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**ORIGINAL RESEARCH ARTICLE**

# An Evaluation of Sub – Chronic Toxicity of Ethanolic Extract of *Cucumis trigonus* Roxb. Fruit on Wistar Albino Rats

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## ABSTRACT

*Cucumis trigonus* Roxb fruit is used for various ailments in Indian traditional system of medicine such as anthelmintic, liver tonic, cardio tonic, appetizer, expectorant and intellect promoting. The present study is designed to evaluate the sub – chronic toxicity of the fixed doses of ethanolic extract of *Cucumis trigonus* Roxb. on albino rats. Animals were treated with different fixed doses of 150,250, 350, 450 and 550 mg/kg b.w. respectively for 90 days. Body weight of the animals was noted at regular intervals, on 91$^{st}$ day the animals were sacrificed and the organ weights were noted. Hematological parameters like RBC, WBC, MCV, MCH, MCHC, PCV, platelets and differential counts were performed. Liver marker enzymes like AST, ALT, ACP and biochemical parameters like urea, uric acid, protein, creatinine etc., were analyzed. The liver of the animals were undergone histopathological studies to ensure the protective activity of the fruit. The results were expressed at $P< 0.05$ level of significance. The results prove that this extract was found to be non toxic at all the dose levels.

**Key words:** *Cucumis trigonus*, hematological parameters, biochemical parameters, sub-chronic toxicity

## INTRODUCTION

Plant is man's friend in survival, giving him food and fuel, shelter and medicine from the days beyond the down of civilization. The use of herbs in treatment of diseases has declined in the west, but it continues to exist throughout the developing world. Many plants synthesize substances that are useful for the maintenance of health of humans and animals. It includes aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Many are secondary metabolites, of which 12,000 metabolites have been isolated [1]. Indian Ayurveda medicine has been using herbs such as turmeric as early as 1900 B.C (Aggarwal, *B.B. et al.*, 2007*).* Many other herbs and minerals used in Ayurveda were later described by ancient Indian herbalists such as Charaka and Sushruta during the 1st millenium BC. The *Sushruta Samhita* in the 6$^{th}$ century BC describes 700 medicinal plants, 64 preparations from mineral sources, and 57 preparations based on animal sources [2].

*Cucumis trigonus* Roxb of family Cucurbitaceae is distributed throughout India and found in areas of Ceylon, Afghanistan, Persia and Northern Australia. It is used for various ailments in Indian Traditional System of Medicine [3]. Fruit and roots have medicinal value. The fruits are used in flatulence, leprosy, fever, jaundice, diabetes, cough, bronchitis, ascites, anaemia, constipation, other abdominal disorders and amentia [4]. In addition, fruit pulp is bitter, acrid, thermogenic, anthelmintic, liver tonic, cardio tonic, appetizer, expectorant and intellect promoting [5]. The title plant is reported to possess analgesic, anti-inflammatory and diuretic activity. Recently it's proteolytic and serine protease activity has been reported [6].

Whenever we administer a chemical substance to a biological system, different types of interactions can occur and a series of dose-related responses result [7]. In most cases these responses are desired and useful, but there are a number of other effects which are not advantageous. The types of toxicity tests

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which are routinely performed by pharmaceutical manufacturers in the investigation of a new drug involve acute, sub -chronic and chronic toxicity. Acute toxicity is involved in estimation of $LD_{50}$ the dose which has proved to be lethal (causing death) to 50% of the tested group of animals. Determination of acute oral toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds [8]. The present study was designed to evaluate the sub chronic toxicity of fixed doses of ethanolic extracts of *Cucumis trigonus* Roxb. fruits on albino rats.

## MATERIALS AND METHODS

### Collection of the plant material

*Cucumis trigonus* Roxb. fruits were collected from Kovanur area of Coimbatore district, Tamil Nadu, India during the month of September to November,2009. The plant was identified and authenticated by taxonomist Dr.K. Arumugasamy, Assistant Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India. Voucher specimen was deposited herbarium centre, Department of Botany, Kongunadu Arts and Science College, Coimbatore.

### Experimental setup

To find out the effective dosage of *Cucumis trigonus* Roxb. final systemic toxicity studies were carried out by the method of Biswas. Thirty six male wistar albino rats weighing 150-200g were used for the final systemic toxicity study. They were randomly distributed into one control group and five treated groups, containing six animals per group and were on standard normal diet provided with water *ad libitum.* They were allowed to acclimatize for seven days to the laboratory conditions before the experiment. The treated group received orally varying doses (150, 250,350,450, 550mg/ kg b.w) at a rate of 1.0ml /rat/day to different sets of animals for 90 days.

### Body weight changes

The body weight of each rat was assessed using a sensitive balance during the acclimatization period, once before commencement of dosing, once weekly during the dosing period and once on the day of sacrifice.

### Mortality and clinical signs

During the dosing periods, all the animals were observed daily for clinical signs and mortality patterns once before dosing, immediately after dosing and upto 4 hr. after dosing [9].

On 91st day, the animals were anaesthetized with light chloroform anesthesia, blood was collected by sino-orbital puncture and centrifuged for 30 min. at 2000rpm to separate serum for biochemical analysis. The liver and kidney were excised immediately and thoroughly washed in ice cold saline and weights were recorded.

### Relative organ weight

On 91st day, all the animals were anaesthetized under light chloroform anesthesia. Different organs namely the heart, kidney, liver, spleen and brain were carefully dissected out and weighed in grams. The relative organ weight of each animal was then calculated as follows,

$$\text{Relative Organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

### Hematological assay

On the 91st day the blood samples were collected from external jugular vein under mild chloroform anesthesia for the estimation of hematological parameters like, Hb, RBC, WBC, MCV, MCH, MCHC, PCV, platelets and differential counts were performed.

### Preparation of tissue homogenate

A 10% tissue homogenate was prepared by homogenizing 1.0g of chopped liver or kidney tissue in 10ml of 0.1M tris HCl homogenizing buffer at pH 7.5. The homogenate was used for assaying the enzyme activities and other biochemical parameters.

### Biochemical parameters assayed

Biochemical parameters such as AST (aspartate transaminase), ALT (alanine transaminase), ACP (acid phosphatase), ALP (alkaline phosphatase) and LDH (lactate dehydrogenase), protein, albumin, cholesterol, urea, uric acid, creatinine, sodium and potassium were assayed.

### Histopathological investigation of liver

The organ was removed, washed with ice cold saline and a small portion of it was quickly fixed in 10% formalin. The tissues were processed by standard histopathological technique

### Statistical analysis

Results were expressed as mean ± SD of six animals in each group. Statistical significance was determined by one-way analysis of variance (ANOVA) and post hoc least-significant difference (LSD) test.

## RESULTS AND DISCUSSION

Increase in the demand of natural products has influenced the direction taken by many studies in pharmacology and toxicology. Study of haematological status is one of the important ways for the diagnosis of root cause of diseases [10]. The body weight of the experimental rats in final systemic toxicity studies were given in the (**Table 1**). The control as well as the experimental rats gained weight throughout the duration of the treatment. There was no statistically significant weight gain or loss in experimental rats when compared to control rats for the doses tested.

The organ weights of the rats treated with ethanolic extract of dried fruits of *C. trigonus* Roxb. for 90 days were given in (**Table 2**). There was no significant weight gain or loss in organ weight for the doses tested in experimental rats when compared to control rats. Our results agrees well with that of [11] who reported that slight changes were found in the weights of internal organs in varying doses that may due to the variation in size of internal organs in each animal.

The effect of administration of *C.trigonus* fruit extracts at different concentrations on haematological parameters at the end of 90 days treatment are shown in (**Table 3**). Administration of the fruit extract at all the selected concentrations did not produce any significant change ($p<0.05$) in all the hematological parameters tested. Our results are in accordance with that of [12], who showed that red blood cell indices were helpful in the differential diagnosis of anemia. In addition, all of the changes were still within the normal limits [13][14].

Liver marker enzymes and biochemical parameters in serum of experimental rats treated with *C.trigonus* extract for 90 days were given in the (**Table 4**). As far as the liver markers are concerned, no index of significant alterations in relation to control group appeared in the 90 days treatment. Toxicity studies indicated that the ethanolic fruit extract of *C.trigonus* was found to be practically nontoxic to all the selected dose levels.

This extract was also found to be non hepatotoxic at all the dose levels, since the serum biochemical parameters are within the normal levels.

**Table 1: Body weight of experimental rats in the systemic toxicity studies due to the ethanolic fruit extract of *C.trigonus* Roxb.**

| Days | Body weight (g) | | | |
|---|---|---|---|---|
| Dose mg/kg b.w | 0th day | 30th day | 60th day | 90th day |
| Control (Group I) | 153.55 ± 0.85 | 165.51 ± 0.35 | 168.01 ± 0.86 | 172.12 ± 0.57 |
| 150 (Group II) | 152.21 ± 0.97 $a^{ns}$ | 166.60 ± 0.17 $a^{ns}$ | 169.14 ± 0.56 $a^{ns}$ | 172.18 ± 1.37 $a^{ns}$ |
| 250 (Group III) | 153.91 ± 0.43 $b^{ns}$ | 165.46 ± 0.17 $b^{ns}$ | 169.24 ± 1.57 $b^{ns}$ | 173.12 ± 0.58 $b^{ns}$ |
| 350 (Group IV) | 152.01 ± 1.37 $c^{ns}$ | 164.06 ± 0.50 $c^{ns}$ | 170.06 ± 0.50 $c^{ns}$ | 173.19 ± 1.00 $c^{ns}$ |
| 450 (Group V) | 152.03 ± 1.01 $d^{ns}$ | 166.33 ± 0.77 $d^{ns}$ | 169.30 ± 0.83 $d^{ns}$ | 172.17 ± 0.88 $d^{ns}$ |
| 550 (Group VI) | 151.04 ± 1.38 $e^{ns}$ | 165.98 ± 0.76 $e^{ns}$ | 169.88 ± 0.49 $e^{ns}$ | 173.02 ± 1.02 $e^{ns}$ |

*Values are expressed as mean ± S.D of six animals*

***Statistical comparisons***

*a - Group II is compared with group I  b - Group III is compared with group I*
*c - Group IV is compared with group I  d - Group V is compared with group I*
*e - Group VI is compared with group I  ns - non-significant at 5% level  * - p < 0.05*

**Table 2: Organ weights of rats treated in the systemic toxicity studies due to the ethanolic extract of *C.trigonus* for 90 days**

| Group/Dose (mg/kg b.w.) | Organ weight (g) | | | | | |
|---|---|---|---|---|---|---|
| Organ weights (g) | I Control | II 150 | III 250 | IV 350 | V 450 | VI 550 |
| Heart | 1.22 ± 0.09 | 1.23 ± 0.08 $a^{ns}$ | 1.12 ± 0.08 $b^{ns}$ | 1.12 ± 0.06 $c^{ns}$ | 1.24 ± 0.05 $d^{ns}$ | 1.23 ± 0.30 $e^{ns}$ |
| Kidney | 1.35 ± 0.04 | 1.33 ± 0.01 $a^{ns}$ | 1.35 ± 0.06 $b^{ns}$ | 1.36 ± 0.05 $c^{ns}$ | 1.42 ± 0.05 $d^{ns}$ | 1.39 ± 0.05 $e^{ns}$ |
| Liver | 5.71 ± 0.13 | 5.79 ± 0.13 $a^{ns}$ | 5.53 ± 0.16 $b^{ns}$ | 5.55 ± 0.24 $c^{ns}$ | 5.63 ± 0.32 $d^{ns}$ | 5.67 ± 0.27 $e^{ns}$ |
| Spleen | 1.12 ± 0.01 | 1.28 ± 0.05 $a^{ns}$ | 1.51 ± 0.04 $b^{ns}$ | 1.21 ± 0.08 $c^{ns}$ | 1.18 ± 0.04 $d^{ns}$ | 1.17 ± 0.05 $e^{ns}$ |
| Brain | 1.51 ± 0.05 | 1.47 ± 0.11 $a^{ns}$ | 1.43 ± 0.04 $b^{ns}$ | 1.33 ± 0.10 $c^{ns}$ | 1.52 ± 0.07 $d^{ns}$ | 1.31 ± 0.10 $e^{ns}$ |

*Values are expressed as mean ± S.D of six animals*

**Table3. Haematological values of the rats treated with *Cucumis trigonus* fruit extract for 90 days**

| Group/Dose mg/kg.b.w.) | I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|
| Hematological Parameters | Control | 150 | 250 | 350 | 450 | 550 |
| RBC( x $10^{12}$/L) | 7.36 ± 0.54 | 7.37 ± 0.53$a^{ns}$ | 7.39 ± 0.29$b^{ns}$ | 7.38 ± 0.25$c^{ns}$ | 7.39 ± 0.29$d^{ns}$ | 7.38 ± 0.48$e^{ns}$ |
| Hb (g/dl) | 13.75 ± 0.45 | 13.77 ± 0.29$a^{ns}$ | 13.78 ± 0.48$b^{ns}$ | 13.79 ± 0.37$c^{ns}$ | 13.81 ± 0.27$d^{ns}$ | 13.82 ± 0.46$e^{ns}$ |
| WBC($10^9$/L) | 5.89 ± 0.23 | 5.91 ± 0.38$a^{ns}$ | 5.93 ± 0.49$b^{ns}$ | 5.94 ± 0.35$c^{ns}$ | 5.95 ± 0.23$d^{ns}$ | 5.96 ± 0.44$e^{ns}$ |
| MCV(µ$m^3$) | 57.77 ± 1.17 | 57.79 ± 1.13$a^{ns}$ | 57.79 ± 1.49$b^{ns}$ | 57.81 ± 1.11$c^{ns}$ | 57.80 ± 0.85$d^{ns}$ | 57.80 ± 0.84$^{ns}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| MCH(pg) | 19.62 ± 0.68 | 19.64 ± 0.69a$^{ns}$ | 19.68 ± 0.58b$^{ns}$ | 19.69 ± 0.48c$^{ns}$ | 19.71 ± 0.65d$^{ns}$ | 19.73 ± 0.44e$^{ns}$ |
| MCHC(g/dl) | 32.63 ± 0.16 | 33.51 ± 0.26a$^{ns}$ | 33.35 ± 0.19b$^{ns}$ | 33.89 ± 0.41c$^{ns}$ | 34.00 ± 0.53d$^{ns}$ | 33.99 ± 0.53e$^{ns}$ |
| PCV (%) | 42.43 ± 0.59 | 43.51 ± 0.29a$^{ns}$ | 44.08 ± 0.31b$^{ns}$ | 44.65 ± 0.18c$^{ns}$ | 44.49 ± 0.18d$^{ns}$ | 44.64 ± 0.29e$^{ns}$ |
| Platelet($10^9$/L) | 723.71 ± 0.09 | 723.67 ± 0.07a$^{ns}$ | 724.76 ± 0.12b$^{ns}$ | 723.65 ± 0.12c$^{ns}$ | 724.69 ± 0.15d$^{ns}$ | 723.69 ± 0.15e$^{ns}$ |
| Neutro (%) | 53.47 ± 0.08 | 53.67 ± 0.16a$^{ns}$ | 53.58 ± 0.27b$^{ns}$ | 53.65 ± 0.11c$^{ns}$ | 53.66 ± 0.12d$^{ns}$ | 53.70 ± 0.18e$^{ns}$ |
| Lympho (%) | 45.56 ± 0.16 | 45.73 ± 0.49a$^{ns}$ | 45.86 ± 0.50b$^{ns}$ | 45.80 ± 0.16c$^{ns}$ | 45.04 ± 0.31d$^{ns}$ | 45.24 ± 0.23e$^{ns}$ |

*Values are expressed as mean ± S.D of six animals*
*Statistical comparisons are as in table 1*

**Table 4: Liver marker enzymes and biochemical parameters in serum of rats treated with *C.trigonus* fruit extract for 30days**

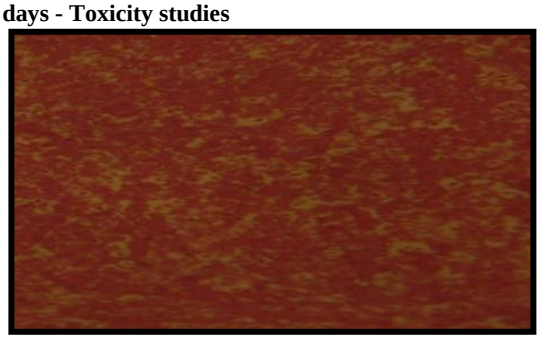
| Group/Dose (mg/kg b.w.) / Biochemical parameters | I control | II 150 | III 250 | IV 350 | V 450 | VI 550 |
|---|---|---|---|---|---|---|
| AST$^{\#}$ | 40.57 ± 0.14 | 41.56 ± 0.60 a$^{ns}$ | 41.53 ± 0.44 b$^{ns}$ | 41.65 ± 0.46 c$^{ns}$ | 41.69 ± 1.57 d$^{ns}$ | 41.15 ± 1.16 e$^{ns}$ |
| ALT$^{\#}$ | 37.50 ± 1.12 | 37.97 ± 1.08 a$^{ns}$ | 39.15 ± 1.04 b$^{ns}$ | 39.23 ± 1.22 c$^{ns}$ | 39.12 ± 1.11 d$^{ns}$ | 39.25 ± 1.26 e$^{ns}$ |
| ACP$^{\$}$ | 57.60 ± 1.11 | 57.62 ± 1.26 a$^{ns}$ | 57.77 ± 1.14 b$^{ns}$ | 58.12 ± 1.07 c$^{ns}$ | 57.21 ± 1.09 d$^{ns}$ | 57.24 ± 1.10 e$^{ns}$ |
| ALP$^{\$}$ | 56.27 ± 2.08 | 56.14 ± 1.04 a$^{ns}$ | 57.43 ± 1.09 b$^{ns}$ | 57.69 ± 1.08 c$^{ns}$ | 57.78 ± 2.23 d$^{ns}$ | 57.80 ± 1.23 e$^{ns}$ |
| LDH$^{\$}$ | 110.43 ± 2.34 | 110.77 ± 2.56 a$^{ns}$ | 110.41 ± 1.08 b$^{ns}$ | 110.41 ± 1.07 c$^{ns}$ | 110.82 ± 2.37 d$^{ns}$ | 110.43 ± 2.06 e$^{ns}$ |
| Protein (g/dl) | 6.30 ± 0.04 | 6.39 ± 0.14 a$^{ns}$ | 6.38 ± 0.34 b$^{ns}$ | 6.35 ± 0.22 c$^{ns}$ | 6.36 ± 0.44 d$^{ns}$ | 6.37 ± 0.96 e$^{ns}$ |
| Albumin (g/dl) | 3.65 ± 0.04 | 3.61 ± 0.13 a$^{ns}$ | 3.73 ± 0.05 b$^{ns}$ | 3.71 ± 0.04 c$^{ns}$ | 3.64 ± 0.01 d$^{ns}$ | 3.52 ± 0.08 e$^{ns}$ |
| Cholesterol (mg/dl) | 94.31 ± 0.07 | 94.65 ± 0.19 a$^{ns}$ | 94.82 ± 0.04 b$^{ns}$ | 95.69 ± 0.40 c$^{ns}$ | 95.79 ± 0.67 d$^{ns}$ | 95.83 ± 0.06 e$^{ns}$ |
| Urea (mg/dl) | 14.51 ± 0.05 | 14.61 ± 0.88 a$^{ns}$ | 14.58± 0.42 b$^{ns}$ | 14.85 ± 0.40 c$^{ns}$ | 14.02 ± 0.80 d$^{ns}$ | 14.69 ± 0.13 e$^{ns}$ |
| Uric acid (mg/dl) | 5.32 ± 0.01 | 5.53 ± 0.01 a$^{ns}$ | 5.52 ± 0.01 b$^{ns}$ | 5.54 ± 0.02 c$^{ns}$ | 5.56 ± 0.04 d$^{ns}$ | 5.46 ± 0.13 e$^{ns}$ |
| Creatinine (mg/dl) | 0.94 ± 0.09 | 0.94 ± 0.01 a$^{ns}$ | 0.94 ± 0.09 b$^{ns}$ | 0.93 ± 0.05 c$^{ns}$ | 0.94 ± 0.01 d$^{ns}$ | 0.95 ± 0.04 e$^{ns}$ |
| Sodium (meq/L) | 142.87 ± 1.94 | 142.92 ± 1.65 a$^{ns}$ | 142.94 ± 1.77 b$^{ns}$ | 142.99 ± 1.99 c$^{ns}$ | 143.05 ± 1.75 d$^{ns}$ | 143.17 ± 2.05 e$^{ns}$ |
| Potassium (meq/L) | 3.96 ± 0.11 | 3.99 ± 0.13 a$^{ns}$ | 3.94 ± 0.19 b$^{ns}$ | 3.99 ± 0.42 c$^{ns}$ | 3.95 ± 0.51 d$^{ns}$ | 3.97 ± 0.59 e$^{ns}$ |

*Values are expressed as mean ± S.D of six animals,*
*Units: $^{\#}\mu$ moles of pyruvate liberated/L; $^{\$}\mu$ moles of phenol liberated/L*

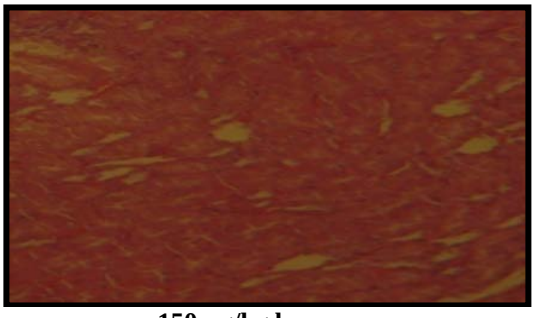
## Histopathological study of liver

All the control and experimental animals showed the normal architecture of the liver with central vein and cords of hepatocytes radiating from the central vein indicating the hepatoprotective activity. There was no cellular injury which indicates that the ethanolic fruit extract have no side effect at all the doses tested.

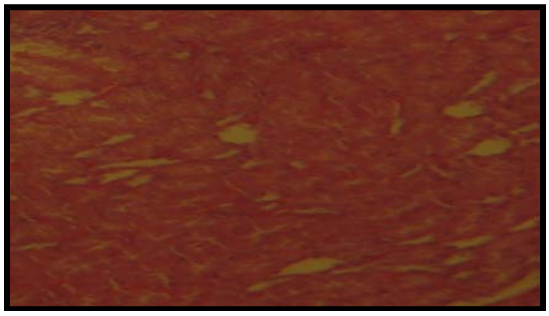
**Histopathological study of liver of fruit extract treated rats for 90 days - Toxicity studies**



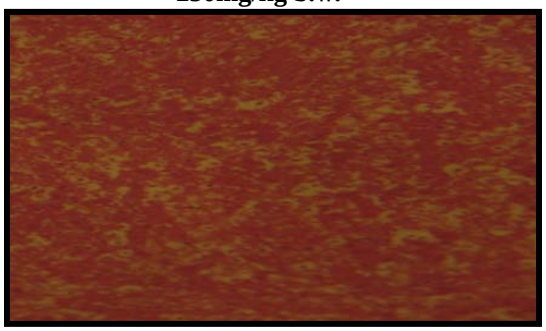
**Control**



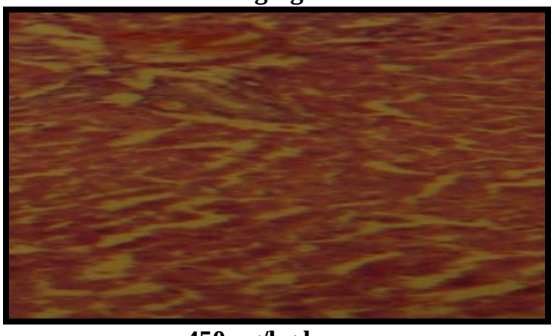
**150mg/kg b.w.**



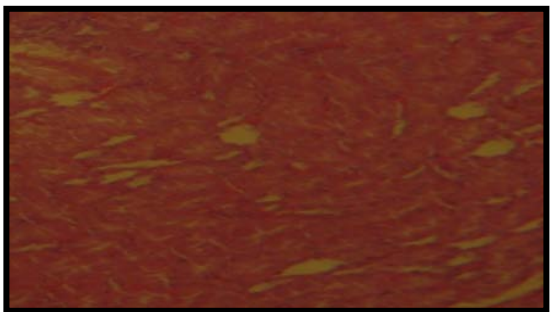
**250mg/kg b.w.**



**350mg/kg b.w.**



**450mg/kg b.w.**



**550mg/kg b.w.**

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