

Ethonalic leaf extract of *Andrographis paniculata*-induced oxidative stress in the testis and epididymis of Male Wistar Rats

Satya narayana K¹, Kumaraswamy Naidu C¹, Trinath D¹, Sreenivasula Reddy P², Srinivasulu Reddy M², Suneetha Y^{3*}

1. Research Scholar, Department of Zoology, Sri Venkateswara University, Tirupati- 517502, India
2. Professor, Department of Zoology, Sri Venkateswara University, Tirupati- 517502, India
3. Assistant Professor, Department of Zoology, Sri Venkateswara University, Tirupati- 517502, India

*Corresponding author: Department of Zoology, Sri Venkateswara University, Tirupati 517502, India, e-mail: ysuneethareddy4@gmail.com

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ABSTRACT

Andrographis paniculata has been used as a traditional medicine and a predominant constituent in 26 Ayurvedic formulations over the last two decades. Its impact on the male fertility is a controversial from the past few years. In the present study we have investigated the antioxidant enzyme status and the indices of lipid peroxidation in the testis and epididymis of male wistar rats. Male wistar rats were exposed to 20, 200 and 1000mg/kg body weight of ethanolic extract of *Andrographis paniculata* (high dose) orally for 65 days daily. Control rats received normal saline only. Tissues were washed, homogenized and the levels of super oxide dismutase, catalase and lipid peroxidation was determined. Results showed an increase in the levels of lipid peroxidation and decrease in the levels of super oxide dismutase and catalase in the testis and epididymis.

Key words: *Andrographis paniculata*, lipid peroxidation, Super oxide dismutase, Catalase

1. INTRODUCTION

Herbal preparations/medicaments have been a treatment option in several parts of the world over the decades. Some of these plant preparations are known to inhibit the male fertility which can be developed into male contraceptives (Akbarsha and Murugaian, 2000). Over the last two decades, use of these herbal products for self-medication by the general public has grown enormously and there is a wide

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tendency to utilize these herbal products to supplement the diet for improving the quality of life and preventing the diseases of elderly people (Sharma and Joshi, 2011).

Andrographis paniculata is one such plant belonging to the family Acanthaceae known to be one an important herbal medicine from centuries in Asia to treat several diseases such as gastro-intestinal tract and upper respiratory infections, fever and herpes. It is known to be a predominant constituent in more than 26 Ayurvedic formulations (Sattayasai et al., 2010). Its leaves and roots are traditionally been used over centuries in the regions of Asia and Europe as a folklore medicine for a wide variety of ailments or as herbal supplements for health promotion. Traditionally it is used as a carminative, Liver stimulant, Pittasaraka, Laxative, anthelmintic, blood purifier, anti-inflammatory, swedajanana, antileprotic, antipyretic and preventive major for malaria etc. (Dey et al., 2013).

Andrographis paniculata contains a number of diterpenoids like andrographolide and several flavonoids such as 5, 7, 2', 3'-tetramethoxyflavone and 5-hydroxy-7, 2',3'-trimethoxyflavone (Sharma et al., 2011). The other chemical constituents that are present in the plant are andrographin, panicolin, andrographolide, diterpene glucosideneoandrographolide, andrographidin, neoandrographolide, chlorogenic, myristic acid, homoandrographolide, andrographiside andropanoside, etc (Dey et al., 2013). Among these constituents, Andrographolide, neoandrographolide, and 14-deoxy-11,12-didehydroandrographolide are known to be viricidal against herpes simplex virus 1 (HSV-1) without having any significant cytotoxicity at viricidal concentrations (Akbar, 2011). Among the other components of *A. paniculata*, Andrographolide is a major one known to show multiple pharmacological properties, such as antipyretic, anti-inflammatory, anti-allergic, anti-platelet aggregation, antiviral, anti-HIV, antithrombotic and antidiabetic activities. Andrographolide has been widely used for the treatment of fever, cold, inflammation, diarrhea and other infectious diseases (Sattayasai et al., 2010; Yadav and Singh, 2012).

Several animal studies showed that *A. paniculata* leaf arrests the spermatogenesis with a decrease in sperm count, disruption of seminiferous epithelium, seminiferous tubules, spermatozoa, sertoli cells (Akbarsha and Murugaian, 2000), weights of the testis, epididymis, sperm motility and seminal vesicle (K.Sathiyaraj et al., 2011), lower level of hormone, female rats have promising percentage of infertility (Sakila et al., 2009) and showing antifertility (Gupta and Sharma, 2006). Another study showed that 50% ethanolic extract of *A. paniculata* did not affect the body weight, reproductive and other internal organs weight changes (M.S. et al., 2013). Andrographolide on the other hand known to effect sexual functions, vascular reactivity and serum testosterone level and in another study it is showed no significant effects on sperm morphology and motility (Sattayasai et al., 2010). Since, previously several controversial results were observed on the antifertility efficacy of *Andrographis paniculata*. In the present study, we have undertaken to investigate the effect of *Andrographis paniculata* leaf extract on the lipid peroxidation and anti oxidative enzyme activities in the testis and epididymis of male wistar rats.

2. MATERIALS AND METHODS

Animals

The experiment was conducted on growing male Wistar albino rats (n=8) of approximately same age-group and body weight with the approval from Institutional Ethical Committee of Sri Venkateswara University (Resolution No: 28/2012-2013/(i)/a/CPCSEA/IAEC/SVU/YS-KSN dt.02.07.2012). The rats were purchased from an authorized dealer (M/S Raghavendra Enterprises, Bengaluru, India). They were housed in polypropylene cages (18" x 10" x 8") lined with sterilized paddy husk, provided tap water *ad libitum* and standard rat feed. They were maintained in well controlled environmental conditions such as 25 ± 2°C temperature, 12 hour light and dark cycles and 50 ± 10% humidity.

Plant material and Chemicals

The fresh whole plants of *Andrographis paniculata* were identified and obtained from the Srinivasa Ayurveda Pharmacy, Srinivasa Mangapuram, Tirupati, Andhra Pradesh, India. Fresh leaves were washed, dried, powdered and stored in air-tight plastic containers. Chemicals used for various assays were obtained from different commercial sources.

Preparation of plant *A.paniculata* extract

Ten grams of *A. paniculata* dry powder was extracted with 100 mL of absolute ethanol (99.9%) by stirring overnight at room temperature. The mixture was then centrifuged at 120 rpm and the supernatant was concentrated in a rotary evaporator at 50°C under vacuum and was then dried.

Experimental protocol

Animals were randomly divided into four groups of eight animals each. Group I was considered as normal control received normal saline only. Group II were administered with 20mg/kg body weight of ethanolic extract of *A. paniculata* (low dose), group III were administered with 200mg/kg body weight of ethanolic extract of *A. paniculata* (medium dose) and group IV were administered with 1000mg/kg body weight of ethanolic extract of *A. paniculata* (high dose) orally for 65 days daily. The doses were selected based on the earlier literature (Allan et al., 2009). On 66th day of experiment, animals were sacrificed. Testis, liver and kidney were isolated, cleared from adhering fluid, weighed to the nearest milligram and used for biochemical analysis. Tissue somatic index (TSI) was calculated using the following formula:

$$TSI = [\text{weight of the tissue (g)} / \text{body weight of the animal (g)}] \times 100.$$

Lipid peroxidation assay

The lipid peroxides in the tissues were determined by using the TBA method (Ohkawa et al., 1979). Isolated tissues were homogenized in 1.15% KCl (20%W/V). For the 1 ml of tissue homogenate, 2.5 ml of 20% trichloro acetic acid (TCA) was added followed by centrifugation at 3500g for 10 minutes. Residue was dissolved in 2.5 ml of 0.05 M sulphuric acid followed by the addition of 3 ml thiobarbituric acid (TBA). The samples were kept in a hot water bath for 30 minutes. The samples were cooled and a standard curve was constructed with the known amount of malondialdehyde (MDA) followed by the measurement of absorbance at 532 nm. The results were expressed as micromoles of MDA formed/mg of tissue/hr.

Superoxide dismutase activity

SOD activity was determined by the method described earlier (Misra and Fridovich, 1972). At alkaline pH, Superoxide anion O_2^- causes the autoxidation of epinephrine to adenochrome; while completing this reaction, SOD decreased the adenochrome formation. One unit of SOD is defined as the amount of extract that inhibits the rate of adenochrome formation by 50%. The testis was homogenized (10% W/V) in 50 mM ice-cold sodium phosphate buffer (pH 7.0) containing 1mM EDTA. The microsomal fraction of the testis was obtained by the differential centrifugation method as described earlier (Chainy et al., 1997). The microsomal fraction was used as enzyme source. The reaction mixture in a final volume of 2.0 ml contained: 0.05 M carbonate buffer (pH 10.2) 30 mM epinephrine (freshly prepared) and the enzyme extract. Changes in absorbance were recorded at 480 nm, measured at 10 sec intervals for 1 min in a spectrophotometer (Hitachi Model No: U 2001). The enzyme activity was expressed as Units /mg protein/min.

Catalase Activity

Catalase activity was determined by the method described earlier (Maehly and Chance, 1954). Testes from control and experimental rats were isolated and homogenized in 50 mM phosphate buffer (pH 7.0) and the microsomal fractions of the testis were obtained by the differential centrifugation method as described earlier (Chainy et al., 1997). The microsomal fraction was used as enzyme source. The reaction mixture in a final volume of 2.5 ml contained: 0.05 M phosphate buffer (pH 7.0) and appropriate amount of enzyme protein. The reaction was initiated by the addition of 19 mM H_2O_2 . The decomposition of H_2O_2 was followed directly by measuring the decrease in absorbance at 240 nm in spectrophotometer (Hitachi Model No: U 2001). The catalase activity was expressed as μ moles of H_2O_2 metabolized /mg protein / h.

Statistical data analysis

All data were expressed as mean \pm SD of eight animals per group. Statistical analyses were performed by using t-test. Probability values $P \leq 0.05$ were taken as statistically significant.

3. RESULTS AND DISCUSSION

In the present research investigation, we are targeted to understand the possible effect of ELAP in human reproductive health. Considering the wide variety of medical applications as a plant herb, its role in tissues oxidative status remains limited. Here in this study, we focused mainly on the levels of lipid peroxidation and anti-oxidant enzyme activities in the testis and epididymis of male rats.

All the rats used for the study were apparently normal and showed no signs of unusual behaviors. None of them were died and excluded from the research. Changes like fur appearance, skin color, salivation, vocalization, lacrimation, urination, respiration, postural, or gait abnormalities were not observed in any of the control and experimental rats. The relative weights of liver, kidney and testis observed in the control and graded doses of ELAP treated rats were represented in the Table 1 given below. Compared to the control no significant difference in the relative weights of liver, kidney and testis was observed in the rats treated with low dose (20 mg/Kg body weight) whereas a significant decrease in the relative weight of testis was observed in the rats exposed to medium (200 mg/Kg body weight) and high (1000 mg/Kg body weight) doses of ELAP compared to the control rats (Table 1). A significant decrease was observed in the relative weight of liver and kidney in the rats exposed to high dose of ELAP compared to the control rats (Table 1).

Generally, Lipid peroxidation is treated as a key process in many pathological procedures and it is assessed by estimating the amount of thiobarbituric acid-reactive substances (TBARS) in a tissue. Lipid peroxidation is defined as oxidation of lipids with carbon-carbon double bonds (Devasagayam et al., 2003). It is considered as a mechanism of cellular damage caused by free radicals having reacted with lipids causing peroxidation resulting in the release of products such as malondialdehyde (Halliwell and Chirico, 1993; Halliwell B and JMC, 2007). Levels of malondialdehyde are an important indicator of lipid peroxidation. Results showed that administration of graded doses of ELAP has a relative increase in testicular and epididymis MDA (Fig. 1) compared to the control rats.

Superoxide dismutases (SOD) and catalase (CAT) are known to be the enzymes that play an important role in protecting the cell against the potentially deleterious effects of reactive oxygen species (Kuthan et al., 1986). SOD is generally considered as a first line of defense against oxidative stress and is known to play a key role in the dismutation of superoxide anions to hydrogen peroxide and catalase is known to neutralize hydrogen peroxides to molecular oxygen and water (Inal et al., 2001; Hassan HM and HE., 1988). Decrease in the activities of SOD and CAT result in the accumulation of highly reactive free radicals thereby eventually generating the reactive oxygen species leading to detrimental or damaging effects in different tissues. These detrimental effects occur due to imbalance between the generation of reactive oxygen species and antioxidant system. Results showed a significant decrease in the levels of SOD and CAT in the rats treated with graded doses of ELAP in the testis and epididymis (Figures 2,3) compared to the control rats.

4. CONCLUSION

In conclusion, the present study demonstrates that the exposure to graded doses of ELAP alters the antioxidant system and increases the lipid peroxidation in testis and epididymis. The results from the present study can tentatively be suggested that the antioxidant system is impaired with the administration of ELAP.

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Table 1

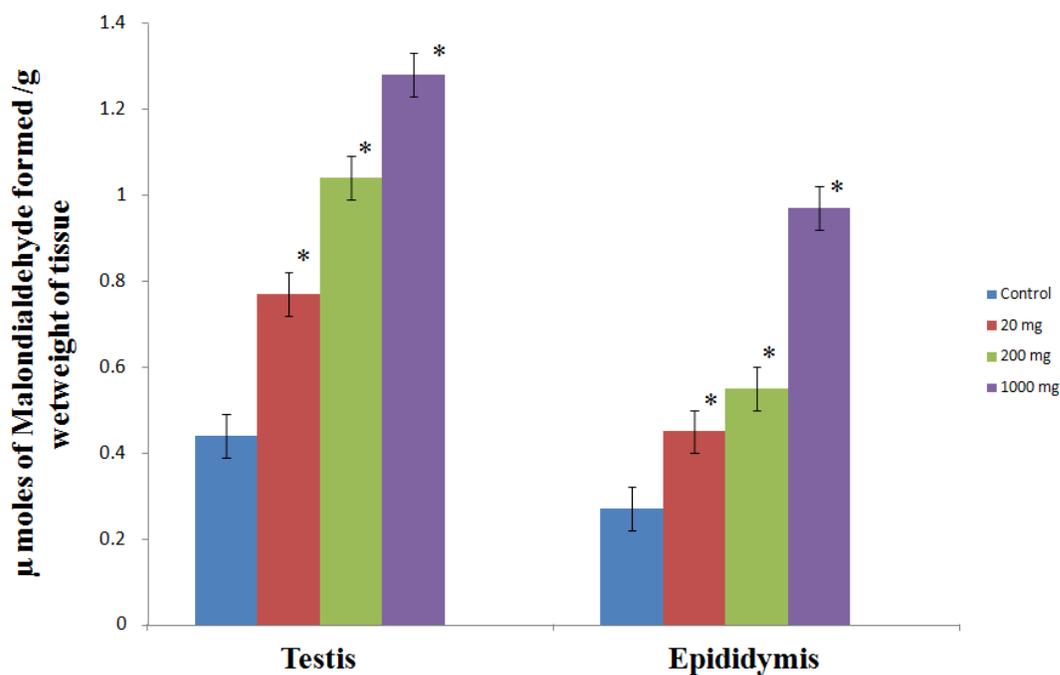
Effect of graded doses of ELAP on the tissue somatic indices (w/w %) in adult male rats

Parameter	Control	Leaf Extract		
		20	200	1000 mg/Kg body weight
Testis	1.259±0.06	1.214±0.13 (-3.54)	1.100*±0.14 (-12.6)	0.951*±0.37 (-24.46)
Liver	3.546±0.15	3.915±0.55 (10.40)	3.164±0.59 (-10.77)	2.846*±0.61 (-19.74)
Kidney	0.763±0.03	0.715±0.08 (-6.29)	0.727±0.09 (-4.71)	0.688*±0.08 (-9.82)

Values are mean ± S.D. of 8 individuals.

Values in the parentheses are percent change from that of control.

* Significantly different from control at P<0.05.

**Figure 1**

Effect of the graded doses of ELAP on the levels of malondialdehyde in testis and epididymis of male rats. Bars are mean ± S.D. of 8 individuals.

*indicates statistically significant from the control at P<0.05.

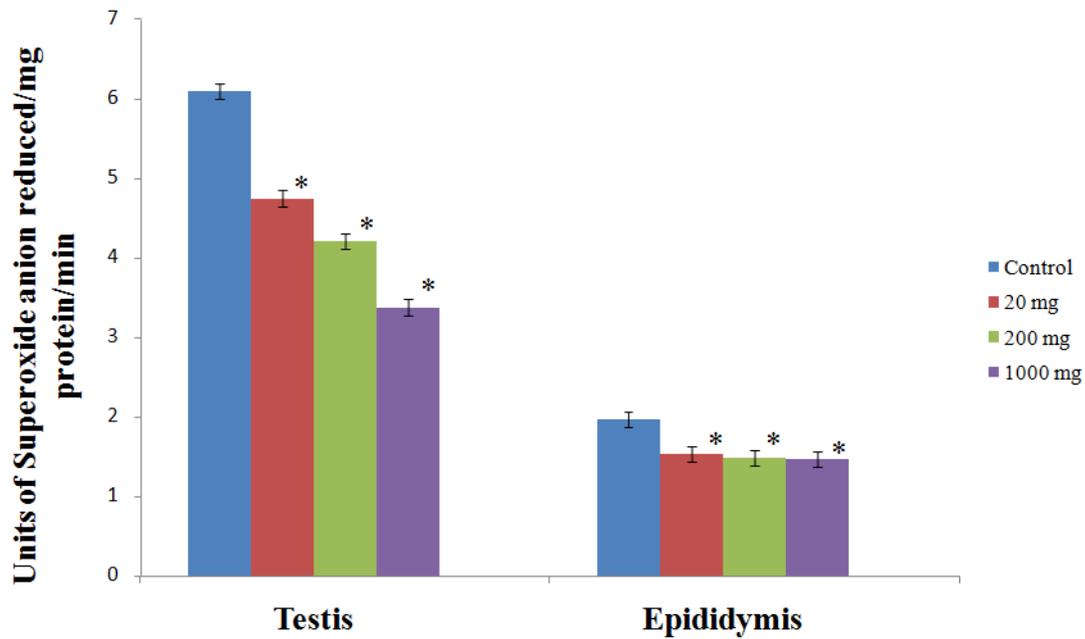


Figure 2

Effect of the graded doses of ELAP on the activity levels of SOD in testis and epididymis of male rats. Bars are mean \pm S.D. of 8 individuals. *indicates statistically significant from the control at $P < 0.05$.

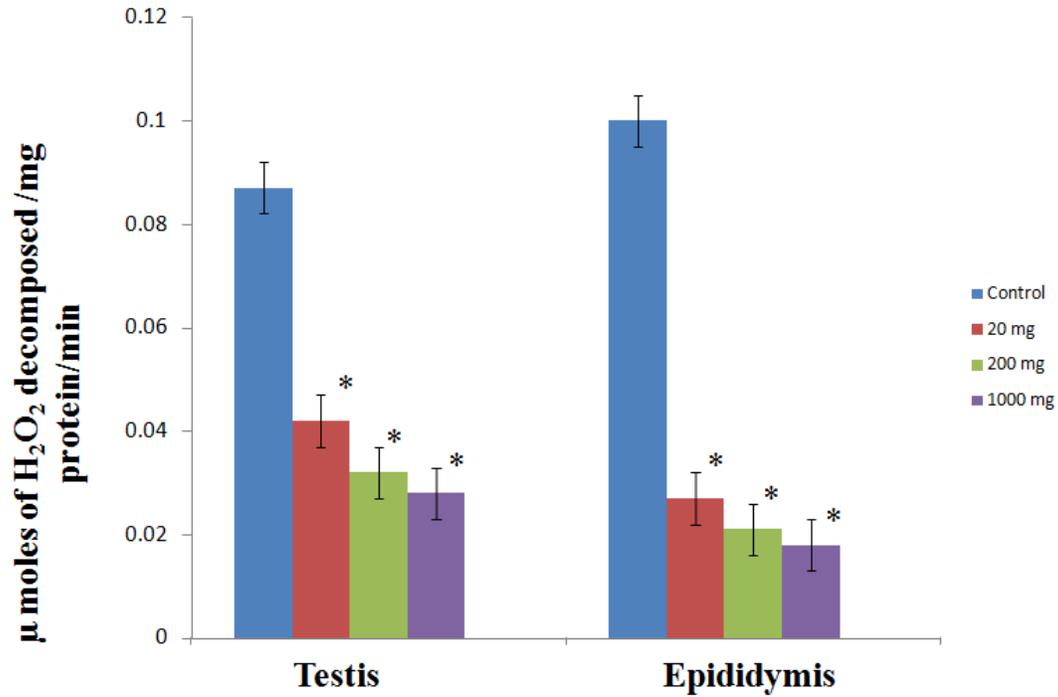


Figure 3

Effect of the graded doses of ELAP on the activity levels of catalase in testis and epididymis of male rats. Bars are mean \pm S.D. of 8 individuals. *indicates statistically significant from the control at $P < 0.05$.