Material & Methods
CARE AND MAINTENANCE OF EXPERIMENTAL ANIMALS

Pathogen free, 6 months age wistar strain male albino rats were used in the present study. The usage of animals was approved by the institutional animal ethics committee resolution No:09(iv)/a/CPCSCA/IAEC/07-08/SVU/Zool/KSR-CHIRK/ dated 26/6/08. The rats were housed in clean polypropylene cages under hygienic conditions with photoperiod of 12 hours light and 12 hours dark. The rats were fed with standard laboratory chow (Hindustan Lever Ltd, Mumbai) and water adlibitum.

Selection and mode of Ethanol Treatment

Alcohol is a generic name for large group of chemical compounds. There are many types of alcohols. Alcohols are named according to the radical to which \(-\text{OH}\) group is attached. The general formula for alcohol is \(\text{R-OH}\), where \(\text{R}\) signifies a hydrocarbon radical attach to an \(-\text{OH}\) group. Ethyl alcohol normally termed as ethanol, appears as colourless liquid solution.
Physical and Chemical Properties

<table>
<thead>
<tr>
<th></th>
<th>Chemical name</th>
<th>Ethyl alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Molecular formula</td>
<td>CH₃CH₂OH</td>
</tr>
<tr>
<td>2.</td>
<td>Molecular Weight</td>
<td>46.04</td>
</tr>
<tr>
<td>3.</td>
<td>Vapor density</td>
<td>1.6</td>
</tr>
<tr>
<td>4.</td>
<td>Solubility</td>
<td>Miscible in water</td>
</tr>
<tr>
<td>5.</td>
<td>State</td>
<td>Liquid</td>
</tr>
</tbody>
</table>

Dosage of ethanol

The dose administration of ethanol was followed as per the protocol given by Husain and Somani, (1997b). 2.0 grams/kg body weight was chosen as the dose, for this study.

Induction of Diabetes:

Streptozotocin (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) or STZ is synthesized by streptomyces achromogenes and is used to induce both insulin-dependent and non-insulin-dependent diabetes mellitus (IDDM and NIDDM, respectively). Single injection of STZ has been given intravenously or intraperitonially to the young rats to induce diabetes. The frequently used single intraperitonial dose in young rats to induce IDDM is between 40 and 60 mg/Kg body weight.

After fasting for 15 hrs, rats were injected intraperitonially with a single dose of Streptozotocin of 50mg/kg body weight obtained from Sigma Chemical Co., St. Louis, MO, USA, freshly dissolved in citrate buffer (pH 4.5). After injection, they had free access to food and water and were given 15% glucose solution to drink overnight to counter hypoglycemic shock. Diabetes in rats was identified by moderate polydipsia and marked polyuria. From the second day onwards fasting blood samples were collected from the
rats by tail vein and the blood glucose was measured by Accu Chek Sensor comfort glucometer (Manufacturer-Roche Germany) to know the induction of diabetes. If the blood glucose levels were more than 300mg/dL, insulin (IIU protamine Zinc insulin) is given to the diabetic rats and the rats are allowed to acclimatize the rats for diabetic condition for one week. After one week the rats with hyperglycemia (blood glucose level 250 mg/dL) were selected and used for the study (All the aspects were standardized in the lab).

Molecular structure of Streptozotocin

![Molecular structure of Streptozotocin](image)

Preparation of *Zingiber officinale*

The fresh rhizomes of *Zingiber officinale* were locally purchased in Tirupati. Two kilograms of air-dried rhizomes of the herb was milled into fine powder mechanically and extracted cold percolation with 95% ethanol for 24h. The extract was recovered and 95% ethanol was further added to the plant material and the extraction was continued. The process was repeated three times. The three extractions were pooled together, combined filtered and the filtrate was concentrated to dryness under reduced pressure in a rotary evaporator. The resulting ethanol extract was air-dried; finally giving 80g of light brown, powdery, crude ethanol extract of *zingiber officinale* dried rhizomes. Without any further purification the plant crude ethanol extract was used in the study. Dose equivalent to 200mg of the crude drug per kg body weight, was calculated and suspended in 2% v/v Tween 80 solution for the experiment.
GROUPING OF ANIMALS

The rats were divided into 6 groups of six in each group and treated as follows: (i) Normal Control, (ii) Ginger treatment (iii) Ethanol treatment (iv) Diabetic (STZ 50mg/kg BW) (v) Diabetic plus ethanol and (vi) Diabetic plus ethanol plus ginger treatment.

Group I – Normal Control (NC)

Six rats were received 0.9% of Nacl / kg bodyweight via orogastric tube for a period of one month.

Group II – Ginger treatment (Gt)

Six rats were received the ethanolic extract of ginger, 200mg/kg bodyweight via orogastric tube for a period of one month.

Group III – Ethanol Treatment (Et)

Six rats were received the 20% of ethanol with a dose of 2.0 grams / kg bodyweight via orogastric tube for a period of one month.

Group IV – Diabetic (Di)

Six rats were used as diabetic control by the induction of STZ intraperforational injection of 50mg/kg BW after of fasting.

Group V – Diabetic + Ethanol (Di + Et)

Diabetic rats received ethanol as described in Group III

Groups VI - Diabetic+ Ethanol +Ginger (Di + Et + Gt)

Diabetic rats received ethanol and ginger as described in group II and group III.

The animals were sacrificed after 24 hrs of the last treatment by cervical dislocation and the liver tissue was excised at 4°C. The tissue was
washed with ice-cold saline, immediately immersed in liquid nitrogen and stored in the deep freezer at -80°C for biochemical analysis and enzymatic assays. Before assay, the tissues were homogenized under ice-cold conditions. Selected parameters were estimated by employing standard methods. A part of the tissue was processed for histopathological studies.

**Procurement of chemicals**

All the chemicals used in the present study were Analar Grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pitrsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

In the present investigation, Barnstead Thermoline water purification plant was used for nano pure water, Kubota KR 200000T centrifuge for centrifugation of the homogenates and Hitachi UV-2000 Spectrophotometer for measuring the optical density values, for high-quality results.

**STANDARD METHOD OF APPRAOCH**

**Biochemical analysis and Enzymatic assays**

1. **ESTIMATION OF BLOOD GLUCOSE**: Estimation of blood glucose was carried out by using accuchek glucometer.

2. **BODY WEIGHT CHANGES**: Body weights of Normal control, ginger treated, ethanol treated, diabetic, ethanol treated diabetic and ethanol treated diabetic rats with ginger supplementation were recorded before and after treatments. The body weights of all groups were recorded at an interval of one week till the completion of the experimental period.

3. **TOTAL PROTEINS**

The protein content was estimated by the method of Lowry *et al.*, (1951). 5% (W/V) tissue homogenates were prepared in 10% (W/V) trichloroacetic acid (TCA) and the contents were centrifuged at 1000g for 15
minutes at 4°C. The residue was dissolved in a known volume of 1N sodium hydroxide. 4 ml of alkaline copper reagent was added to the known amount of this solution and the mixture was kept at room temperature for a few minutes. Then 0.4 ml of folin phenol reagent was added to the above content. The colour developed was read in spectrophotometer at 600 nm against the reagent blank. Protein content was calculated from a standard graph prepared with bovine serum albumin. The total protein content was expressed in mg of protein /gram wet weight of the tissue.

4. TOTAL FREE AMINO ACIDS

The total free amino acids were estimated by the method of Moore and Stein, (1954). 5% (W/V) homogenates of the tissues were prepared in 10% (W/V) trichloro acetic acid (TCA) and centrifuged the contents at 1000g for 15 min at 4°C. To 0.5 ml of supernatant, 2.0ml of ninhydrin reagent was added and the contents were exactly boiled for 6½ minutes in a boiling water bath. The contents were cooled to laboratory temperature. The samples were made upto 10 ml with glass double distilled water and the colour intensity was read at 570 nm in a spectrophotometer against the reagent blank. The total free amino acid content was expressed in mg of free amino acids/gram wet weight of the tissue.

5. ASPARTATE AMINOTRANSFERASE (EC. 2.6.1.1) (DL-Aspartate-2-oxoglutarate aminotransferase)

Aspartate aminotransferase activity was assayed by the method of Reitman and Frankel, (1957).

5% tissue homogenates were prepared in 0.25 M ice cold sucrose solution and centrifuged at 1000 x g for 15 min. The supernatant fraction was used as an enzyme source. The reaction in a total volume of 2.0 ml contained 100 μ moles of phosphate buffer (pH 7.2), 40 μ moles L-aspartic acid, 2 μ moles of α - ketoglutarate and 15 mg of tissue as enzyme source. After
incubating the reaction mixture at 37°C for 30 minutes, the reaction was arrested by the addition of 1.0ml of 0.001N 2-4 dinitrophenyl hydrazine and allowed to stand at room temperature for 20 min. The color developed with 10ml of 0.4 N sodium hydroxide was read at 545 nm in a spectrophotometer against the reagent blank. The enzyme activity was expressed as μ moles of pyruvate formed /mg protein / hr.

6. ALANINE AMINOTRANSFERASE (EC. 2.6.1.2) (DL-Alanine 2-oxoglutarate aminotransferase)

Alanine aminotransferase activity was assayed by the method of Reitman and Frankel, (1957).

5% tissue homogenates were prepared in 0.25 M ice cold sucrose solution and centrifuged at 1000 x g for 15 min. The supernatant fraction was used as an enzyme source. The reaction in a total volume of 2.0 ml contained 100 μ moles of phosphate buffer (pH 7.2), 60 μ moles α – alanine, 2 μ moles of α – ketoglutarate and 10 mg of tissue as enzyme source. After incubating the reaction mixture at 37°C for 30 minutes, the reaction was arrested by the addition of 1.0ml of 0.001N 2-4 dinitrophenyl hydrazine and allowed to stand at room temperature for 20 min. The color developed with 10ml of 0.4 N sodium hydroxide was read at 545 nm in a spectrophotometer against the reagent blank. The enzyme activity was expressed as μ moles of pyruvate formed /mg protein / hr.

7. BRANCHED-CHAIN AMINOTRANSFERASES (2.6.1.42)

(Leucine, Isoleucine and Valine aminotransferases)

Branched chain aminotransferases (LAT, ILAT and VAT) were estimated by the method of Taylor and Jenkins, (1966).

5% tissue homogenates were prepared in 0.02 M ice cold tris buffer and centrifuged at 1000 x g for 15 min. The supernatant fraction was used as
an enzyme source. The reaction in a total volume of 1.5 ml contained 20 μ moles of α - ketoglutarate, 20 μ moles of substrate (Leucine for LAT, Isoleucine for ILAT and Valine for VAT) and 10 mg of tissue as enzyme source. After incubating the reaction mixture at 37°C for 30 minutes, the reaction was arrested by the addition of 1.0ml of acidic diphenyl hydrazine and the contents were shaken well. Exactly after 10 minutes the precipitate was extracted with 5 ml of toluene by shaking vigorously for 20 seconds. After 10 minutes, the two phases of the mixture were separated with a separating funnel. To 4 ml of the upper phase 1.5 ml of 10% sodium hydroxide was added. The amount of keto acid was determined by mixing 1 ml of aliquot with 2 ml of 1 N sodium hydroxide. The intensity of the color developed was read against the reagent blank at 440 nm in a spectrophotometer. The enzyme activity was expressed as μ moles of keto acid formed /mg protein / hr.

8. GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PDH) (E.C: 1.1.1.49)

Glucose-6-phosphate dehydrogenase activity was assayed by the method of Lohr and Waller, (1965) as modified by Mastanaiah et al., (1978). 10% (W/V) tissue homogenates were prepared in 0.25 M ice cold sucrose solution and centrifuged at 1000 g for 15 min at 4°C. The reaction mixture in a total volume of 2 ml contained 100 μ moles of sodium phosphate buffer (pH 7.4), 20 μ moles of glucose-6-phosphate, 2 μ moles of INT and 0.3 μ moles of NADP. The reaction was initiated by adding 0.5ml containing 50 mg of tissue as an enzyme source. The reaction mixture was incubated at 37°C for 30 min and the reaction was stopped by the addition of 5 ml of glacial acetic acid. The formazan formed was extracted with 5 ml of toluene at 5°C. The optical density of the formazan was read at 495 nm against the toluene blank. The activity was expressed in μ moles of formazan formed/ mg protein / hour.
9. ISOCITRATE DEHYDROGENASE (ICDH)

(Isocitrate: NADP⁺ oxidoreductase E.C: 1.1.1.42)

Isocitrate dehydrogenase activity was assayed by the method of Korenberg and Pricer, (1951) as modified by Mastanaiah et al., (1978).

Ten percent homogenates (W/V) of liver tissues were prepared in 0.25M ice cold sucrose solution and centrifuged at 1000g for 15 minutes at 4°C. The supernatant was used for the enzyme assay. The reaction mixture in a final volume of 2.0 ml contained 40 μ moles of DL-isocitrate, 100 μ moles of magnesium chloride, 100 μ moles of sodium phosphate buffer (pH-7.4), 4 μ moles of INT (2-P-iodophenyl 3-P-nitrophenyl 5-phenyl tetrazolium chloride), 0.2 μ moles of ADP and 0.2 μ moles of NADP (for NADP⁺-ICDH).

The reaction was initiated by the addition of 0.2 ml supernatant containing 20mg of the tissue as an enzyme source and the contents were incubated at 37°C for 30 minutes. After incubation, the reaction was stopped by adding 5.0 ml of glacial acetic acid and the formazan formed was extracted overnight at 5°C into 5.0 ml of toluene. The colour was measured at 495nm in a spectrophotometer against toluene blank. The enzyme activity was expressed as μ moles of formazan formed/mg protein/hour.

10. MALATE DEHYDROGENASE (MDH) (L-malate NAD⁺ oxidoreductase-E.C: 1.1.1.37)

The specific activity of MDH was measured by the method of Nachlas et al., (1960) as suggested by Prameelamma and Swami, (1975) with slight modifications. 10% (W/V) homogenates of the liver tissues were prepared in ice cold 0.25 M sucrose solution and centrifuged at 1000g for 15 minutes at 4°C. The supernatant fraction was used for enzyme assay. The reaction
mixture in a total volume of 2 ml contained 100 μ moles of phosphate buffer (pH 7.0), 40 μ moles of sodium malate, 0.1 μ mole of NAD and 4 μ moles of INT. The reaction was initiated by the addition of 0.2 ml of homogenate containing 20 mg of tissue as an enzyme source. The incubation was carried out at 37°C for 30 minutes and the reaction was arrested by adding 5 ml of glacial acetic acid. The rest of the procedure was same as described earlier for G6PDH. The activity was expressed in μ moles of formazan formed / mg protein / hour.

11. GLUTAMATE DEHYDROGENASE

(GDH-L-Glutamate; NAD oxidoreductase - EC: 1.4.1.3)

Glutamate dehydrogenase (GDH) activity was assayed by the method of Lee and Lardy (1965). 5% (W/V) of tissue homogenates were prepared in ice cold sucrose (0.25M) solution and the contents were centrifuged at 1000g for 15 minutes at 4°C. The supernatant part was used as an enzyme source. The reaction mixture in a total volume of 2 ml contained 100 μ moles of phosphate buffer (pH 7.4), 40μ moles of sodium glutamate, 0.1 μ mole of NAD, 2 μ moles of INT and 0.2 ml containing 10 mg of tissue as an enzyme source. The reaction mixture was incubated at 37°C for 30 minutes. The reaction was arrested by the addition of 5ml glacial acetic acid and the formazan formed was extracted into 5 ml of toluene. The intensity of the color was read at 495 nm against the toluene blank. The enzyme activity was expressed as μ moles of formazan formed/mg protein/hour.

12. SUPEROXIDE DISMUTASE : (SOD-EC: 1.15.1.6)

Superoxide dismutase activity was determined according to the method of Misra and Fridovich, (1972) at room temperature. The liver tissue was homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 10 min at 4°C in ice cold centrifuge. The supernatant was
separated and used for enzyme assay. 100 μl of tissue extract was added to 880 μl (0.05 M, pH 10.2, containing 0.1 mM EDTA) carbonate buffer and 20 μl of 30 mM epinephrine (in 0.05% acetic acid) was added to the mixture and measured the optical density values at 480 nm for 4 min on a Hitachi U-2000 Spectrophotometer. Enzyme activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit activity.

13. CATALASE (CAT-EC 1.11.1.16)

Catalase activity was measured by a slightly modified version of Aebei, (1984) at room temperature. The Liver tissue was homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 10 min at 4°C in ice cold centrifuge. The resulting supernatant was used as enzyme source. 10 μl of 100% ethyl alcohol was added to 100 μl of tissue extract and then placed in an ice bath for 30 min. After 30 minutes the tubes were kept at room temperature followed by the addition of 10 μl of Triton X-100 RS. In a cuvette containing 200 μl of phosphate buffer and 50 μl of tissue extract was added 250 μl of 0.066 M H₂O₂ (in phosphate buffer) was added and decrease in optical density measured at 240 nm for 60 s in a UV Spectrophotometer. The molar extinction coefficient of 43.6 M cm⁻¹ was used to determine CAT activity. One unit activity is equal to the moles of H₂O₂ degraded / mg protein / min.

14. SE-DEPENDANT GLUTATHIONE PEROXIDASE (Se-GSH-Px, EC: 1.11.1.9)

Se-Dependant Glutathione Peroxidase (Se-GSH-Px) was determined by a modified version of Flohe and Gunzler, (1984) at 37°C. 5% (w/v) of liver tissue homogenate was prepared in 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenate were centrifuged at 10,000 rpm
for 10 min at 4°C in refrigerated centrifuge. The resulting supernatant was used as enzyme source. The Reaction mixture consisted of 500 µl of phosphate buffer, 100 µl of 0.01 M GSH (reduced form), 100 µl of 1.5 mM NADPH and 100 µl of GR (0.24 units). 100 µl of tissue extract was added to the reaction mixture and incubated at 37°C for 10 min. Then 50 µl of 12 mM t-butyl hydroperoxide was added to 450 µl of tissue reaction mixture and the colour developed was measured at 340 nm for 180s. The molar extinction coefficient of $6.22 \times 10^3 \text{ M cm}^{-1}$ was used to determine the activity. One unit of activity is equal to the mM of NADPH oxidized / mg protein / min. The enzyme activity was expressed in µmoles of NADPH oxidized / mg protein / min.

15. GLUTATHIONE REDUCTASE (GR-EC: 1.6.4.2)

Glutathione reductase activity was determined by a slightly modified method of Carlberg and Mannervik, (1985) at 37°C. The Liver tissue was homogenized (5%-w/v) in 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenates were centrifuged at 10,000 rpm for 10 min at 4°C in refrigerated centrifuge. The separated supernatant part was used as enzyme source. NADPH (50 µl, 2mM) in 10mM Tris buffer (pH 7.0) was added to the cuvette containing 50 µl of GSSG (20mM) in phosphate buffer (0.5 M, pH 7.0 containing 0.1 mM EDTA) and 800 µl of phosphate buffer. The tissue extract (100 µl) was added to the NADPH-GSSG buffered solution and measured at 340 nm for 3 min. The molar coefficient of $6.22 \times 10^3 \text{ M cm}^{-1}$ was used to determine GR activity. One unit of activity is equal to the mM of NADPH oxidized / mg protein / min. The enzyme activity was expressed in µmoles of NADPH oxidized / mg protein / min.

16. GLUTATHIONE-S-TRANSFERASE (GST- EC: 2.5.1.18)

Glutathione-S-transferase activity was measured with its conventional substrate, 1-chloro 2, 4-Dinitro Benzene (CDNB) at 340 nm as per the method
of Habig et al., (1974). The liver tissue was homogenized in 50 mM ice cold Tris-HCL buffer (pH 7.4) containing 0.2 M sucrose and centrifuged at 16,000 g for 45 min at 4°C and the resulting supernatant was again centrifuged at 1,05,000 g for 1 hour at 4°C. The pellet was discarded and the supernatant was used as the enzyme source. The reaction mixture in a total volume of 3 ml contain 2.4 ml of 0.3 M potassium phosphate buffer (pH: 6.9), 0.1 ml of 30 mM CDNB, 0.1 ml of 30 mM GSH and 0.4 ml of enzyme source. The reaction was initiated by the addition of glutathione and the absorbance was read at 340 nm against the reagent blank and the activity was expressed in μ moles of thioether formed / mg protein / min.

17. GLUTATHIONE (GSH) CONTENT

Glutathione content was determined according to the method of Theodorus et al., (1981). The liver tissue was homogenized in 0.1 M ice cold phosphate buffer (pH 7.0) containing 0.001M EDTA and protein is precipitated with 1 ml of 5% sulfosalicylic acid (w/v) and the contents were centrifuged at 5000 g for 15 min at 4°C. The resulting supernatant was used as the enzyme source. The reaction mixture in a total volume of 2.5 ml contained 2.0 ml of 0.1M potassium phosphate buffer, 0.005 ml of NADPH (4 mg / ml of 0.5% NaHCO₃), 0.02 ml of DTNB (1.5 mg / ml), 0.02 ml of glutathione reductase (6 units / ml) and require amount of tissue source. The reaction was initiated by adding 0.41 ml of enzyme source and change in absorbance was recorded at 425 nm against the reagent blank. The glutathione content was expressed in nano moles / gram wet weight of the tissue.

18. MDA content [Lipid Peroxidation (LP)]

This assay is used to determine MDA levels as described by Ohkawa et al., (1979). The Liver tissue was homogenized (5% - w/v) in 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenates were centrifuged at 10,000 rpm for 10 min at 4°C in refrigerated centrifuge. The
separated supernatant part was used for the estimation. 200 µl of the tissue extract was added to 50 µl of 8.1% sodium dodecyle sulphate (SDS), vortexed and incubated for 10 min at room temperature. 375 µl of twenty percent acetic acid (pH 3.5) and 375 µl of thiobarbituric acid (0.6%) were added and placed in a boiling water bath for 60 min. the samples were allowed to cool at room temperature. A mixture of 1.25 ml of butanol: phyridine (15:1) was added, vortexed and centrifuged at 1000 rpm for 5 min. The colored layer (500 µl) was measured at 532 nm using 1, 1, 3, 3-tetraethoxypropane as a standard. The values were expressed in µ moles of malondialdehyde formed / gram wet weight of the tissue.

HISTOLOGICAL STUDIES

Liver tissues from Normal control, Ginger treated, ethanol treated, diabetic, diabetic plus ethanol, and diabetic plus ethanol treated with ginger treated rats were collected after sacrificing the rats with cervical dislocation. The specimens were fixed in neutralized formalin, dehydrated with ethanol and embedded in paraffin wax (56°C). Serial sections (5µm) were taken and stained with haematoxylin and eosin following the earlier described methods of Ross and Reith (1989).

Validity of Experimental Procedures

General:

For all the enzyme studies in the present investigation, the assays were standardized by conducting preliminary test to determine the optimal pH, temperature, enzyme and substrate concentration and these optimal conditions were subsequently followed for each enzyme assay.
Aliquots for assay

Aliquots were selected such that initial rates were approximated as nearly as possible yet providing sufficient product to fall in a convenient range of spectrophotometric measurement.

Enzyme Units

Enzyme activities were expressed in standard units i.e., μ moles of product formed or substrate cleaved / mg protein / hour.

Substrate Requirement

All the enzyme assays were done under the conditions following zero order kinetics unless otherwise stated.

Lambert-Beer Law

All most all the products of the reactions were measured by the spectrophotometric procedures in which the optical density (absorbance) of the resulting colored complex was proportional to the concentrations of the reaction products.

Enzyme nomenclature

The nomenclature of enzymes used in the present study is according to the report of the commission of the “International union of Biochemistry” (IUB).

Assay of dehydrogenases using INT

Tetrazolium salts are unique class of oxidation-reduction indicators in the study of dehydrogenases. The advantages of using tetrazolium salts as electron acceptors are:

i) The tetrazolium salts give a stable color or reduction.
ii) They are highly soluble in aqueous solutions.

iii) They can be reduced both aerobically and anaerobically.

iv) They have high redox potential which makes the reduction easier.

v) They are freely permeable through membranes.

The first developed tetrazolium salt was triphenyl tertazolium chloride (TTC). Following the application of TTC, new tetrazolium salts were developed. Various tetrazolium salts receive electrons from various sites of electron transport system (Nachlas et al., 1960), which is due to the inherent difference in the redox potentials of various tetrazolium salts. The phenyl ring was observed to increase its redox potential. Karmaker et al., (1959) reported that INT (2-parainophenyl 3-paranitophenyl 5-phenyl tetrazolium chloride) was superior to the most of the tetrazolium salts as electron acceptor for the assay of various dehydrogenases.

Statistical treatment of the Data:

The mean and standard deviation (SD) were calculated by using the method of Pillai and Sinha (1968). The formulas used for calculating SD, percent deviation were as follows:

\[ \text{SD} (\sigma) = \sqrt{\sum X^2 - (\sum x/n)^2 / n - 1} \]

Where, \( X \) = individual observations
\( n \) = total number of observations

Percent deviation = \( \frac{CM - EM}{CM} \times 100 \)

Where, CM = mean of control value
EM = mean of experimental value
ANOVA

The data are expressed as Mean values with their SD. Readings of the six different groups were compared using one-way ANOVA analysis with DUNNETT'S MULTIPLE COMPARISION TEST. Statistical analysis was performed using SPSS (Version 11.5; SPSS Inc., Chicago, IL, USA). Using M.S. Office, Excel Software the data has been analyzed for the significance of the main effects (factors), and treatments along with their interactions.

The results were presented with the F-value. In most of the cases F-value was found to be significant with p value less than 0.001** and 0.05*. This indicates that effects of factors are significant. Those effects which are not significant have been indicating in the ANOVA table with ‡ mark.