groups was compared with 500mg treated groups are greater. Despite this, significant increase in the triglyceride and total cholesterol at 500mg/kg *Clerodendrum serratum* than that of other two groups.

Effects of *Clerodendrum Serratum* extracts on anti-oxidant enzyme in testicular tissues

Catalase

The mean activity of Catalase in testicular tissues of methanolic extract in group IV was significantly lower than that in group II and group III. Similarly, a lower mean activity of Catalase was noted in group IV (p<0.05) then in group I control rats (Fig.1).

Superoxide Dismutase (SOD)

The mean activity of SOD in testicular tissues of methanolic extract in group IV was significantly lower than that in group II and group III. Similarly, a lower mean activity of SOD was noted in group IV (p<0.05) then in group I control rats (Fig.2).

Glutathione Reductase (GSH)

The mean activity of GSH in testicular tissues of methanolic extract in group IV was Significantly lower than that in group II and group III.Similarly, a lower mean activity of GSH was noted in group IV(p<0.05) then in group I control rats (Fig.3).

Glutathione Peroxidase (GP_X)

The mean activity of GPX in testicular tissues of methanolic extract in group IV was significantly lower than that in group II and group III. Similarly, a lower mean activity of GPX was noted in group IV (p<0.05) then in group I control rats (Fig.4).

Fig.1. Determination of Catalase

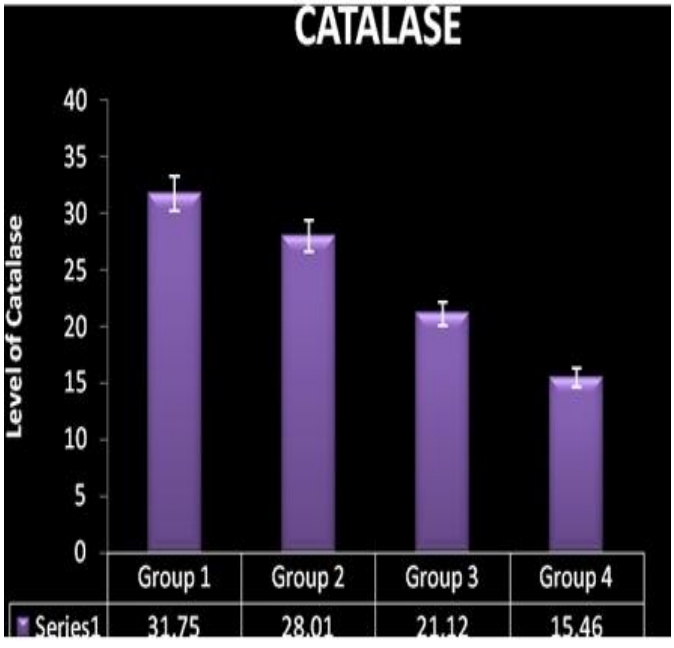


Fig.2. Determination of Superoxide dismutase (SOD)

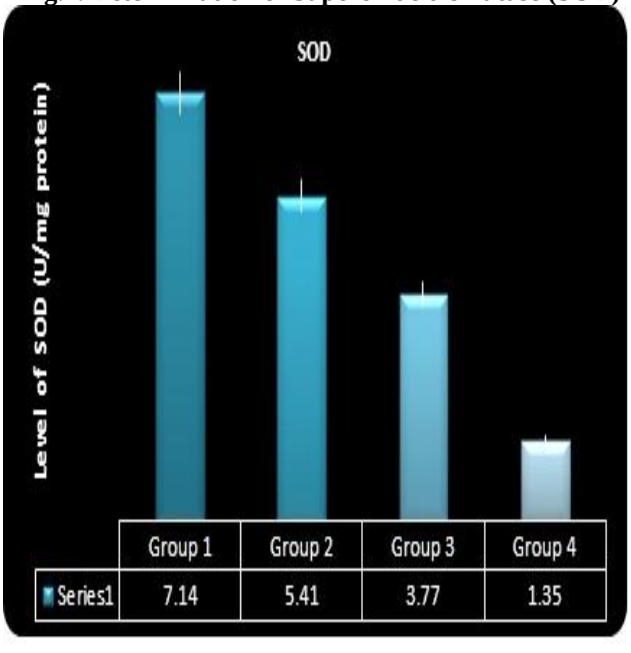


Fig. 3. Determination of Glutathione reductase (GSH)

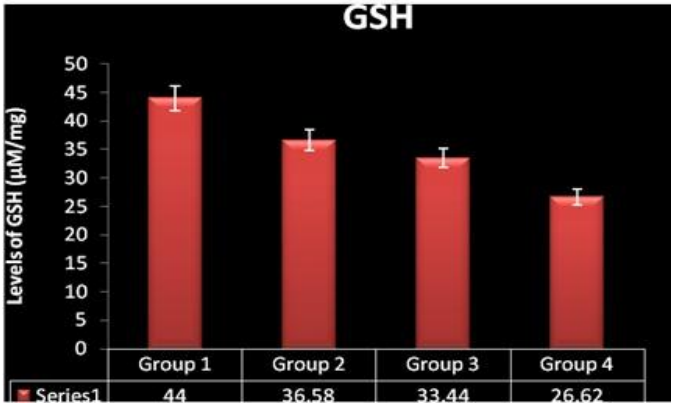


Fig. 4. Determination of Glutathione peroxidase (GPx)

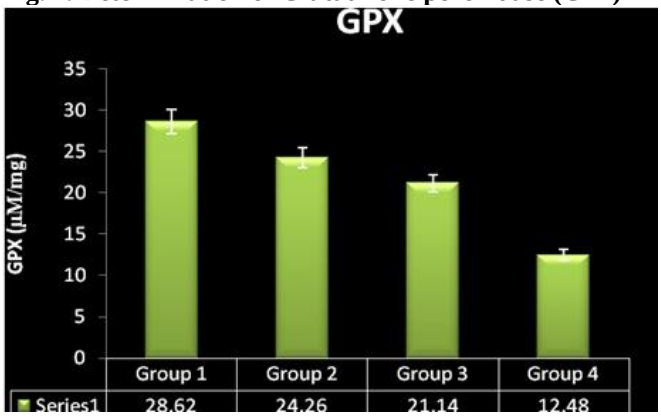


Table 1. Effect of *Clerodendrum serratum* on Lipid Profile

Groups	Triglyceride (mg/dL)	Total Cholesterol (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)
Group-I (Control)	196.29±1.86	26.80±7.99	38.12±1.13	175.99±3.78	82.66±2.23
Group-II MECS (100mg/kg)	245.97±1.28	150.61±1.33*	53.69±0.91*	170.39±3.07*	107.41±3.84*
Group-III MECS (300mg/kg)	526.42±1.92	209.70±3.32*	110.31±1.69*	154.16±3.59*	124.37±3.88*
Group-IV MECS (500mg/kg)	547.12±2.55	229.43±4.65*	118.59±5.73*	135.01±3.48*	174.63±4.57*

MECS-Methanolic extract of *Clerodendrum serratum*. Values are expressed as mean ± SEM (n = 5). The mean differences between the values bearing *P< 0.05 are statistically significant compare with control group.

DISCUSSION

Evaluation of herbs for anti-fertility effects has been in progress worldwide for several decades to identify effective and safe substances for control of population explosion. The anti-fertility effect of methanolic extract of *Clerodendrum serratum* was confirmed by following measures. Cholesterol is involved in steroidogenesis in testes. It is the most important precursor in synthesis of steroid hormones and its level is related to fertility of individuals (Eik-Nes KB, Hall PF, 1962). Increased level of cholesterol may be due to decreased androgen production, which results in accumulation of cholesterol in testes and impaired spermatogenesis (Bedwal RS *et al*., 1994).

Reactive Oxygen Speices (ROS) such as superoxide anions (O_2-) hydrogen peroxide (H_2O_2), hydroxyl radical (OH-) and nitric oxide (NO) are directly or indirectly involved in DNA damage leading to mutations. Some antioxidant defences are present in the plants and their byproducts mainly edible vegetables and spices, have a key role in chemopreventers in human diet. For example, *Pleurotus florida*, possessed significant antioxidant enzymes activity (Nayana J & Janardhanan KK, 2000). *Indigofera tinctoria* had strong antioxidant effect (Sreepriya M *et al*., 2001) and *Coriandrum sativum* increased the antioxidant enzyme activity (Chithra V *et al*., 1999). *Clerodendrum serratum* is widely used for various ailments. But the available literature on *Clerodendrum serratum* does not reveal the effect of its antioxidant enzyme activities in testis. Since *Clerodendrum serratum* is one of the commonly used plant, we studied the role of *Clerodendrum serratum* on lipid peroxidation and antioxidant defense mechanism in rat testis.

CONCLUSION

In conclusion, from the overall results, it could be inferred that aerial parts of *Clerodendrum serratum* showed potent male antifertility effect. Further long term studies are in progress for the evaluation of complete and reversible fertility with this extract and also other effects of this important plant.

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