MATERIALS AND METHODS
ANIMAL ETHICAL CLEARANCE:

Local Institutional Animal Ethical Committee of our University, obtained ethical clearance for conducting experiments on animals from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Regd. No 470/01/a/CPCSEA, dt.24th Aug 2001).

PROCUREMENT AND MAINTENANCE OF RATS:

The selection of rats is based on its ability of survival, its withstanding capacity in a fairly wide range of stress conditions and its maintenance and handling is quite comfortable for study. Male albino winstar strain rats of body weight 160±10 g were procured from Sri Raghavendra Enterprises, Banglore. The rat colony was maintained in laboratory at 24±2°C and 12 hrs light and 12 hrs darkness. Rats were fed on standard rat diet and water was supplied ad libitum. Before experimentation, rats were acclimatized for 2 weeks.

SYSTEMATIC POSITION OF GINGER

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
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<tbody>
<tr>
<td>Division</td>
<td>Magnoliopsida</td>
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<tr>
<td>Class</td>
<td>Liliopsida</td>
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<tr>
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<td>Zingiberales</td>
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<tr>
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<td>Zingiberaceae</td>
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<tr>
<td>Genus</td>
<td>Zingiber</td>
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<tr>
<td>Species</td>
<td>officinale</td>
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PREPARATION OF ETHANOLIC EXTRACT OF RHIZOME OF *Zingiber officinale*

The ginger was collected from local market and identified by Department of Botany, Sri Krishnadevaraya University, Anantapur. The ginger was cut into small pieces and dried under ceiling fan for 5 to 6 days. The dried ginger was ground in an electronic grinder and powder was collected. 50g of powder was extracted in 250mL of ethanol for 18 hrs in a soxhlet apparatus. The extract was dried at reduced pressure, stored at 0-4°C and used for the experimentation (Debanka Sekhar Misra, et al., 2005).

EXPERIMENTAL DESIGN

In present study, thirty rats were divided into five groups; each group consists of six rats.

**Group I** : Control

**Group II** : 300mg of Pb(NO₃)₂/Kg body weight/one week

**Group III** : 300mg of Pb(NO₃)₂/Kg body weight/one week + 150mg of ginger extract/Kg body weight/one week

**Group IV** : 300mg of Pb(NO₃)₂/Kg body weight/three weeks

**Group V** : 300mg of Pb(NO₃)₂/Kg body weight/three weeks + 150mg of ginger extract/Kg body weight/three weeks
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Lead nitrate Pb(NO$_3$)$_2$ was dissolved in distilled water and given through gastro-intestinally to Group I, II, III, IV, V with the help of a gavage. The ethanolic extract of ginger was dissolved in 5% Tween-80 and given to Group III and V gastro-intestinally with the help of a gavage after six hours of lead nitrate incubation.

LD$_{50}$ of lead nitrate orally in rats is 3613mg/kg bodyweight (Chisolm, 1971). In present study, the dose given to albino rats was around the sublethal dose.

SAMPLE COLLECTION AND PREPARATION FOR BIOCHEMICAL ESTIMATIONS AND ASSAYS

ANIMAL SACRIFICE AND ORGAN COLLECTION

After experimental period, from Group I three rats, Group II all six rats and Group III all six rats were sacrificed after one week of exposure. From Group I remaining three rats and Group IV all six rats and Group V all six rats were sacrificed after three weeks of exposure. Animals were sacrificed by cervical dislocation and immediately kidneys were removed and washed with ice cold 0.9% NaCl (saline) and suspended in 0.15M KCl in polypropylene containers, sealed with parafilm, labeled carefully and frozen at -80°C until assays were carried out.
PREPARATION OF TISSUE EXTRACTS FOR GLUTATHIONE

Immediately after separation of kidney, 10% tissue homogenate was prepared in 0.15M KCl using a homogenizer at 0°C. The whole homogenate was used for estimation of glutathione.

SAMPLE PREPARATION FOR ANTIOXIDANT ENZYME ASSAY:

10% Kidney homogenate in 0.15M KCl was prepared using a homogenizer at 0°C and centrifuged in cold (0-4°C) at 12,000 rpm for 45 minutes. The supernatant thus obtained was distributed into eppendorf tubes, labeled and stored at -20°C and all the antioxidant enzymes were assayed at the earliest.

1. GLUTATHIONE (GSH):

**Principle:** Total reduced glutathione content was measured by following the method of Ellman’s (1959). This method was based on the development of a yellow color, when 5,5′-dithio-2-nitrobenzoic acid (DTNB) reacts with the compounds containing sulfhydryl groups with a maximum absorbance at 412nm.

**Reagents:**
1. 0.2 M Phosphate buffer, pH 8.0
2. 5% TCA
3. Ellman’s reagent: 19.8mg DTNB in 100ml of 0.1% Sodiumcitrate
4. GSH Standard 20μg/ml
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**Procedure:** 0.5 ml of the 10% kidney homogenate was deproteinized with 3.5 ml of 5% TCA and centrifuged. To 0.5 ml of supernatent, 3.0 ml phosphate buffer and 0.5 ml of Ellman’s reagent were added and the yellow color developed was read at 412 nm. A series of standards (4-20 µg) were treated in a similar manner along with a blank. Values are expressed as µg of GSH/mg protein.

2. **GLUTATHIONE PEROXIDASE (EC 1.11.19):**

**Principle:** A known amount of the enzyme preparation was allowed to react with H₂O₂ in the presence of GSH for a specific time period according to the method of Rostruck (1973) and remaining GSH was measured by Ellman’s method (1959) as described for GSH estimation.

**Reagents:**

1. 0.4M Phosphate buffer (pH 7.0), containing
   0.4 mM EDTA, 10 mM sodium azide
2. 2mM GSH
3. 10% TCA
4. 0.2mM H₂O₂

**Procedure:** To 0.5 mL buffer, 0.2 mL 10% kidney homogenate, 0.2 mL GSH, 0.1 mL H₂O₂ were added and incubated at room temperature for 10 min along with a control tube containing all reagents except enzyme source. The reaction was arrested by
adding 0.5mL of 10% TCA, centrifuged at 4000rpm for 5min and GSH content in 0.5mL of supernatent was estimated. The activity was expressed as µg of GSH consumed/min/mg protein.

3. GLUTATHIONE-S-TRANSFERASE (E.C.2.5.1.18):

**Principle:** Glutathione-S-transferase activity was measured by monitoring the increase in the absorbance at 340nm using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate according to the method of Habig et al. (1974).

**Reagents:**
1. 0.14M Sodium phosphate buffer, pH 6.5
2. 0.01M CDNB dissolved in 50% ethanol
3. 30mM GSH

**Procedure:** The assay system contained 1.7 mL of buffer, 0.2mL GSH and 0.04mL kidney homogenate (40 µg protein). The reaction was initiated by 0.06mL CDNB. The change in absorbance was recorded at 1 min intervals at 340nm for 5 min and the activity was calculated using an extinction coefficient of CDNB-GSH conjugate as 9.6 mM$^{-1}$ cm$^{-1}$ and expressed as m moles of CDNB-GSH conjugate formed/min/mg protein.

4. CATALASE (E. C. 1.11.1.6):

**Principle:** Catalase catalyses the break down of H$_2$O$_2$ to H$_2$O and O$_2$ and the rate of decomposition of H$_2$O$_2$ was measured
spectrophotometrically at 240nm following the method of Beers and Sizer (1952).

**Reagents:**
1. 0.05M Sodium phosphate buffer, pH7.0
2. 0.059M $\text{H}_2\text{O}_2$ in buffer

**Procedure:** The assay system contained 1.9mL buffer, and 1.0mL $\text{H}_2\text{O}_2$. The reaction was initiated by addition of 0.1mL kidney homogenate (45 µg protein). The decrease in absorbance was monitored at 1 min intervals for 5 min at 240nm and activity was calculated using a molar absorbance coefficient of $\text{H}_2\text{O}_2$ as 43.6 M$^{-1}$cm$^{-1}$. The activity was expressed as m moles of $\text{H}_2\text{O}_2$ decomposed/min/mg protein.

**HISTOLOGY**

The histological sections of the kidneys of male rats were taken by adopting the procedure as described by Humason (1972). The tissues were isolated and gently rinsed with physiological saline solution (0.9% NaCl) to remove mucus and other debris adhering to them. They were fixed in Bouin’s fluid (75 mL saturated aqueous picric acid, 25mL 40% formaldehyde and 5mL glacial acetic acid) for 24 hours. The fixative was removed by washing through running tap water for overnight. Then the tissues were processed for dehydration. Ethyl alcohol was used as the dehydrating agent, as it is the most suitable and economical besides its hardening effect. The alcoholic transfer schedules were so
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arranged as to utilize both dehydration and hardening effect. The tissues were passed through successive series containing 30%, 50%, 70%, 80%, 90%, 95% and absolute alcohols. Then the tissues were cleaned in methyl benzoate and embedded in paraffin wax. Sections of 5μ thickness were cut using "SIPCON" rotatory microtome. The sections were stained with Harris hematoxylin (Harris, 1900) and counter stained with eosin, dissolved in 95% alcohol. After dehydration and cleaning, the sections were mounted in Canada balsam. Photomicrographs of the section preparations were taken using Olympus (PM-6 model) photomicrographing equipment.

STATISTICAL ANALYSES

DMR (Duncan’s Multiple Range Test) had been employed for the statistical of the data and P value (level of significant) is significant at less than 0.05.