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Anti-fertility activity and lipid peroxidatation effects of *Clerodendrum serratum* in male rats

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ABSTRACT

Aim: The objective of the present study is to explore anti-fertility and lipid peroxidation properties of methanolic extract of *Clerodendrum Serratum* aerial plant. **Methods:**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 biochemical and testicular lipid peroxidation levelwere analyzed. **Result:**Finding of this study explored a significantly decrease ($p<0.05$) the Alanine amino transferase, Aspartate aminotransferase and Alkaline phosphatase levels in methanolic extract of *Clerodendrum Serratum* treated rats when compared to control. But Malondialdehyde levels were significantly increased in methanolic extract of *Clerodendrum Serratum* treated rats testicular tissues in dose dependent manner when compared with control. **Conclusion:**The present study, it concluded that methanolic extract of *Clerodendrum Serratum* aerial parts have antifertility effects in male rats.

Key words: Anti fertility, *Clerodendrum Serratum*, Malondialdehyde.

1. INTRODUCTION

The future of life on the planet is under the pressure of the population explosion the world's population estimate, for mid-year 2011, is estimated at 6,928,198,253[1] and continues to grow by 83 million people per year. During the last half-century, the world's population more than doubled[2].Fertility is an issue of global and national public health concern. Fertility regulation comprising contraction and management of infertility forms an important component of reproductive health. With recent progress towards a better understanding of male reproductive physiology there is a need to develop new contraceptive modalities for male. Several potential approaches for induction of infertility have been investigated over a long period including hormonal and chemical approaches. The chemical compounds affecting testicular function include different groups like steroidal and non-steroidal among them are Diazole, Depot medroxy progesterone acetate (DMPA), Cyproterone acetate (CPA), Levenogestral, Melatonin, Serotonin. But application of above compounds has been seriously questioned owing to various hazards as they proved toxic on both the short as well as long term use in the reproductive system[3]. The traditional use of medicinal plants to treat different sorts of diseases, including fertility related problems is widespread throughout the world as many plant substances are known for their interferences with the male reproductive system[4]. Some of the plants had spermicidal effects; others caused reduction in sperm counts and alter the mobility of the sperms. Some of the plants caused testicular change and altered hormone levels[5].

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Clerodendrum serratum (Verbenaceae) is a tropical medicinal plants distributed in the forest of western ghates of india. In Indian system of medicine, that plant is well known as bharangi (Sanskrit) and commonly known as blue glory (English) and Gantubharangi (Kannada)[6]. As per the traditional claims roots are the potential source of drugs for aliments such as asthma, bodyache, bronchitis, fever, cholera dropsy, eye disease, inflammation, malaria, snake bite, rheumatism, tuberculosis wounds and ulcer[7].

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 ß-sitosterol, γ-sitosterol and compesterol[8].

2. MATERIALS AND METHODS

2.1 Plant material

The aerial parts of the *Clerodendrum serratum* were collected from tirumala hills belong to Thirupathi, Andrapradhesh, India. Taxnomicalindetification was made from botanical survey of medici-

nal plant unit, Sri Venkateswara University Thirupathi, Andrapradhesh. 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.

2.2 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 (SaiDurga 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.

2.3. Design of experiment

Twenty healthy male albino rats 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) Methanolic extracts of *Clerodendrum serratum* (MECS), 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 serum was separated form blood samples for the enzyme estimation and testis were collected and stored at -20°C for determination of lipid peroxidation levels.

2.4 Determination of activities of enzymes (ALT,AST and ALP) in serum[9]

2.4.1. Alanine Aminotransferase (ALT/GPT)

Principle

Alanine aminotransferase (ALT or GPT) catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm; by means of the lactate dehydrogenase (LDH) coupled reaction.

Reagents:

Reagent A: 8 x 60 mL Tris 150 mmol/L, L-alanine 750 mmol/L, lactate dehydrogenase > 1350 U/L, pH 7.3.

Reagent B: 8 x 15 mL NADH 1.3 mmol/L, 2-oxoglutarate 75 mmol/L, sodium hydroxide 148 mmol/L, sodium azide 9.5 g/L.

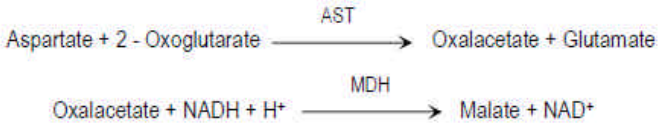
Method:

25µl of tissue samples were added to 240µl of reagent A, mixed well and incubated for 3 – 5 minutes at 37°C. Then 60 µl of reagent B was added to the above content and incubated for 1 minute at 37°C. Initial absorbance was read at 340nm and intervals thereafter for 3 minutes.

2.4.2. Aspartate Aminotransferase (AST/GOT)

Principle

Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the malate dehydrogenase (MDH) coupled reaction.



Reagents

Reagent A: 8 x 60 mL.Tris 121 mmol/L, L-aspartate 362 mmol/L, malate dehydrogenase >460 U/L, lactate dehydrogenase > 660 U/L, pH 7.8.
Reagent B: 8 x 15 mL. NADH 1.3 mmol/L, 2-oxoglutarate 75 mmol/L, sodium hydroxide 148 mmol/L, sodium azide 9.5 g/L.

Method:

25µl of tissue samples were added to 240µl of reagent A, mixed well and incubated for 3 – 5 minutes at 37°C. Then 60 µl of reagent B was added to the above content and incubated for 1 minute at 37°C. Initial absorbance was read at 340nm and intervals thereafter for 3 minutes.

2.4.3.Alkaline Phosphatase (ALP)

Principle

Alkaline phosphatase (ALP) catalyzes in alkaline medium the transfer of the phosphate group from 4-nitrophenylphosphate to 2-amino-2-methyl-1-propanol (AMP), liberating 4-nitrophenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405nm

Reagents

Reagent A: 4 x 60 mL. 2-Amino-2-methyl-1-propanol 0.4 mol/L, zinc sulfate 1.2 mmol/L, N-hydroxyethylethylenediaminetriacetic acid 2.5 mmol/L, magnesium acetate 2.5 mmol/L, pH 10.4.Reagent B: For 4 x 15 mL.4-Nitrophenylphosphate 60 mmol/L.

Method:

25µl of tissue samples were added to 240µl of reagent A, mixed well and incubated for 3 – 5 minutes at 37°C. Then 60 µl of reagent B was added to the above content and incubated for 1 minute at 37°C. Initial absorbance was read at 340nm and intervals thereafter for 3 minutes.

2.5. 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.

2.5.1. Determination of lipid peroxidation:[10]

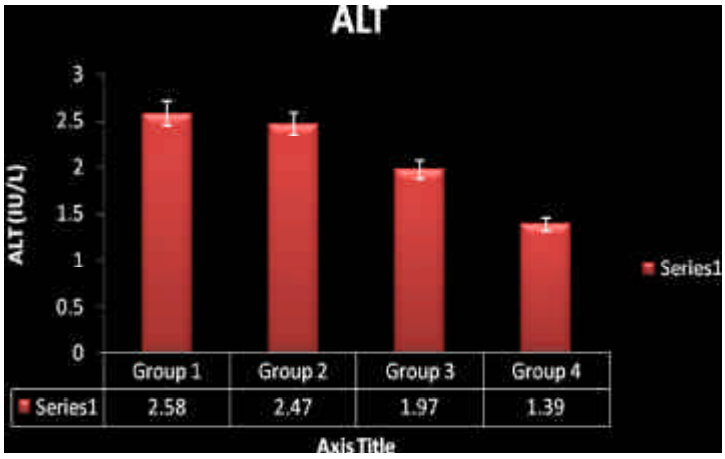
Malondialdehyde (MDA) (nmol/mg protein), a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid-reacting substances (TBARS) by this method. Briefly, to 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.81% thiobarbituric acid aqueous solution were added in succession. To this reaction mixture, 0.2 ml of the tissue sample was added. The mixture was then heated in boiling water for 60 min. After cooling to room temperature, 5 ml of butanol: pyridine (15: 1 v/v) solutions were added. The mixture was then centrifuged at 2,000 g for 15 min. The upper organic layer was separated, and the intensity of the resulting pink color was read at 532 nm. Tetramethoxypropane was used as an external standard. The level of lipid peroxides was expressed as nanomole of MDA formed/mg protein.

3. RESULTS

3.1. Bio Chemical analysis

3.1.1. Effect of *Clerodendrum Serratum* extract on Alanine aminotransferase (ALT)

The mean activity of Alanine Amino Transferase(ALT) in serum of methanolic extract in group IV was significantly lower than that in group II and group III.Similarly, a lower mean activity of ALT was noted in group IV($p<0.05$) then in group I control rats (Fig.1).

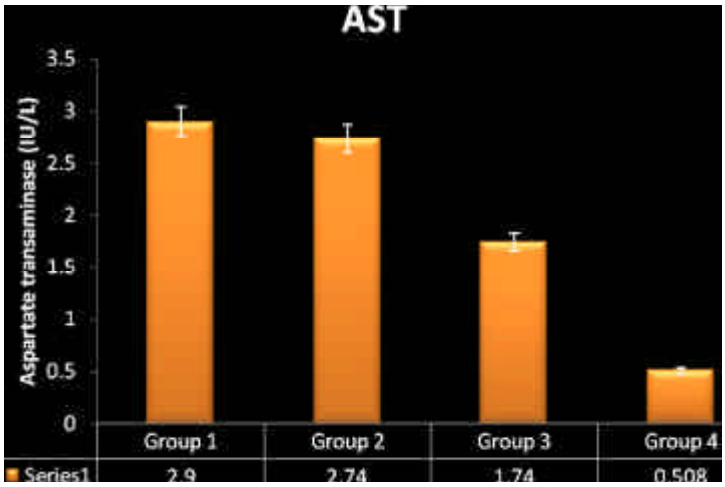


Values in figures are expressed as mean ±SEM (n = 5).The Alanine aminotransferase (ALT) enzymes and Shows the mean differences between the values bearing P< 0.05 are statistically significant compare with control group.

Fig.1. Determination of Alanine aminotransferase (ALT)

3.1.2. Effect of *Clerodendrum Serratum* extract on Aspartate aminotransferase (AST)

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

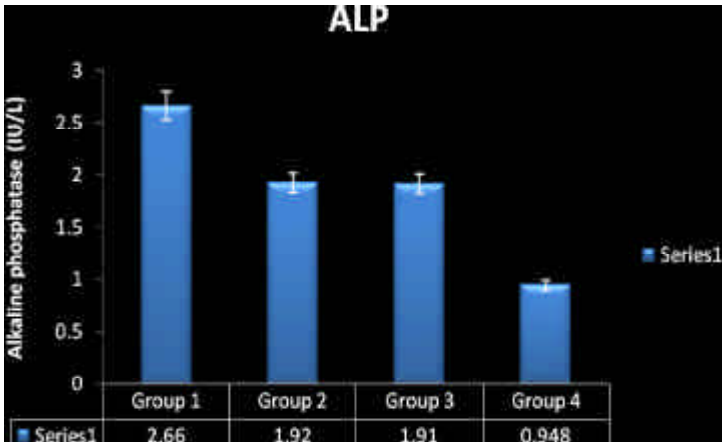


Values in figures are expressed as mean ±SEM (n = 5).The Aspartate aminotransferase(AST) enzymes and Shows the mean differences between the values bearing P< 0.05 are statistically significant compare with control group.

Fig. 2. Determination of Aspartate Aminotransferase (AST)

3.1.3. Effect of *Clerodendrum Serratum* extract on alkaline phosphatase (ALP)

The mean activity of alkaline phosphatase (ALP) in serum of methanolic extract in group IV was significantly lower than that in group II and group III. Similarly, a lower mean activity of alkaline phosphatase (ALP) was noted in group IV ($p<0.05$) then in group I control rats (Fig.3).



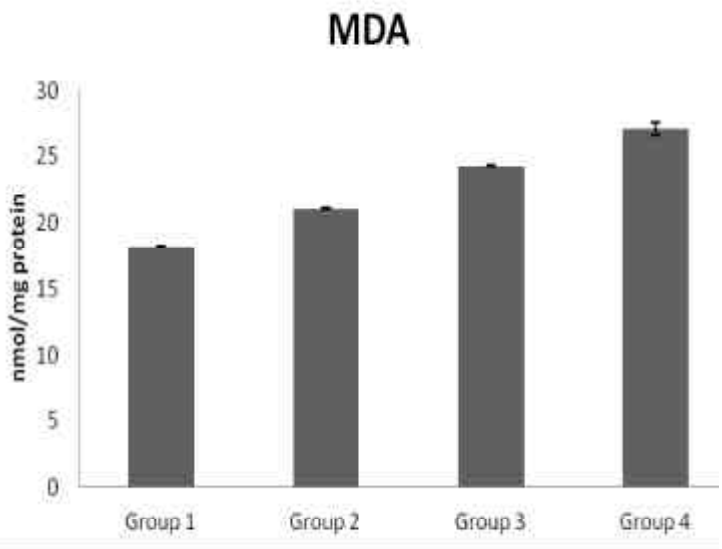
Values in figures are expressed as mean ±SEM (n = 5).The Alkaline phosphates (ALP) enzymes and Shows the mean differences between the values bearing P< 0.05 are statistically significant compare with control group.

Fig.3. Determination of Alkaline phosphates (ALP)

3.2.Effects of *Clerodendrum Serratum*extracts on lipid peroxidation in testicular tissues

3.2.1. Effect of *Clerodendrum Serratum* extract on Malondialdehyde (MDA)

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



Values in figures are expressed as mean ±SEM (n = 5).The Malondialdehyde (MDA) Enzymes and Shows the mean differences between the values bearing P< 0.05 are statistically significant compare with control group.

Fig. 4. Determination of Malondialdehyde (MDA)

4. DISCUSSION

ALP plays a role in the transport of sugars and otherorganic molecules across biological membranes, and also contributes in the epididymal histoarchitecture, in the transport of molecules between the epithelium principal cells andcapillaries of the sub-epithelial connective tissue[11]. ALP is related to the maturation ofspermatozoa into the luminal compartment of the epididymis[12].Alkaline phosphates is membrane bound enzyme which is involved in the synthesis ofnuclear protein nucleic acids and phospholipids. It is also involved in the cleavage of phosphate esters and also in mobilizing carbohydrates and lipid metabolites. These areutilized either within the cell of reproductive organ or by spermatozoa in theseminal fluid. The activity of ALP is concerned with energy metabolic activities andprocesses in the body and the decrease in its activity may indicate impaired energy processingof the cells[13]. The long-term feeding of fresh tulsi leaves have shown to increase the body weight, while decrease the weights of testes, prostate, and adrenal gland in rats. The results suggested that infertility in male rats seems to be due to impairment of spermatogenesis as well as changes like decrease in pH, hypotonic environment, and chemical substances like mucoproteins, alkaline phosphatase and acid phosphatase in spermatogenic cells leading to formation of non-viable spermatozoa.[14].

Evaluation of liver function can be made by estimating the activities of serum ALT, AST and acid phosphatase which are enzymes originally present in cytoplasm. When there is any liver damage, these enzymes drip into the blood stream in assenting with the extent of liver damage.

ALP is generally dispersed in the testes and is essential in the sperm physiology. Decrease in the activity of ALP suppress spermatogenesis and exchange of materials between germinal , sertoli cells and extensive lytic activity. The findings of the present study showed that administration of *Clerodendrum Serratum* at the dose of 100, 300 and 500mg/kg bwdecrease the ALP activity.

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. Superoxide dismutases (SOD) scavenge extracellular and intracellular superoxide anion and prevent lipid peroxidation of the plasma membrane. In order to act against H_2O_2 it must be conjugated with catalase or glutathione peroxidase[15] . It is plausible that higher rate of ROS production may inhibit the action of these antioxidant enzymes or otherwise the reduced expression of these antioxidant enzymes may cause increased oxidative stress [16].The increased levels of MDA suggested that potential sources of ROS production were the morphologically or functionally abnormal spermatozoa and other cells, including the Sertoli cells or Leydig cells[17]. The methanolic extract of *Clerodendrum Serratum* increased MDA in dose dependent manner. Which also responsible for anti-fertility effects of *Clerodendrum serratum* in male rats.

5. CONCLUSION

In conclusion, from the overall results, it could be inferredthat aerial parts of *Clerodendrum Serratum*showed potent antifertility activities in a dose-dependent manner in male rats . 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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