

An Investigation of the Effect of *Garcinia kola* (Heckel) and *Cola nitida* (Vent.) Schott & Endl. on Sperm Motility with Two Vital Organs of Male Wistar Rats

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Abstract

A total number of 30 Wistar rats were used for the experiment and acclimatized for two weeks prior to the commencement of the consumption of the seeds (*Garcinia kola* and *Cola nitida*). The rats were grouped into four (4) groups A, B C and D. Group A serves as the control having just 5rats which were fed with feeds without any treatment. Group B contained 15 rats feed with *Garcinia kola* seeds orally and the rats in this group were sub divided into 3 sub-groups having 5 rats each which were feed varying amounts of *Garcinia kola* seeds in 25kg, 50kg and 75kg concentration per subgroup. These same procedures were applied to Group C which was feed with *Cola nitida* in the same variation as group B. However, group D were feed with an average combination of both *Garcinia kola* and *Cola nitida* meaning that for 25kg treatment concentration, 12.5kg of each powdered seeds were used and likewise for the 50kg and 75kg treatment concentrations. The animals were feed in these rotations for 6weeks. The weight of each rat were measured and recorded at the end of each week. At the end of the treatments, the Wistar albino rats were sacrifice by cervical dislocation to test for the effect of the treatment on the Full Blood Count, Liver, Kidney and Sperm. The results showed that ingestion of G. Kola seed resulted in significantly reduced sperm count, motility, mild changes in the full blood count and abnormalities present in the liver and kidney.

Keywords: wistar rat; garcina kola; cola nitida; acclimatization; sperm motility; vital organs

Introduction

The use of plants has a long drawn history in health care delivery in Africa. Several plants are now being used in parts or whole to treat and manage many diseases (Garba et al., 2007). Also, scientific evaluation of traditional drug of plant seed origin and screening of more effective and safe hypoglycemic agents has continued to gain medicinal importance (Biswas, 2012). *Garcinia kola*, commonly called bitter kola is found mainly in tropical rain forest region of Central and Western Africa (Cheek, 2007). *Cola nitida* is among various species of cola, they are eating by elderly people. Kola contains about two percent caffeine and is chewed by many people as a stimulant. The fruit, seeds, nuts and bark of the plant have been used for centuries in folk medicine to treat ailments from coughs to fever (Cheek, 2007). *Garcinia kola* is traditionally used by African medicine men who believe that it has purgative, antiparasitic, and antimicrobial properties. The seeds are used for bronchitis, throat infections, colic, head or chest colds, and cough. It is also used for liver disorders and as a chewing stick.

Cola nitida nuts contain caffeine, theobromine and tannin. Along with the closely related *Cola acuminata*, which is also native to West Africa, the trees are cultivated commercially in tropical regions of the world and the nuts used in the manufacture of "cola" drinks. Other ingredients of these drinks include spice oils, other aromatic compounds (sometimes including the leaves of the tree), caramel for colouring, sweeteners, phosphoric or citric acid, and carbon dioxide to provide effervescence (Prohp et al., 2009). The nut is traditionally been used as a stimulant when chewed. It is reported to lessen fatigue, prevent hunger pangs, increase mental activity and reduce the need for sleep.

There is endless need for budding scientists to continue to explore novel medicinal and microbial importance of these seeds, thus this project research presents and exhaustive antimicrobial analysis and effects of *Garcinia kola* and *Cola nitida* on male rat organs (Kidney, Liver and Testes).

Wistar rat is currently one of the most popular rats used for laboratory research. It is characterized by its wide head, long ears, and having a tail length that is always less than its body length. The Sprague Dawley rat and Long-Evans rats were developed from Wistar rats. Wistar rats are more active than others like Sprague Dawley rats. The spontaneously hypertensive rat and the Lewis rat are other well-known stocks developed from Wistar rats (Saad et al., 2009).

Materials and Methods

Fresh seed of Cola nitida and Garcinia kola were bought from Ago-Iwoye market, Ijebu North Local Government of Ogun State. These seeds were authenticated at Forestry Research institute of Nigeria, Jerico, Ibadan (FRIN) and voucher number: 110940 and 110941 were assigned to Garcinia kola (Heckel) and Cola nitida (Vent) respectively. The Wistar rats were purchased from an animal house in Ibadan and acclimatized for two weeks prior to the commencement of the consumption of the seeds. The seeds were chopped and air-dried at room temperature for two weeks after which dried seeds materials were milled with an electric blender (Lexus MG2053, India). The milled seeds were then mixed with feed in varying treatment quantities of 25kg, 50kg and 75kg and fed to the different treatment groups for a period six (6) weeks.

Collection of blood samples

Blood samples were collected through the orbit (eyes) using capillary tube, and dispensed into a test tube that contained an anticoagulant ethylene diaminetetra acetic acid (EDTA).The sample was transported to a laboratory immediately for analysis.

Semen analysis

Semen were collected from both treated and untreated rats (control), using the electro-ejaculation method as described by Pant and Srivastava, 2003.

Laboratory procedure for histological processing

The appropriately labeled samples were brought to the laboratory and subjected to the following procedure. Dissected, and appropriately labeled according to their grouping, fixed in 10% neutral buffered formalin for further fixing before processed in automatic tissue processor, embedded in paraffin wax and sectioned at 5 microns on a rotary microtome (Leica RM2245, Leica Biosystem, Iceland) mounted on glass slides. The stepwise protocol for the automatic tissue processor for histological examination slide as described by Akpokodje et al., (2005).

Results and Discussion

Sample	Pcv %	Hb g/dL	Rbc cell/mm3	Wbc cell/mm3	Platelet mL	Lym %	Neut %	Mon %	Eos %	
Control	39	12.6	6.20	6000		172000	72	25	2	1
BKR1	42	13.8	6.79	6000		227000	64	32	2	2
BKR2	53	17.2	8.24	6250		177000	73	25	2	0
BKR3	45	14.7	7.32	6700		147000	69	26	3	2
KNR1	47	15.4	7.42	5800		253000	73	24	2	1
KNR2	53	17.4	8.27	5700		130000	62	36	1	1
KNR3	48	15.6	8.19	6350		213000	69	27	1	3
CBR1	48	15.5	8.13	9100		229000	67	31	1	1
CBR2	30	9.6	4.83	9550		203000	70	26	2	2
CBR3	51	16.5	8.19	6500		149000	68	27	2	3

Keys: Pcv- Pack cell volume, Hb- Hemoglobin, Rbc- Red blood cell, Wbc- White blood cell, Lym- Lymphocyte, Neut- Neutrophils, Mon- Monocyte, Eo- Concent of eosinophils.

NOTE: Male samples were used.

Table 1: Full blood count

The table above is showing the effects of GK and CN on the full blood count of the male rats due to the chemical constituents present in the seeds which show mild change in the blood cells. These show mild changes in the full blood count of the tested rats. The effect of G.kola and C.nitida on PCV, HB, RBC and WBC are within normal range. BKR2, KNR2 and

CBR3 were observed to have polycythemia in RBC, the cells are bigger while others are within normal range. The seeds had no apparent effects on platelet count but there were significant dose dependent increase compare to control, this result as a remarkable reflection of anti-aggregation ability of antioxidants and these is in collaboration with the work done by Percy M. J. et al., (2006).

S	M(%)	L/d	SC	B T	CT	A H	HT	TT	AM	DH	TC	TAS	% ABN	
BKR1	75	92	274	-	-	-	-	-	-	-	441	-	-	
BKR2														
BKR3	TOO	FEW												
KNR1	20	80	168	1	3	2	-	-	-	1	-	452	7	1.55
KNR2	30	85	184	2	3	-	-	-	-	1	-	415	6	1.45

KNR3	65	75	242	1	2	1	-	-	1	-	406	5	1.33
CBR1											412		
CBR2	50	75	174	1	2	2	1	-	1	-	414	7	1.67
CBR3													
CON	60	80	261	2	2	-	1	-	-	-		5	1.21
TROL	NIL												
	60	70	235	2	1	-	-	-	1	-	400	4	1
	75	95	342	-	-	-	-	-	-	-	322	-	-

Key: S- sample,% M- percentage motility, L/D- live: dead, SC- sperm count, BT- bent tail, CT- coiled tail, AH- abnormal head, HT- headless tail, TT- tailless tail, AM- abnormal mid piece, DH-double head, TC- total count, TAS- total abnormal sperm,% ABN- percentage abnormality.

Table 2: Morphological features of the sperm cells

Semen were collected from both treated and untreated rats (control), using the electro-ejaculation method as described by Pant and Srivastava, 2003. The consumption of GK and CN resulted in different defects in the morphological features of the sperm cells as shown in the table above. The results showed reduction in the count, motility, and caused abnormalities like bent tail, headless tail, and abnormal head. The seed also marked reduction in the testosterone in male rat according to Naiho

(2004) who demonstrated that chronic consumption of Garcinia Kola seed resulted in the disruption in the basement membrane of seminiferous tubules, near absence of sperm in the lumen and loss of intestinal cells of Leydig. It is possible that the observed reduction sperm count and motility may be due to direct spermicidal action of the components of G. Kola seed, as reported by Udoh, (1998).

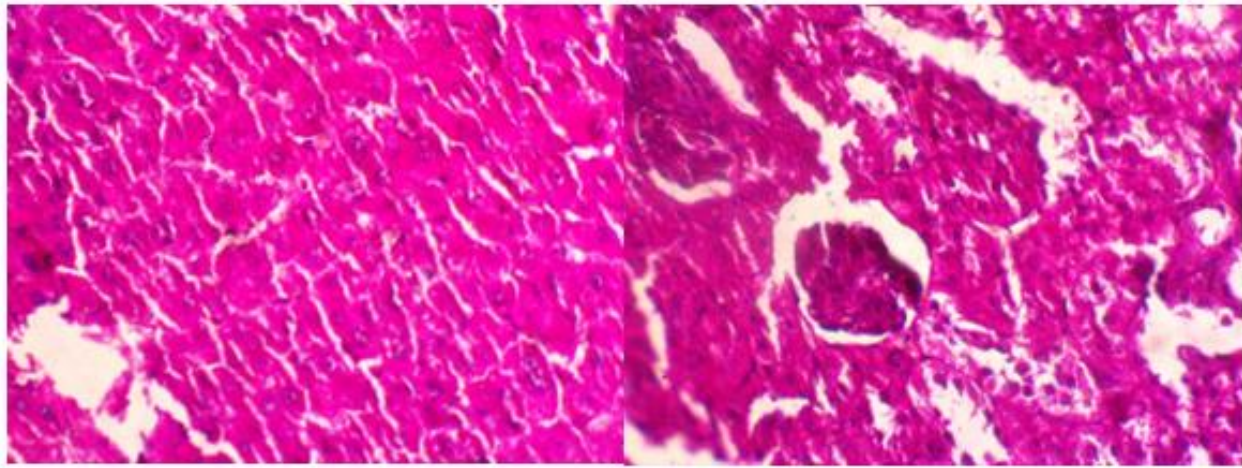
Sample	LIVER	KIDNEY
Control	No observable lesion	No observable lesion
BKR1	(a) diffuse hepatocellular atrophy (b) Moderate diffuse hepatocellular degeneration	there is mild tubulaepithelial necrosis
BKR2	(a) Mild cord atrophy	multifocal degeneration and necrosis of tubular epitheliacells.
BKR3	(a) severe centrilobular degeneration and necrosis of hepatocytes and marked cord atrophy (b) severe centrilobular degeneration and necrosis of hepatocytes.	no observable lesion
KNR1	(a) centrilobular degeneration and necrosis of hepatocytes (b) Diffuse hepatocellular degeneration and necrosis	no observable lesion
KNR2	(a) periportal to diffuse hydropic degeneration and coagulation necrosis of hepatocytes. (b) Multifocal hepatocellular degeneration and atrophy. (c) Diffuse hepatocellular atrophy	no observable lesion
KNR3	(a) mild hepatocellular atrophy	
CBR1	(a) centrilobular to multifocal coagulation necrosis of hepatocytes (b) a few Kupffer cells in the sinusoids (c) centrilobular atrophy of hepatocytes	no observable lesion

CBR2	(a) diffuse vacuolar degeneration (b) coagulation necrosis of hepatocytes with foci of inflammatory infiltrates.	no observable lesion
CBR3	(a) diffuse severe hepatocellular degeneration and atrophy	no observable lesion

Table 3: Effects of the two plants on the vital organs of the rats

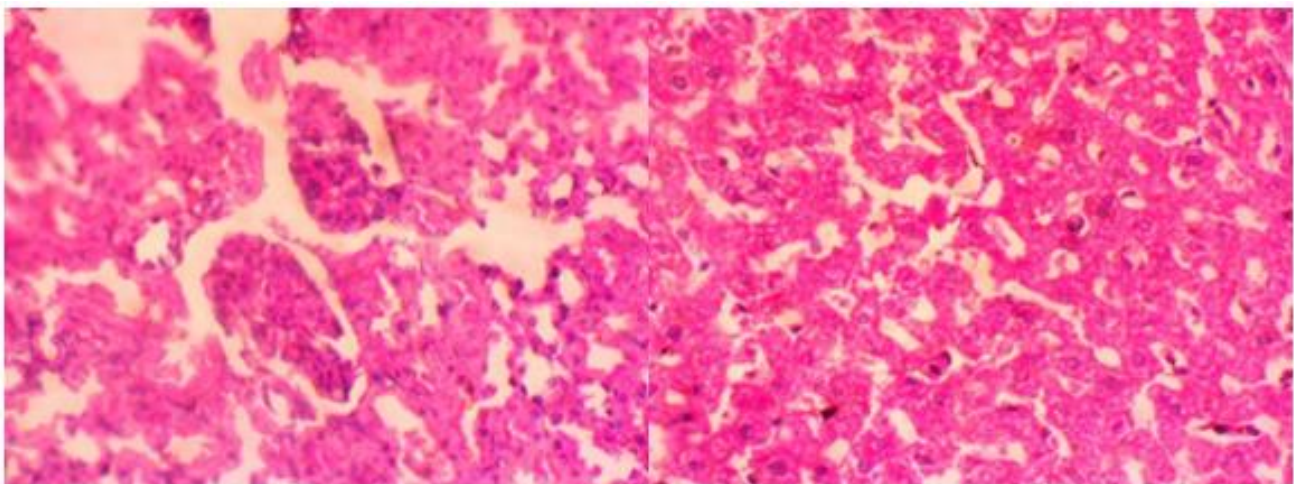
The table above shows the observed effects of the treatments on the liver and kidney tissue of the rats. The significant effects of G.kola on the vital organs of the tested rats could be attributed to the presence of

anthraquinone glycoside as also reported by Hassan et al., (2007) that its presence cause adverse effects on liver and kidney in Ficussycomorus extracts.



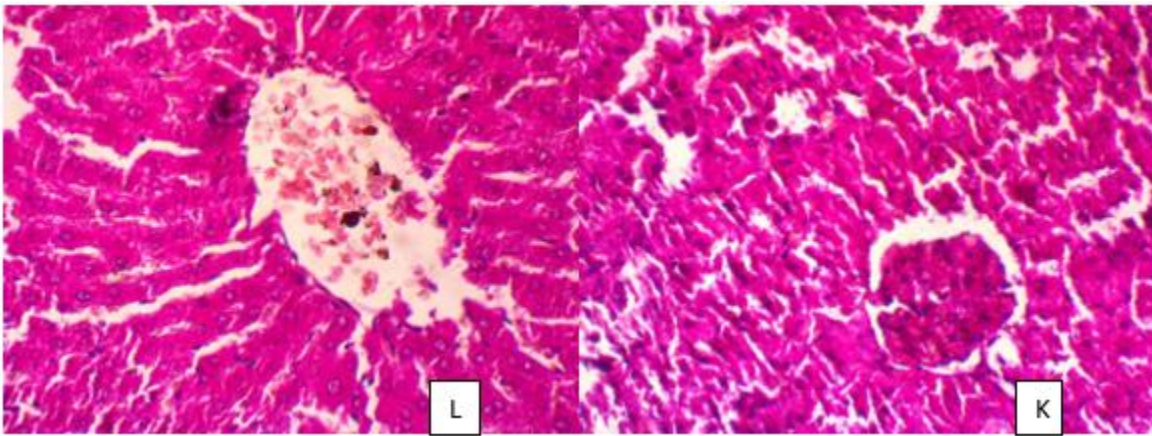
LIVER- no observable lesion
KIDNEY- no observable lesion

Figure 1. Control



KIDNEY- no observable lesion

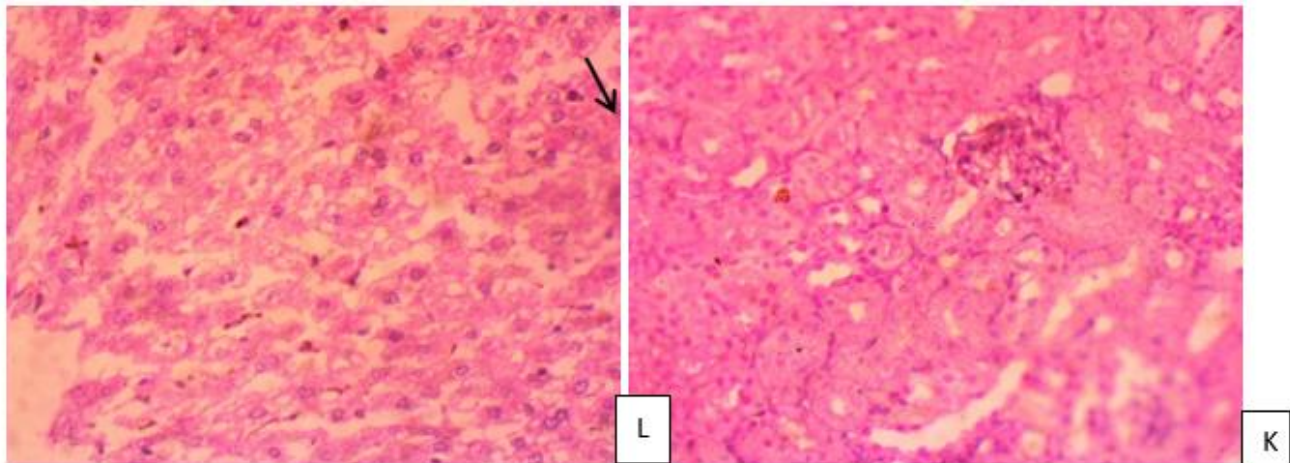
LIVER- there is centrilobular to multifocal coagulation necrosis of hepatocytes and a few Kupffer cells in the sinusoids



LIVER- there is centrilobular atrophy of hepatocytes

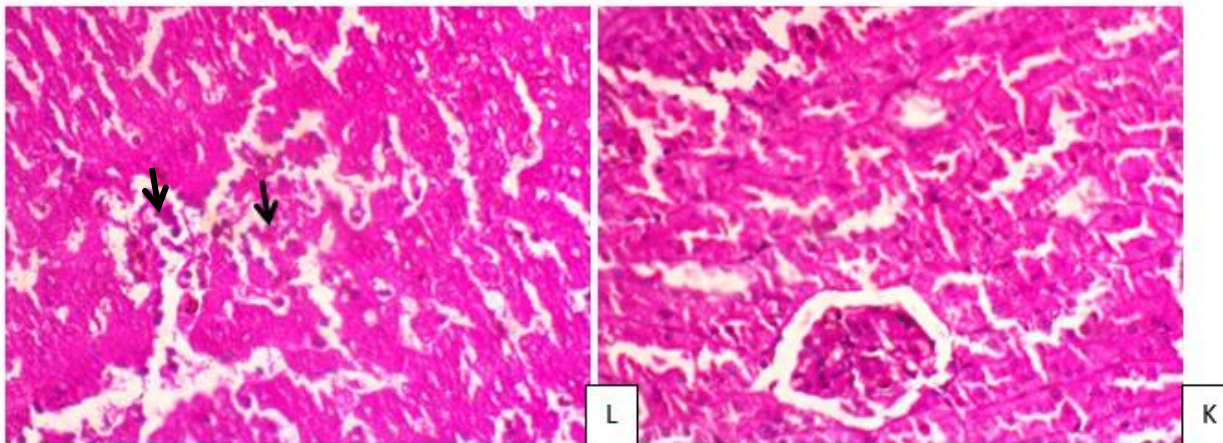
KIDNEY- no observable lesion

Figure 2. CBR1



LIVER- there is diffuse vacuolar degeneration and coagulation necrosis of hepatocytes (arrow)

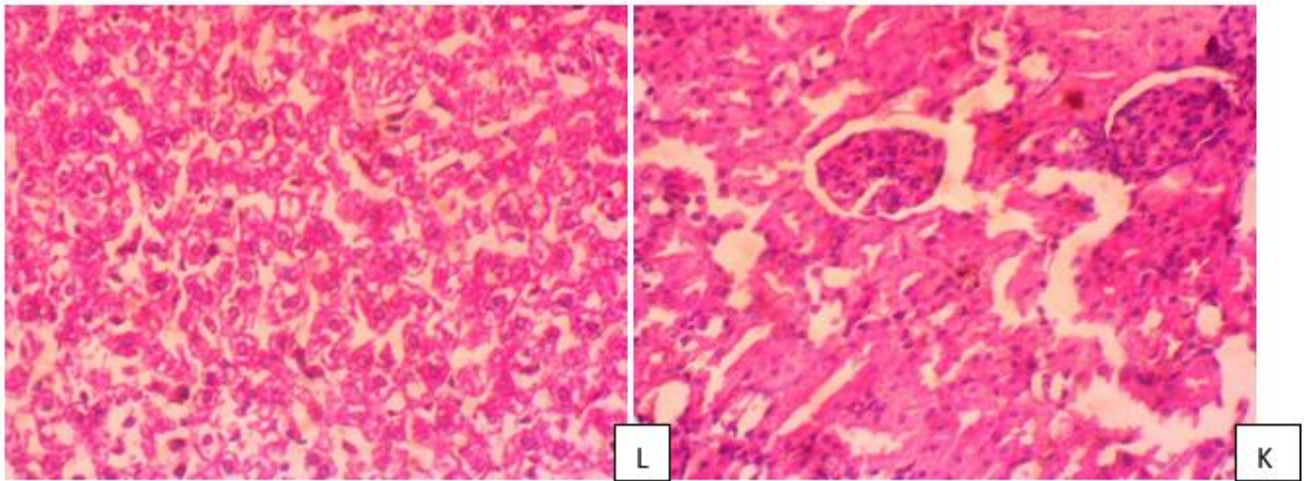
KIDNEY- no observable lesion



LIVER- there is diffuse vacuolar degeneration and coagulation necrosis of hepatocytes with foci of inflammatory infiltrates (arrow)

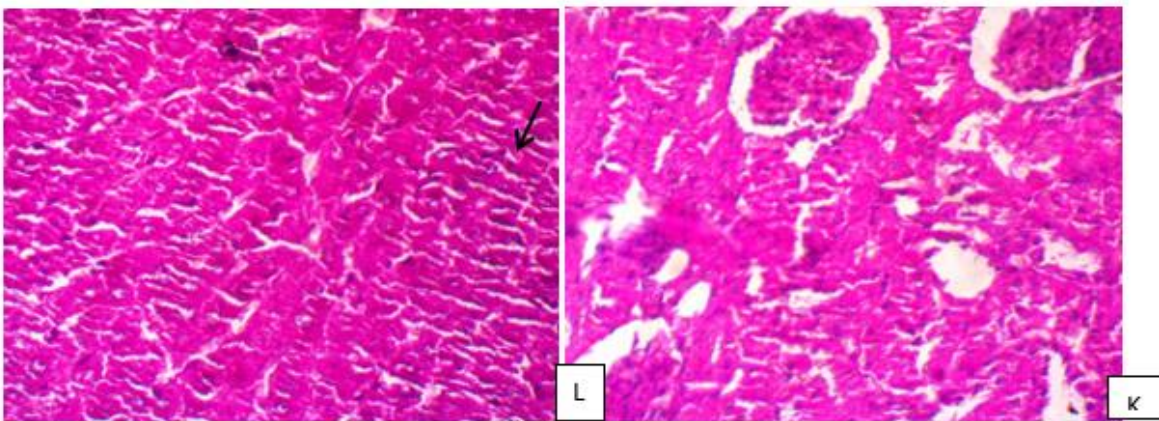
KIDNEY- no observable lesion

Figure 3: CBR2



LIVER- diffuse severe hepatocellular necrosis and atrophy

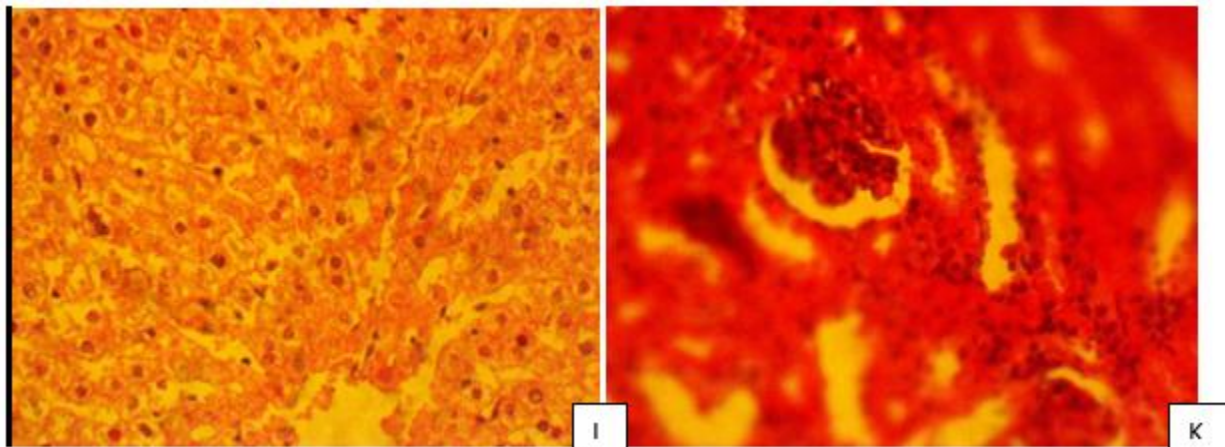
KIDNEY- no observable lesion



LIVER- diffuse severe hepatocellular necrosis (arrow)

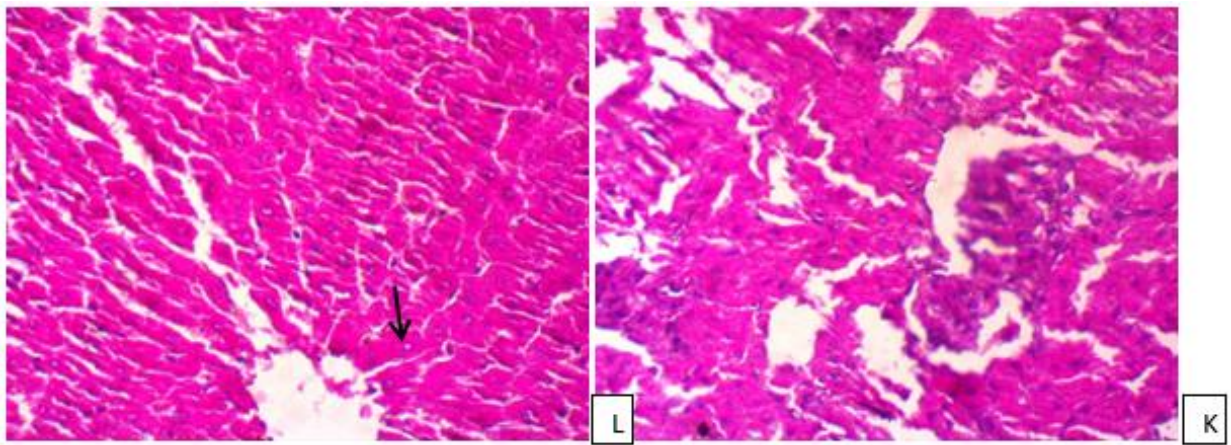
KIDNEY- no observable lesion

Figure 4: CBR3



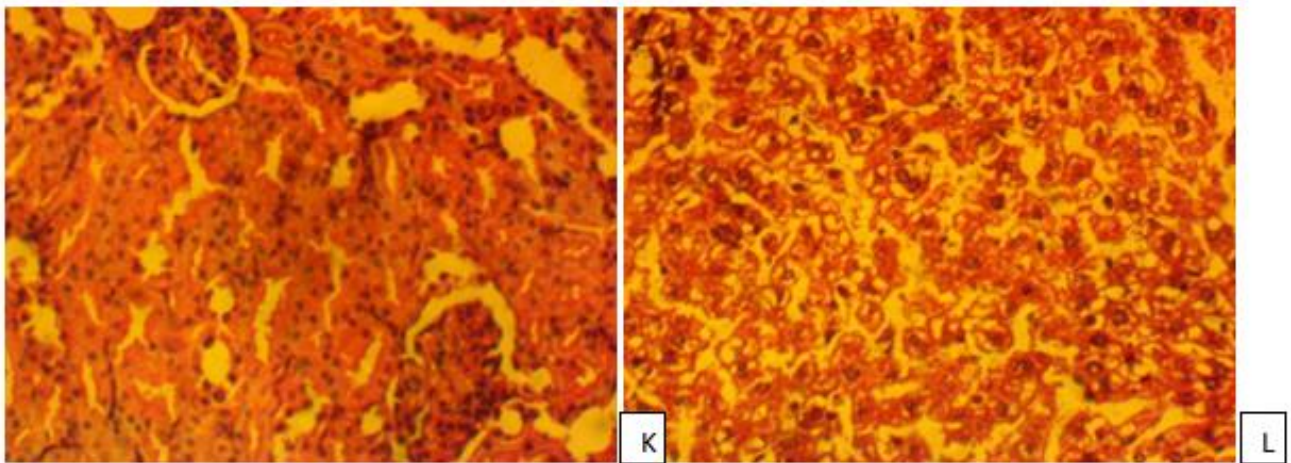
LIVER- there is centrilobular degeneration and necrosis of hepatocytes

KIDNEY- no observable lesion

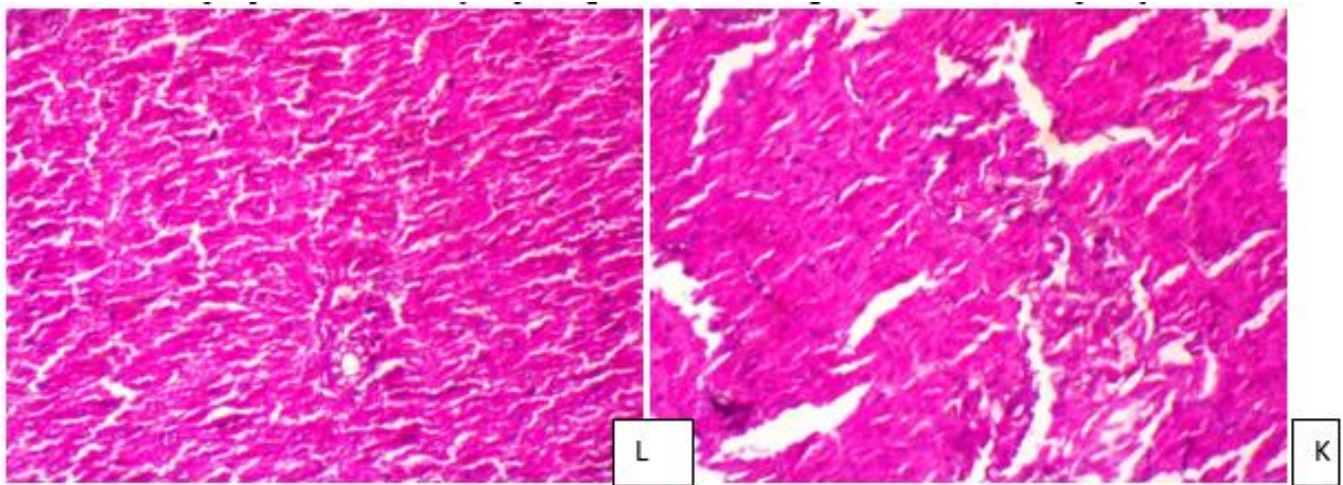


LIVER- there is centrilobular degeneration and necrosis of hepatocytes (arrow)
KIDNEY- no observable lesion

Figure 5: KNR1

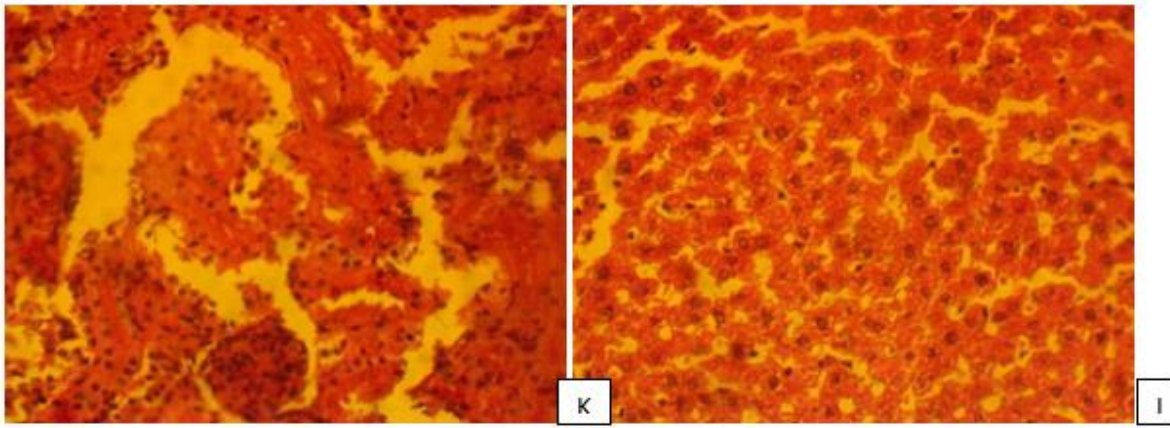


KIDNEY- No observable lesion
LIVER- there is periportal to diffuse hydropic degeneration and coagulation necrosis of hepatocytes.



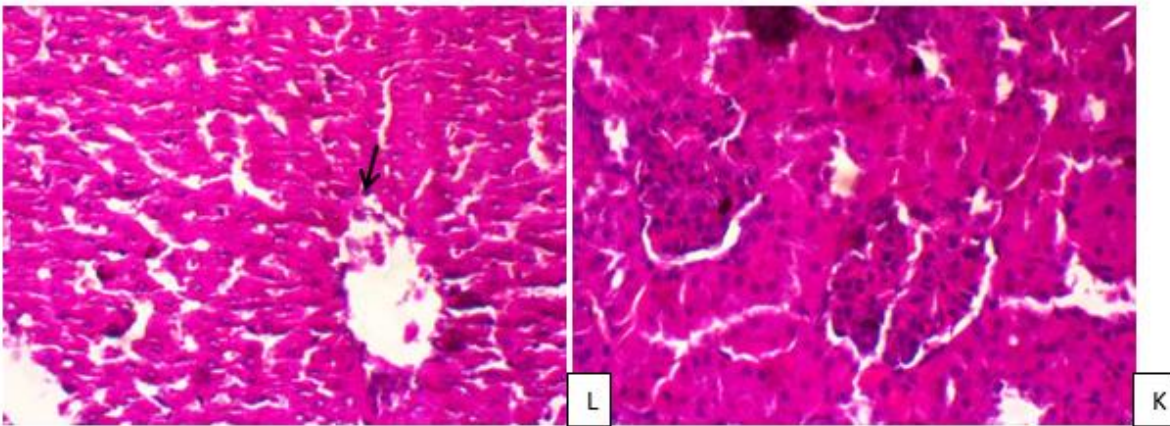
LIVER- there is diffuse atrophy and necrosis of hepatocytes.
KIDNEY- No observable lesion

Figure 6: KNR2



KIDNEY- no observable lesion

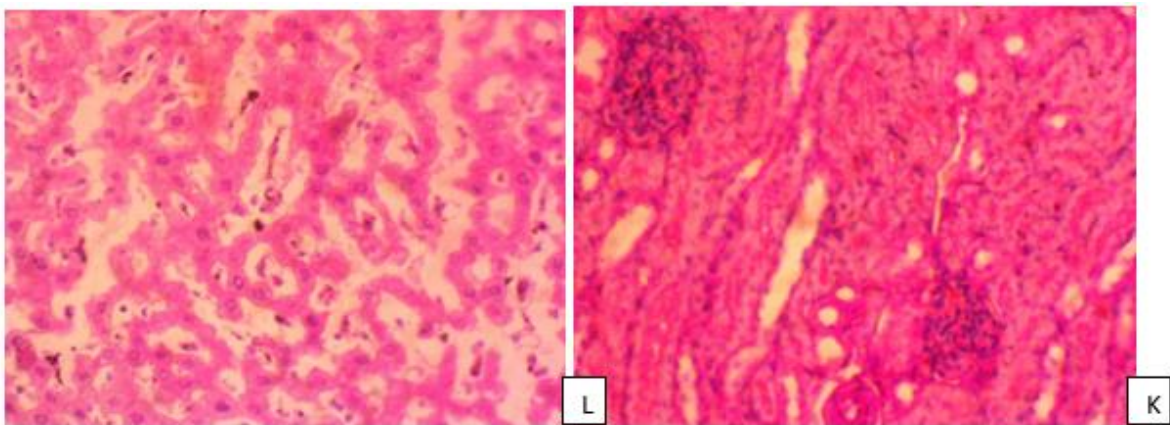
LIVER- there is mild hepatocellular atrophy



KIDNEY- no observable lesion

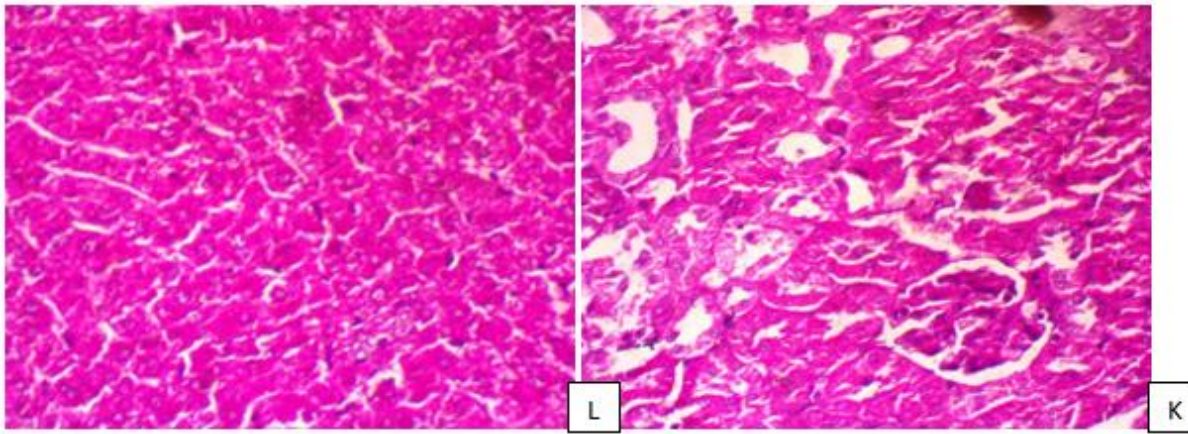
LIVER- there is mild hepatocellular atrophy (arrow)

Figure 7: KNR3



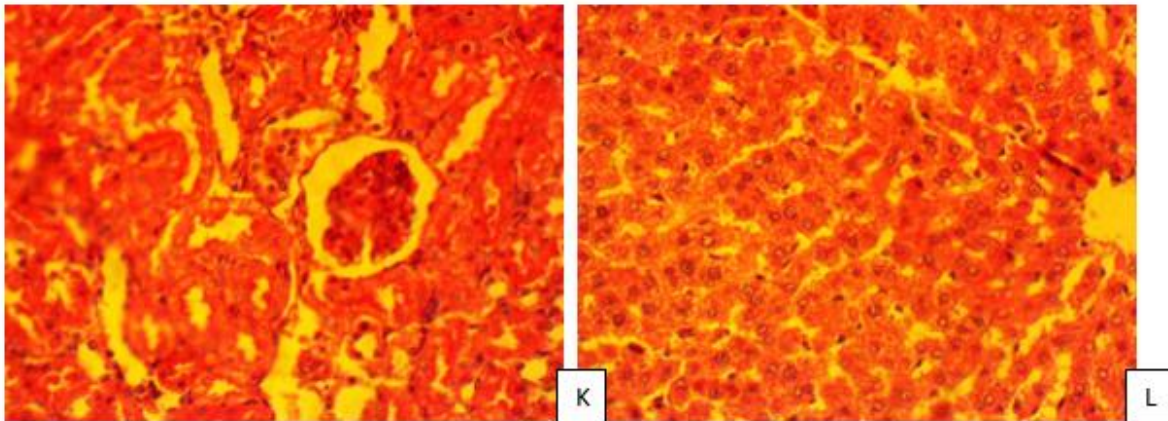
LIVER- there is diffuse hepatocellular atrophy

KIDNEY- no observable lesion

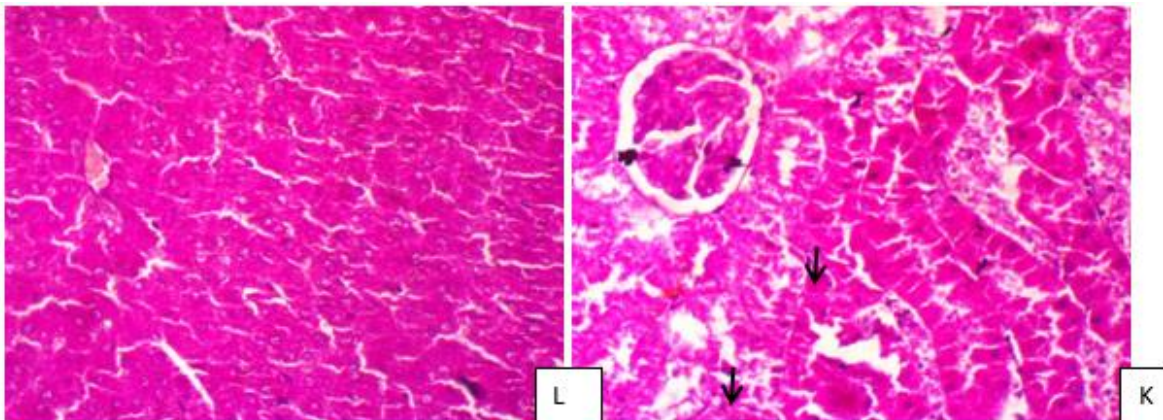


LIVER- there is moderate diffuse hepatocellular degeneration
KIDNEY- there is mild tubular epithelial necrosis

Figure 8: BKR1

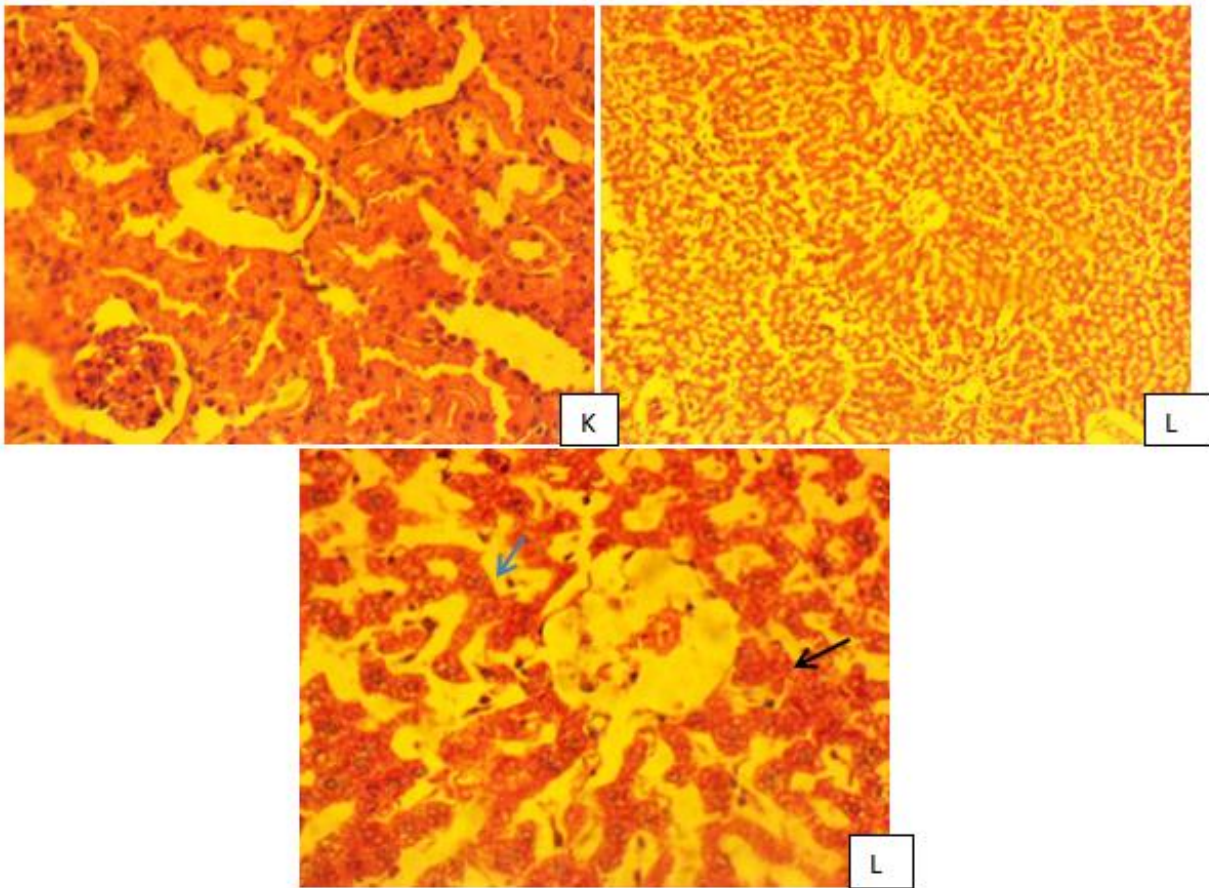


KIDNEY- no observable lesion
LIVER- no observable lesion



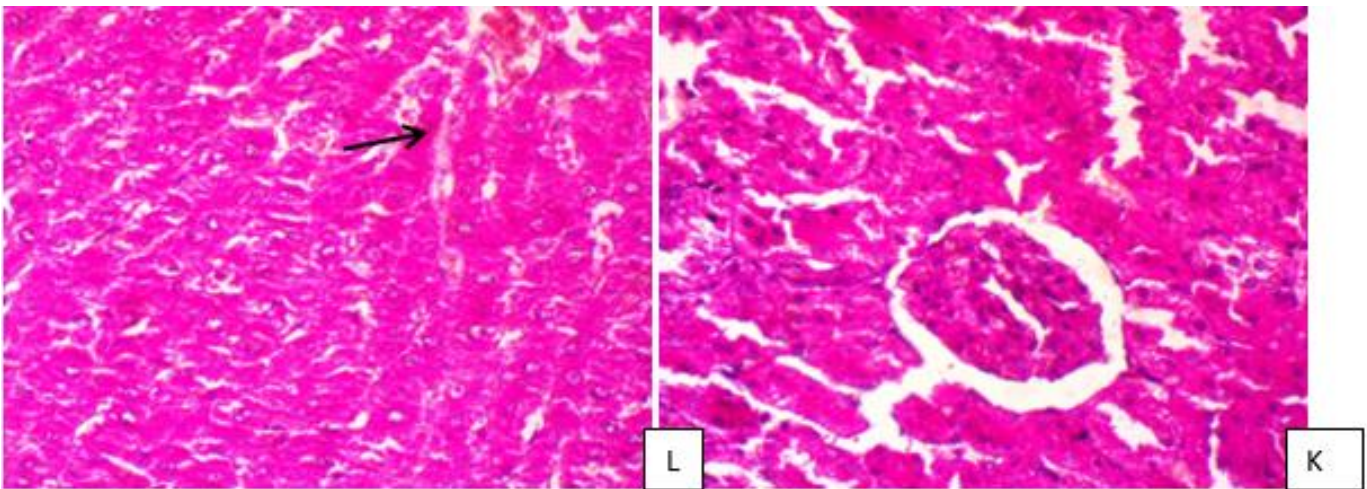
LIVER- no observable lesion
KIDNEY- multifocal degeneration and necrosis of tubular epithelial cells (arrow)

Figure 9: BKR2



KIDNEY- no observable lesion

LIVER- severe centrilobular degeneration and necrosis of hepatocytes (black arrow) and marked cord atrophy (blue arrow)



LIVER- severe centrilobular degeneration and necrosis of hepatocytes (arrow)

KIDNEY- no observable lesion

Figure10: BKR3

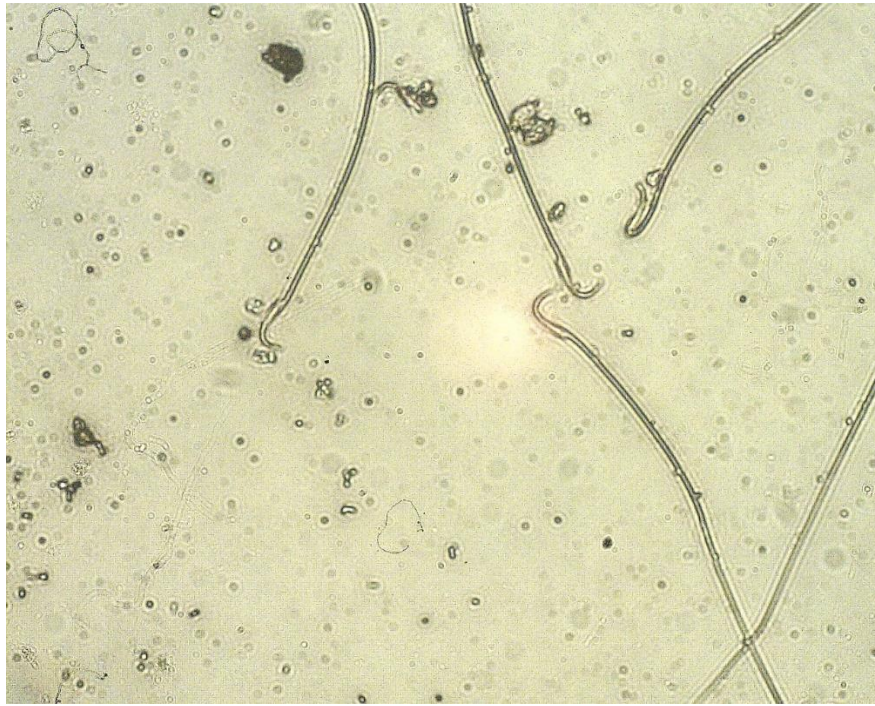


Figure 11: Normal sperm sample



Figure 12: Bent sperm tail

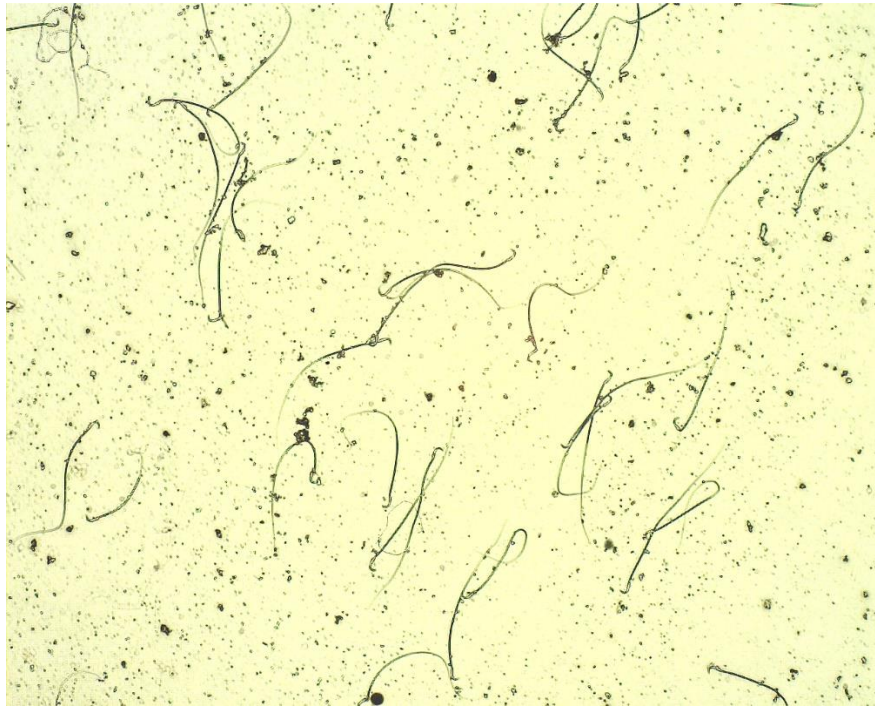


Figure 13: Headless sperm

Conclusion

The analysis showed significant changes on the tissues most especially the liver. On the testes, there were less morphological abnormalities. Hence, the morphological effect of *G. cola* and *C. nitida* on male rat organs proved that, there were negative effects on the tested organs which may impair reproductive function. These suggested that the seeds action relating to increase in blood cell lines may be effected through an influence on the stimulant cytokine erythropoietin.

Wistar rat is currently one of the most popular rats used for laboratory research. It is characterized by its wide head, long ears, and having a tail length that is always less than its body length. The Sprague Dawley rat and Long-Evans rats were developed from Wistar rats. Wistar rats are more active than others like Sprague Dawley rats. The spontaneously hypertensive rat and the Lewis rat are other well-known stocks developed from Wistar rats (Saad et al., 2009).

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There is endless need for budding scientists to continue to explore novel medicinal and microbial importance of these seeds, thus this project research presents and exhaustive antimicrobial analysis and effects of *Garcinia kola* and *Cola nitida* on male rat organs (Kidney, Liver and Testes).

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