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RESEARCH ARTICLE

ORAL TOXICITY STUDY OF KARANJA SEED CHURANA (PONGAMIA PINNATA PIERRE) ON ALBINO RATS.

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Abstract

The acute toxicity studies of crude seed suspension of Pongamia Pinnata was studied in Charles Foster strain albino mice. The studies included the gross observation such as changes in body weight, ponderal changes in liver and heart; spleen and kidney were observed. Haematological and histopathological studies were also done. Rats are grouped as 6 rats in each group. The rats treated with suspension Karanja seed churana (Pongamia pinnata powder) orally with dose of 1600mg/kg body weight daily for 20 days and observed for any toxicity. On 21st day, rats are sacrificed and blood samples and organs were sent to laboratory for various studies. The test drug did not produce significant toxic effect on the organs mentioned but mild to moderate pathological changes were observed in spleen and liver, but these changes are not severe. All the haematolological parameters were non-significant except haemoglobin percentage which was highly significant in comparison to control group. It is proved that the test drug is safe for human use at this dose but should be administered cautiously in impaired liver functions.

Introduction:-

Toxicology is a discipline, overlapping with biology, chemistry, pharmacology, medicine, and nursing, that involves the study of the adverse effects of chemical substances on living organisms [1]. For this purpose various toxicity studies are carried out in animals, with assumption that man will behave in the same manner as the animals. WHO estimates that 80% of world's population presently uses herbal medicines for some aspect of primary health care [2] . Natural does not mean safe or even low risk. Our medicines offer very little actionable data on toxicological profile. Depending on the duration of drug exposure to animal's toxicological studies may be three types such acute, sub-acute and chronic toxicological studies. These are designed to capture toxic effects elicited by administration of single (or rarely multiple) doses to experimental animals within 24 hrs. To be described as **acute toxicity**, adverse effects should occur within 14 days of administration of substance. The purpose of this study is to determine median lethal dose (LD50 or LD90, dose required to kill 50% or 90% resp.) of lab animals ,preliminary identification of target organs of toxicity, selection of starting doses for preliminary human studies. It provides basis on which to design further testing programs[3] . In **sub-acute toxicity studies**, repeated doses of drug are given in sub-lethal quantity for a period of 15 to 20 days. Sub acute toxicity studies are used to determine effect of drug on biochemical parameters of tissues[3]. The purpose of this study is to identify target organs, major toxic effects, to establish dose levels for chronic exposure studies and for establishing safety criteria for human. In **chronic toxicity**

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studies[3], drug is given in different doses for a period of 90 days to over a year to determine carcinogenic and mutagenic potential of drug the purpose of this study is to determine carcinogenic potential and general toxicity.

Materials and Methods:-

Drug material:-

The drug Pongamia pinnata Pierre, seed churana sample was supplied by Pharmacy, Gujrat Ayurveda University, Jamnagar and identified and authenticated in Pharmacognosy laboratory. For administering to the experimental animals, a drug suspension was made with requisite quantity of distilled water according to the dose required.

Animals:-

Swiss albino mice and Charles Foster strain albino rats were obtained from the animal house attached to the I.P.G.T.R.A, Gujrat Ayurveda University, Jamnagar. The animals were maintained on Navchakan Oil Mills, "Amrut" Brand rat pellets feed and tap water given *ad libitum.* The animals were maintained under normal ambient conditions. Each experimental group consisted 6 rats of either sex. Control group received equal quantity of the Vehicle (distilled water) used for the preparation of the test drug suspension.

Chemicals:-

Analytical grade chemicals were used for bio-chemical estimation.

Instruments used:-

Plethysmograph, weighing scale, monopane balance, incubator, sterilizer, surgical instruments, cotton, syringes, needles, centrifuge, refrigerator, feeding syringes and tubes, serological water bath, vortex mixer, haem agglutination titer tray, microtiter plates , picric acid.

Route of administration:-

The drug was administered by oral route with the help of a gastric catheter sleeved on to a syringe. The dose of the drug was calculated by extrapolating the human dose to animals based on the body surface area ratio by referring to the standard table of Paget and Barnes (1994) [4].

Statistical analysis:-

Student's't' test for unpaired data has been used for analyzing the data generated during the study[5].

Toxicity Study:-

According to Charaka, the best drug is one, which cures the aimed disease and does not provoke any other diseases [6]. As the trial drug was selected for clinical study for oral use, hence it was considered necessary to carry out its toxicity study to assess its effect on the body organs and also on haematological and biochemical parameters.

Testing protocol:-

12 Charles Foster strain albino rats weighing between 180-220mg were taken for the present study. They were divided into two groups having six rats in each group. Group I was control and was administered tap water, while the second group was given suspension of the trial drugs orally in a dose of 1600mg per Kg body weight daily for twenty days. The animals were kept carefully and observed for any toxicity or side effects.

On the 21st day rats were again weighed and sacrificed by cervical dislocation. Blood was collected immediately by serving the neck blood vessels in two different bulbs one containing anti-coagulant and the other without anti-coagulant. After the collection, the blood samples were sent for pathological and biochemical investigations.

The rats were dissected and the organs heart, liver, spleen, kidney, testes and jejunum were taken out with care and kept in normal saline (0.9%). All the organs were cleaned of extraneous tissue and weighed on a monopan balance and transferred to a glass bottle containing 10% formalin. These samples were sent to the laboratory and were processed for hosto-pathological studies, which were carried out as per standard procedure (NIN Manual 1983). Then the prepared slides were viewed under microscope at various magnifications to note down the changes, if any, in their cyto – architecture. The photomicrographs of the sections were taken with the help of a carl-zeiss binocular microscope with photo – micrographic attachment.

Results And Observations:-

A) Effect on body weight:-

The data pertaining to the effect of the drug treatment on the changes observed in the body weight of rats is presented in table 1.

Table 1:- Effect of Karanja Seed Churana on body weight of albino rats

Group	Dose (g/kg)	Liver weight		Weight change (g)	Percentage change
		B.T.	A.T.		
Control	-	174±9	220±11	46±4.83	26.43
Karanja seed churana	1.6	198±9	226±12	28±2.71*	14.14

Data : Mean ± S.E.M. *P< 0.02

A statistically significant decrease in body weight of rats was observed in drug administered group at the dose level studied.

B) Ponderal changes:

Liver and Heart

The effect of the drug treatment on the weight changes observed in the liver and heart are included in table 2.

Table 2:- Effect of Karanja Seed Churana on liver and heart weight of albino rats

Group	Dose (g/kg)	Liver weight		Heart weight	
		Absolute (g)	Relative g/100 body weight	Absolute (g)	Relative g/100 body weight
Control	-	**9.63±0.21**	**3.14±0.04**	**0.72±0.03**	**0.27±0.013**
Karanja seed churana	**1.6**	**7.58±0.21***	**3.40±0.13**	**0.78±0.03**	**0.35±0.013****

Data : Mean ± S.E.M. *P< 0.001 **P<0.01

Moderate to significant increase in the absolute and the relative heart weight was observed in drug treated group in comparison to control group. However, only increase in relative weight was statistically significant at the level of p<0.01. there was highly significant (p<0.001) decrease with respect to data of liver weight in drug treated group, when presented as relative value, increase was found to be statistically non-significant.

Spleen and Kidney:-

The data of weight changes observed in spleen and kidney after drug treatment are presented in table 3.

Table 3:- Effect of Karanja Seed Churana on spleen and kidney weight of albino rats

Group	Dose (g/kg)	Spleen weight		Kidney weight	
		Absolute (g)	Relative g/100 body weight	Absolute (g)	Relative g/100 body weight
Control	-	0.489±0.032	0.18±0.04	1.58±0.062	0.60±0.03
Karanja seed churana	1.6	0.471±0.169	0.21±0.004	1.46±0.039	0.66±0.019

Data : Mean ± S.E.M.

Marginal decrease in absolute values of spleen and kidney weights was observed in drug administered group, where as in terms of relative values marginal increase was observed with respect to both spleen and kidney data. However, none of the observed changes were statistically significant.

C) Haematological studies

Haemoglobin

The data related to the effect of test drug on blood haemoglobin level can be found in table 4.

Table 4:- Effect of Karanja Seed Churana on Haemoglobin level in albino rats

Group		**Haemoglobin (g%)**
Control	-	12.5±0.15
Karanja seed churana	1.6	15.6±0.19*

Data : Mean ± S.E.M. *P< 0.001

A highly significant (P,0.001) increase in haemoglobin percentage was observed in Karnaja seed churana treated group in comparison to control group.

Total and differential leucocyte count

The data of the effect of test drug on Total and differential leucocyte count are included in table 5.

Table 5:- Effect of Karanja Seed Churana on Total and differential leucocyte count in albino rats

Group	**Dose (g/kg)**	**Total leucocyte count**	**Differential leucocyte count**	
			neutrophil	Lymphocyte
Control	-	4800±825.0	43.5±3.1	55.0±3.25
Karanja seed churana	1.6	5050±296.38	44.67±3.6	54.33±3.56

Data : Mean ± S.E.M.

Increase in Total leucocyte count, mild increase in neutrophil percentage and a mild increase in lymphocyte percentage were observed in test drug administered group. However, these changes were found to be statistically non- significant.

Biochemical studies

The data on the effect of test drug on blood urea level can be found in table 6.

Table 6:- Effect of Karanja Seed Churana on Blood urea in albino rats

Group	**Dose (g/kg)**	**Blood urea (mg/100 ml)**
Control	-	44.2±1.63
Karanja seed churana	1.6	46.33±1.50

Data : Mean ± S.E.M.

Blood urea level remained unaffected in the drug administered group at the dose level studied.

Histopathological Study

1. Jejnum

This drug did not affect the cyto- architecture of jejunum at the dose level studied. Photomicrographs of representative sections are presented in Fig. 1.

2. Spleen

Photomicrographs of representative sections are presented in Fig. 2. Increase in the proportion of white pulp was observed in test drug treated group in comparison to sections from control group.

3. **Heart**

Photomicrographs of representative sections of heart are presented in Fig. 3. The test drug did not produce any disturbance in the cyto-architecture of heart in majority of rats. However, in one rat mild inflammatory changes were observed.

4. **Liver**

Microscopic scanning of liver sections from test drug administered group showed moderate fatty degenerative changes. Representative photomicrographs are shown in Fig. 4.

5. **Kidney**

The test drug did not produce any histo pathological changes in kidney. Representative photomicrographs are shown in Fig. 5.

6. **Testis**

Microscopic scanning of testis section from drug treated group did not show any significant changes in cyto architecture. Fig.6

7. **Uterus**

The test drug did not produce any histo pathological changes in the uterus. Fig.7

Discussion:-

Toxicity studies showed mild increase in relative weights of liver, heart, spleen and kidney. Increase in weight may be indicative of stimulation of activity. Liver and kidney weight gain may be indicative of enzyme induction. Spleen weight gain may be indicative of non-specific stimulation of its activity. This further corroborates by histo-pathological studies show mild increase in the proportion of white pulp in spleen and moderate fatty degeneration in liver. The weight gain may sometimes due to oedema. Since it is not corroborated by histological examinations, hence , it can be ruled out.

The test drug did not produce any significant disturbances in the cyto- architecture of testies, heart, jejunum, uterus and kidney. This indicates that at the dose level studied, the drug do not produce significant toxic effect on these organs. Though mild to moderate pathological changes were observed in spleen and liver, but these changes are not severe. One should carefully monitor the patient, if the drug is given in very high doses for prolonged period. Overall considerations of haematological and biochemical parameters do not show any toxic activity in the drug administered group in comparison to control group. This indicates that the drug is devoid of marked toxic effect especially on kidneys. There was statistically highly significant increase in haemoglobin content of the blood. TLC, DLC were not much affected. This clearly shows that the test drug does not produce any significant adverse effect on blood forming tissues.

Conclusion:-

Histopathologically no significant disturbance in the cyto-architecture of heart, kidney, uterus, testis and thymus was revealed. Increase in the proportion of white pulp in spleen and moderate fatty degenerative changes in the liver were observed. But, because these changes are not severe and haematological and biochemical parameters of these animals do not show any toxic activity, hence reveals that the drug is devoid of marked activit at the mentioned dose. However caution should be exercised when the drug is administered for longer duration to the patients with impaired liver functions.

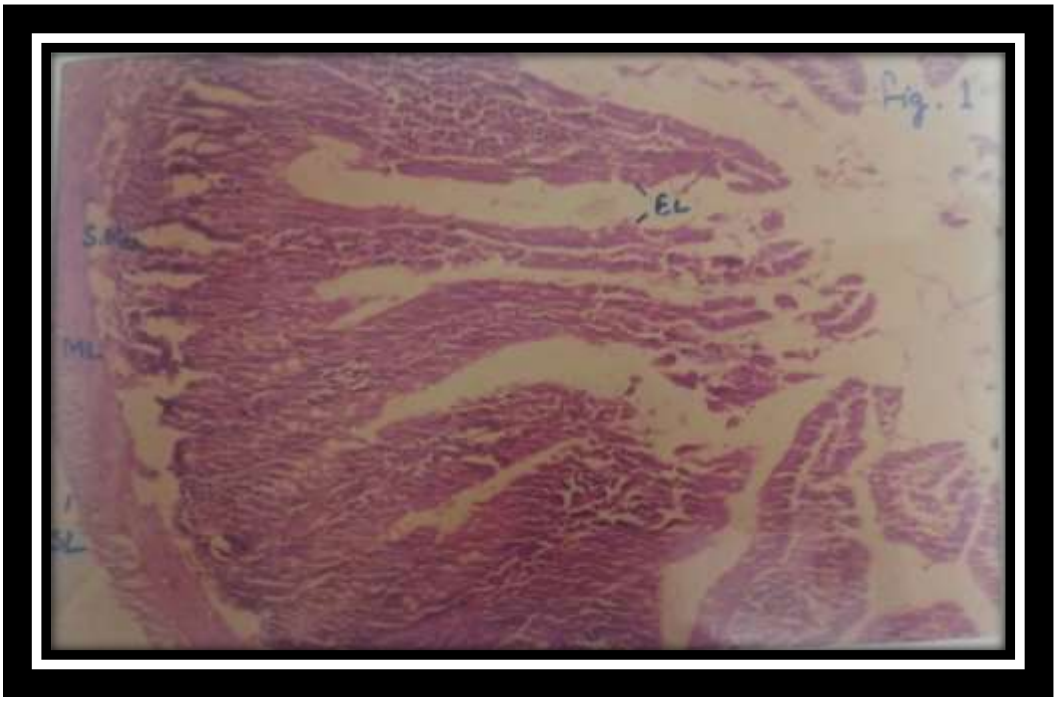

Fig 1:- Photomicrograph of section of jejunum obtained from Karanja seed treated rats. (1 X 100 magnifications)

EL: Epithelial layer SMu: Submucosa ML: Muscular layer SL: Serous layer

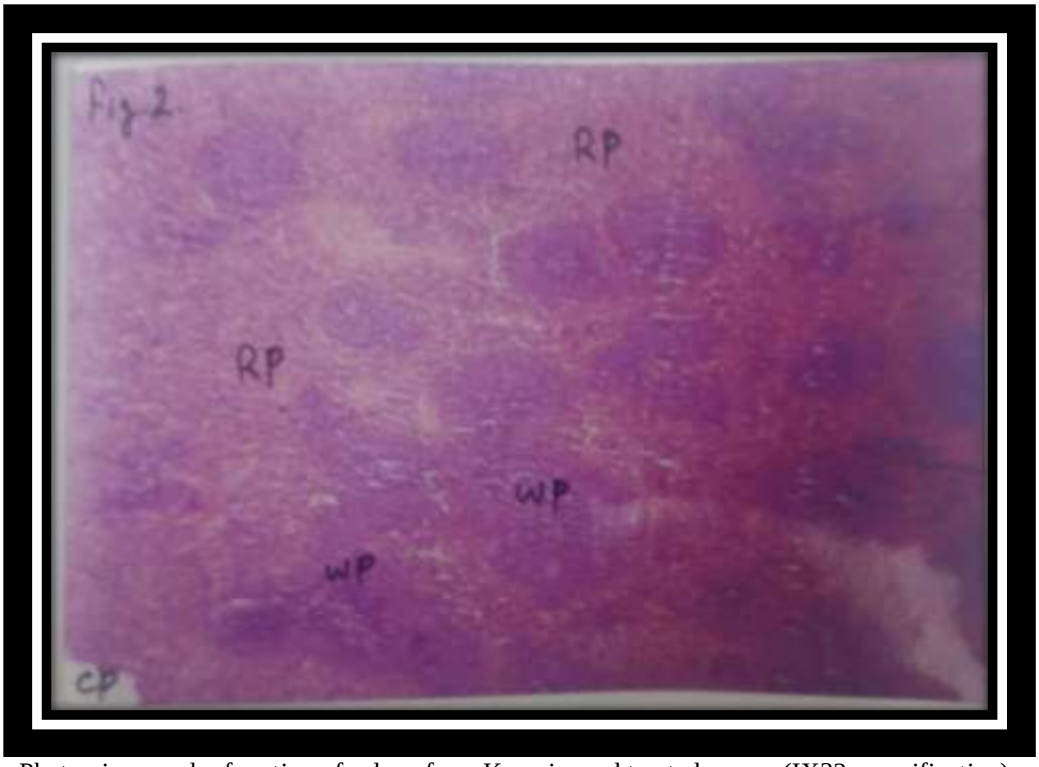

Fig 2:- Photomicrograph of section of spleen from Karanja seed treated group. (IX32 magnification)
Cp: Capsule WP: White Pulp RP: Red Pulp (Note : Increased proportion of white pulp)

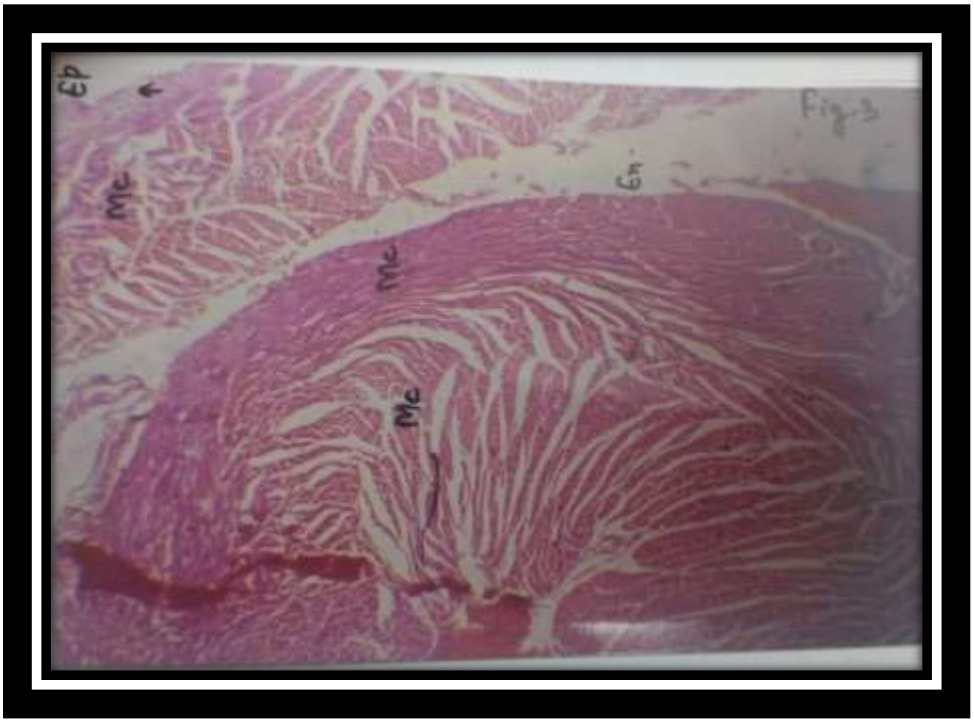

Fig 3:- Photomicrograph of section of heart from Karanja seed administered rats. (IX32 magnification)
Ep: Epicardium Mc: Myocardium En: endocardium
(Note : Cell infiltration and degenerative changes increased)

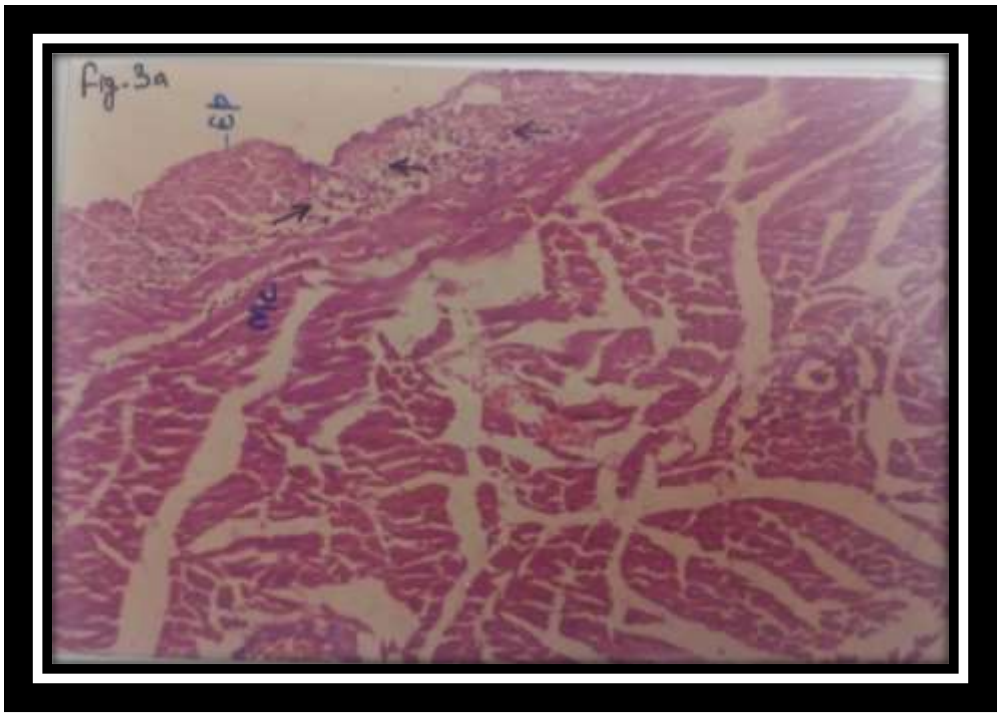

Fig 3 a:- same as Fig. 3 but at (I X 100 magnification).

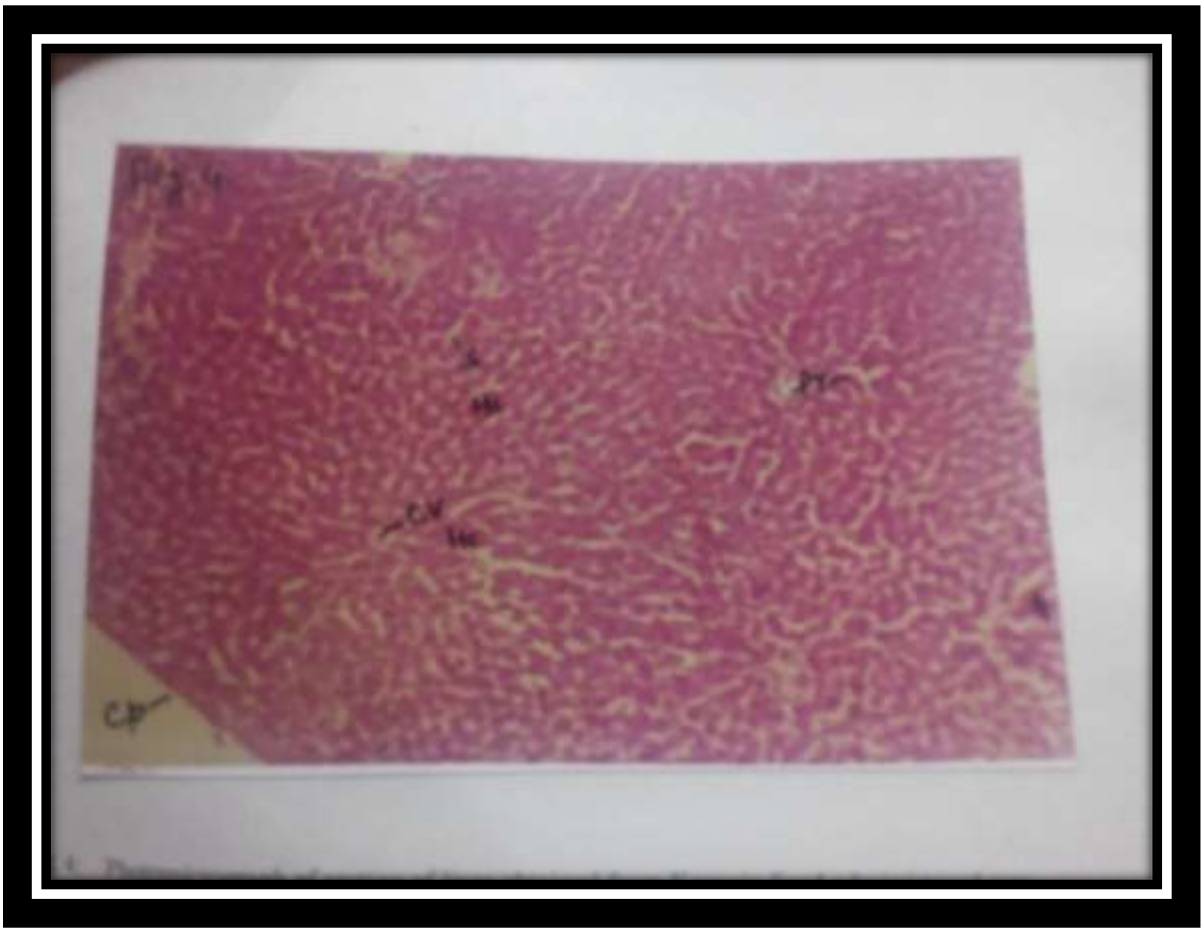

Fig 4:- Photomicrograph of section of liver from Karanja seed treated group. (I X 100 magnification)

Cp: Capsule CV: Central Vein HC: Hepatic cells PT: Portal Traid S: Sinusoid
(Note: Diffused fatty degenerative changes increased)

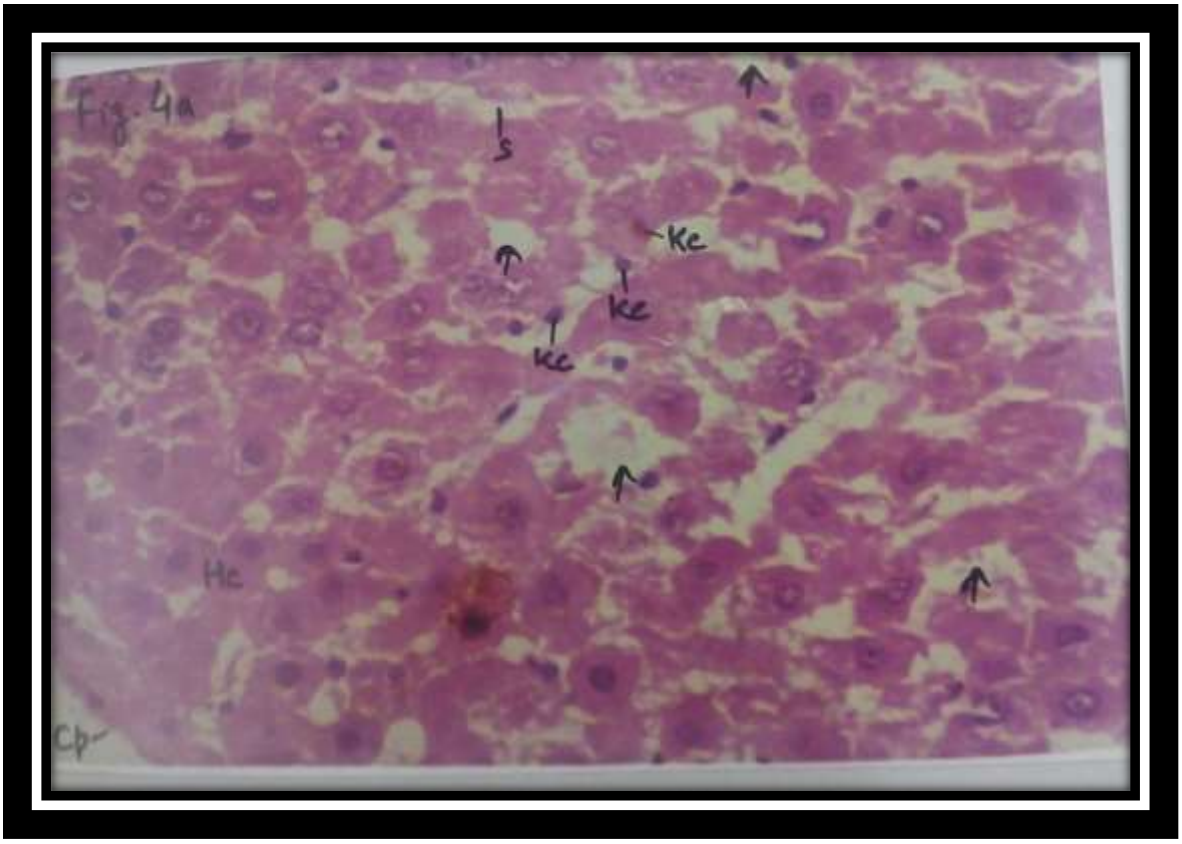

Fig 4a:- Same as Fig. 4 but at 1X 400 magnification.
Kc: Kupffer cells (Note: Fatty degenerative changes increased)

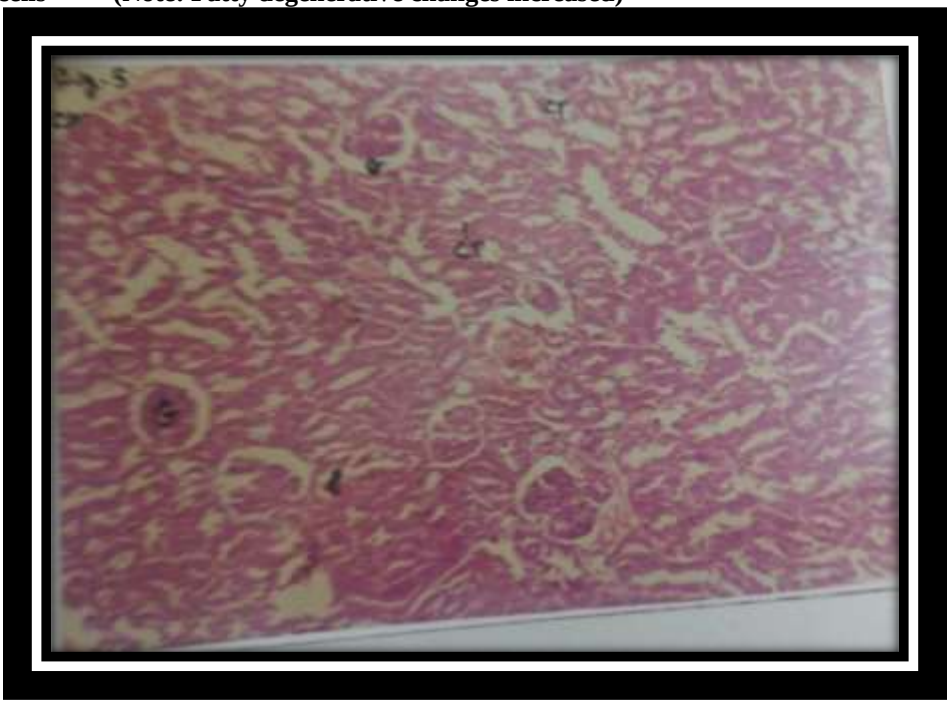

Fig 5: Photomicrograph of section of kidney from Karanja seed treated group. (I X 100 magnification)

Cp: Capsule G: Glomerus CT: Convolvulated tubule
(Note: Normal Cyto- architure)

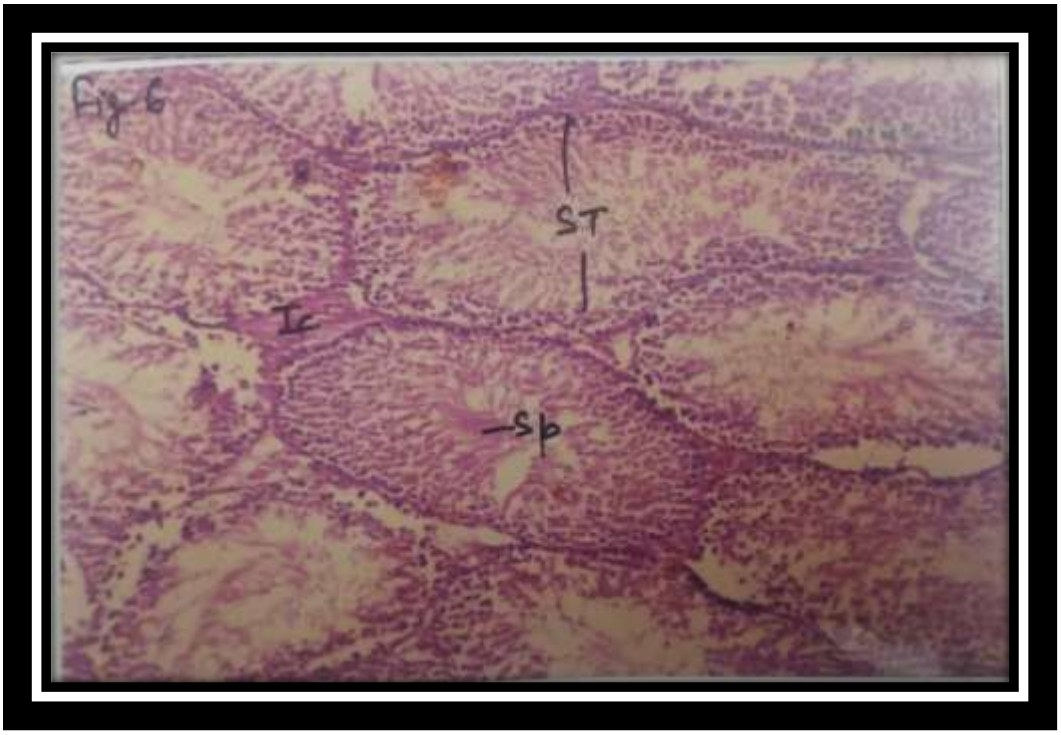

Fig 6:- Photomicrograph of section of testis from Karanja seed treated group. (I X 100 magnification)
ST: Semeniferous tubule IC: Interstitial cells Sp: Sperm
(Note: Normal Cyto- architecture with average spermatogenesis)

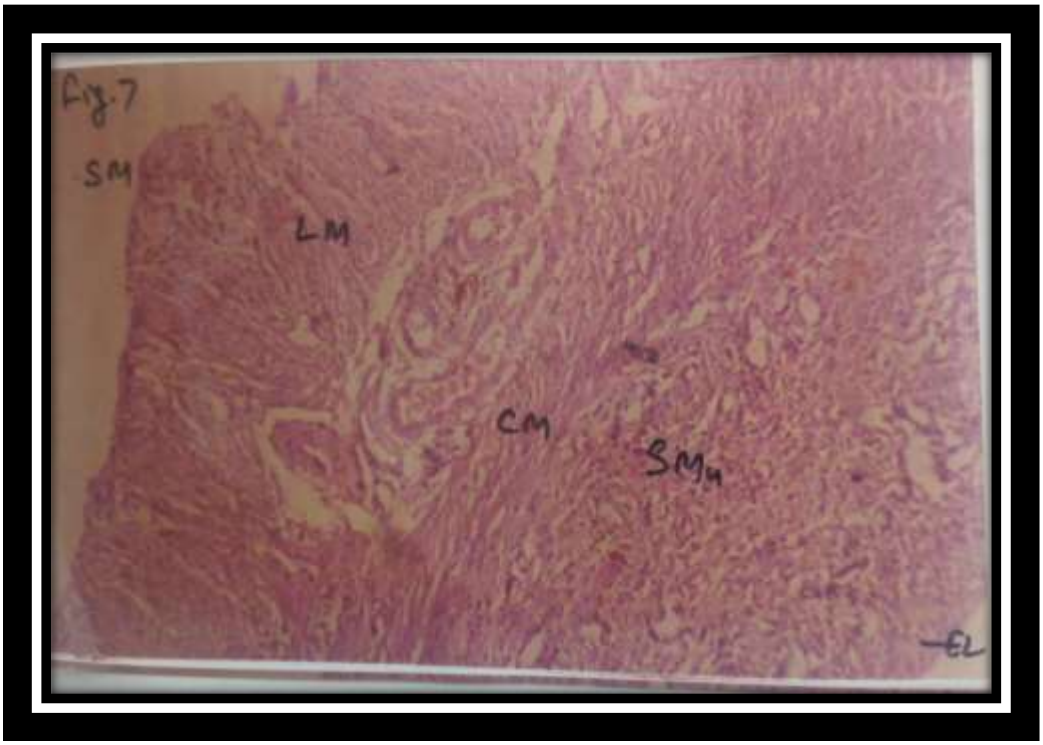

Fig 7:- Photomicrograph of section of uterus from Karanja seed treated group. (I X 100 magnification)

EL: Epithelial Layer SMu: Submucosa CM:Circular muscle layer LM: Longitudinal muscle layer SM:Serous membrane
(Note: Normal Cyto-architecture)

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