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Full Length Research Paper

Acute and sub-acute toxicities of hamegonorrhea, a herbal gonorrhea mixture

***Otimenyin, S. O. +Olorunfemi, P.O., Sabo, Y, S., and Edache J. N.**

*Department of Pharmacology, +Department of Pharm. Tech., Faculty of Pharmaceutical Science, University of Jos, Plateau State, Nigeria.
*Corresponding Authors E-mail: sundayotimenyin@gmail.com

Abstract

Hamegonorrhea herbal preparation is used in Nigeria, West Africa, for the management of gonorrhea and related infection. The safety profile of Hamegonorrhea was studied in wistar rats. Safety profiles were investigated by determining the acute and sub-acute toxicity of Hamegonorrhea. Oral LD_{50} obtained from this study revealed that the herbal preparation is relatively safe at 5000 mg/Kg. Animals subjected to daily doses of this Hamegonorrhea for 28 days did not show any sign of toxicity or death. The heamatological indices obtained revealed that the PCV, clotting time, blood cells counts were not significantly altered during the treatment period. Liver enzymes, ALT, AST, and ALP were not affected by the medicinal plant preparation. Kidney function, which was evaluated using creatinine clearance and histology was found to be preserved. The gastro-intestinenal integrity was also preserved after the 28 days treatment period. The result obtained suggests that heamagonnorhea herbal preparation has good safety profile.

Keywords: Liver enzymes, LD50, safety profiles, herbal Toxicity, Hamegonorrhea.

INTRODUCTION

Man used natural products for the management of disease conditions before the advent of orthodox medicine. They did this with the belief that most herbal preparations are safe for human and animal consumption. The ascribed safety is probably due of their belief that most herbal products from natural origin are harmless. Toxic plants, or plants that caused serious complication or death in patients or animals are often discontinued and reserved for hunting wild animals or for war situations. This screening approach has helped in screening medicinal plant for human use before the advent of Science. The general belief now is that harmful plants have been withdrawn from traditional herbal pharmacopeia and that all medicinal plants in the pharmacopeia are harmless. Research into medicinal plants has supported this view to a greater extent, except for the presence of harmful microorganisms that may contaminate the products because of the unhygienic production environment.

Hemagonnorrhea is used for the management of Gonnorrheal infection in Nigeria. The herbal mixture (200ml) is manufactured by Hameko Naturalist Hospital Ltd. (Plot 2 and 3 Morolab street Kagini district, Abuja). The herbal mixture contains six medicinal plants, (*Sesamum Indicum*, *Gossypium hirsithium*, *Linum usitatissiumum*, *Polygonum avlulare*, *Plantago ovata*, and *Panax ginseng*), and Gamma globulin.

Sesamum indicum, also known as Sesame, is used in folk medicine as Hypoglycemic, Liver tonic, tinnitus remedy and in the management of Amenorrhea, Dysmenorrhea, Burn, Cholera, Constipation, Cough, Dysentery, and Gonorrhea. It is reported to have abortificient properties, and to contain acethylcholine like substance, (Galini *et al.,* 1992). Scientifically, most pharmacological studies on *Sesamum indicum* seed has reported hypoglycemic effect, (Nakano and Kwak, 2006), hepatoprotective effect, (Munish, K., 2011) antitumor effect, (Xuh and Pio 2003), antiestrogenic activity, (Lee *et al.,* 2005), antihypertensive effect, (Lee *et al.,* 2005; Munish, 2011), and increases vitamin E concentration with out use of vitamin E supplements (Lee and Chen, 2004; Munish, 2011),

Gossypium Hirsitum, is commonly known as upland cotton, it contain low levels of gossypol, a compound that

has been reported to cause male infertility, (Angela *et al., 2005*). Gossypol has also been shown to have *in vitro* and *in vivo* inhibitory activities against diverse pathogenic agents, such as *Trypanosoma cruzi,* (Montamat *et al*., 1982; Abe et al., 2004), *Plasmodium falciparum,* (Royer *et al*., 1986; Tripathi *et al*., 2004), *Entamoeba histolytica,* (González-Garza *et al.,* 1989), *Trichomonas vaginalis,* (González-Garza *et al*., 1995), *Giardia lamblia,* (Mata-Cárdenas *et al*., 1998), *Taenia taemiaeformis,* (Rikihisa *et al*., 1990), *Edwardsiella ictaluri,* (Yildirim-Aksoy *et al*., 2004), Herpes simplex virus II, (Radolf *et al.,* 1986), and the Human Immunodeficiency Virus, (Royer *et al*., 1991).

Linum usitatissiumun is locally known as Flax, or linseed. It is used for the management of constipation, hypercholesteronemia, arthritis, hot flushes and pain. It is claimed to have laxative and antimicrobial activity, especially against gonococci species, (Blumenthal *et al*., 2000).

Plantago ovate is known locally in most part of the world as Desert Indian wheat, or Blond Psyllium. It is reported to have hypocholesterolaemic action, (Ganji, and Kies, 1994), decrease plasma level of LDL-C and apolipoprotein B, (Moreyra *et al*., 2005), uric acid, total and LDL cholesterol, (Sierra *et al*., 2002), reduce fat deposition (a known side effect of some anti retroviral drugs) in HIV patients, (Hendricks *et al.,* 2003), lower blood pressure in hypertensive patients, (Burke *et al*., 2001), and decrease Glucose absorption, (Moreyra *et al*., 2005).

Polygonum avlulare is commonly known as Knot-grass. It has anti oxidant, (Chin-yuan, 2006), antiobesity, (Sung, 2013), antimicrobial, (Hediat *et al.,* 2010), anti-inflammatory, (González, 2001), and anticancer activities, (Habibi, 2013). Scientific investigations showed that it increase the vitality and motility of sperm cells, (Milan *et al*., 2011), heals gingivitis, (González, 2001), and inhibit proliferation and apoptotic gene expression of breast cancer cell line, (Habibi *et al.,* 2011).

Panax Ginseng is commonly called Man root, root of immortality, Tartar root and life root. It is used in Aromatherapy and as essential oil for the management of body weakness or fatigue, impotence, as well as an immune booster. It has been reported to have Antibacterial, antioxidant, radiopretective activities. it is stimulates learning, memory, and physical capabilities, radioprotection,

This work seeks to investigate and ascertain the safety profile of the herbal gonorrhea mixture (hamegonorrhoea).

MATERIALS AND METHOD

The Kit test methods was used for biochemical assay, kits used include; urea test kit (fortress diagnostics), creatinine test kit (fortress diagnostics), AST test kit (fortress diagnostics), ALT test kit (fortress diagnostics), protein test kit (fortress diagnostics), sodium test kit (linear chemicals, s. l.), calcium test kit (linear chemicals, s.l.), potassium test kit (fortress diagnostics), triglycerides test kit (fortress diagnostics), and albumin test kit (rx monza). The Kits were used are described by their manufacturer.

Collection of herbal preparation

Freshly prepared herbal product (Hamegonorrhea) was obtained from Hameko naturalist Hospital LTD in 2010 by Mr. Patrick O. Olurunfemi of the Department of Pharmaceutical Microbiology, University of Jos, Jos, Plateau State, Nigeria. Hamegonorrhea was stored in the laboratory cabinet till use. Composition of Hamegonorrhea was obtained from the manufacturer and compared with listed contents on the product label. The product contains eight (6) plants, gamma globulin and water.

Animals: Male wistar albino rats (160 - 230 g) obtained from the animal house of the Department of Pharmacology University of Jos was used for this study. The rats were fed on standard laboratory diet, given water *ad libitum* and maintained under laboratory conditions of temperature 28 ± 1°C, relative humidity 11 ± 1% and 12 h light and 12 h dark cycle.

Acute toxicity (LD50) study

Acute toxicity study was evaluated using the method describe by Lorke (1983) as described by Otimenyin *et al.,* 2013. Daily for fourteen days, the rats were observed for signs of toxicity, which include but not limited to paw-licking, salivation, stretching on the floor and wall of cage, and death.

Sub-acute toxicity study

Sub-acute toxicity study was evaluated using the method described by Otimenyin *et al*., (2010). Group of 5 male rats received Hamegonorrhea (1000 mg/Kg) orally daily for 28 days and the second group (control) of 5 male rats were given 10 ml/kg of distilled water daily for 28 days. Daily body weights of the rats were monitored during the period of administration. After 28 days, animals were allowed to fast overnight, anaesthetized with petroleum ether and blood samples were collected from the rats via cardiac puncture into heparinized tubes for hematological analysis and non-heparinized centrifuge tubes for biochemical analysis. The animals were then sacrificed and liver, kidneys, lungs, testis, stomach and heart isolated, weighed and preserved in 40 % formalin for histopathological studies.

Haematological analysis

Haematological analysis was carried out using standard physiological methods, (Rinder and Dabieh, (1979), as briefly described by Otimenyin, *et al.,* 2013.

Biochemical Studies

Blood samples were collected by cardiac puncture with the aid of syringe, transferred into centrifuge bottle, and centrifuged at 4000 rpm for 15 minutes until the serum was petitioned from blood cells. The serum was separated and stored in the refrigerator till use. Biochemical assay were carried out using kit methods, (Otimenyin *et al*., 2010).

Histopathological studies

The Liver, Kidney, Heart, Lungs, Spleen, and Stomach were isolated from sacrificed animals, grossly examined for any pathological changes and then fixed in 10 % saline for 5 days at room temperature. The method described by Otimenyin *et al.,* 2013, was used to obtain slides of the different organs for examination under the microscope.

Statistical analysis

Results were expressed as mean ± SEM. Statistical analysis of data was carried out using one –way analysis of variance and students T- test. Significant differences were determined using a Student's t-test and the differences were considered significant if $p < 0.05$.

RESULTS

Acute toxicity studies

The LD_{50} was found to be greater than 5000 mg/kg

Sub-acute toxicity studies

Table 1. Effect of herbal blood tonic mixture (Hamegonorrhea) on changes in body weight of rat

Days	Control (g)	Hamegonorrhea, 1000 mg/kg
Day 1	0.21±0.12	0.42±0.31
Day 7	1.09±0.53	1.74±0.94
Day 14	1.74 ± 1.03	1.99± 1.83
Day 21	2.05 ± 2.21	2.10 ± 1.53
Day 28	2.93 ± 1.99	2.32 ± 1.38

Values are expressed as mean ± S.E.M. $P < 0.05$ when rats weight on day one was compared to the weight on days 21 and 28.
N= number of animals = 5

Slight increase ($P < 0.05$) in the daily weight of animals treated with Hamegonorrhea was observed. This observation was similar to weight changes observed in control group.

Table 2. Effect of herbal blood tonic mixture (Hamegonorrhea) on weight of internal organ of rats

Parameters	Control	Hamegonorrhea 1000 mg/kg
Heart	0.75±0.064	0.68 ± 0.09
Liver	6.30 ±0.21	6.03 ±0.12
Lungs	1.45 ± 0.22	1.40 ± 0.14
Spleen	0.72 ± 0.62	0.79 ± 0.08
Kidney	0.75 ± 0.95	0.70 ± 0.45
Stomach	2.42 ± 0.24	2.31 ± 1.20

Value are expressed as mean ± S.E.M
N = Number of animals per group = 5
* Significant difference from control $p < 0.05$

The blood tonic mixture (Hamegonorrhea) did not alter the integrity ($P < 0.05$) of internal organs of rats

Table 3. Effect of herbal blood tonic mixture (Hamegonorrhea) on haematological parameters

Parameters	Control	Test (1000 mg/kg)
RBC	4.27 ± 0.22	4.18 ± 0.20
WBC	5.22 ± 0.85	5.41 ± 0.18
PLT	85.75 ± 2.01	81.45 ± 2.71
CT (Mins)	40.00 ± 10.00	44.56 ± 1.28
PCV	51.75 ± 0.47	49.02 ± 1.43
Hb	17.25 ± 0.16	16.55 ± 0.59
NEUt	44.50 ± 0.64	40.30 ± 3.67
LYMP	54.50 ± 1.19	57.31 ± 3.54
EOST	0.50 ± 0.28	0.56 ± 0.43
Mono	0.50 ± 0.28	0.43 ± 0.09

Data are expressed as mean ± S.E.M
N = Number of animals per group = 5
*Significant difference from control $P < 0.05$
RBC = Red blood cells ($x10^6mm^{-3}$)
WBC = White blood cells ($X10^0mm^{-3}$)
PLT = platelet ($X10^3mm^{-3}$)
CT = Clotting time (mins)
PCV = Packed cell volume (%)
Hb = Hemoglobin concentration (gldl)
Neut = Neutrophiles (%)
Lymp = Lymphocyte (%)
Eosi = Eosinophile (%)
Mono = Monocyte (%)
Baso = basophile (%)

Hamegonorrhea significantly ($P > 0.5$) increased the clotting time of the treated animal when compared to control rats (table 3).

Table 4. Effect of Hamegonorrhea on biochemical parameters of rats.

Parameters	Control	Hamegonorrhea 1000 mg/kg
Urea (mg/dl)	38.70 ± 4.53	34.30 ± 5.07
Albumin (gldl)	4.64 ± 0.55	4.67 ± 0.34
Cholesterol (mg/dl)	153.82 ± 7.25	140.30 ± 5.16*
Triglyceride (mg/dl)	70.69 ± 6.52	60.42 ± 3.45*
Creatinine (mg/dl)	0.36 ± 0.27	0.43 ± 0.25
Total protein (g/dl)	12.41 ± 0.35	11.12 ± 0.31

Values are expressed as mean ± S.E.M
N = Number of animals per group
*Significant difference (P>0.05) from control

Hamegonorrhea significantly (P>0.05) reduced blood cholesterol and triglyceride level in rats.

Table 5. Effect of Hamegonorrhea on rat`s liver enzymes

Parameters	Control	Test (mg/kg)
ALT (u/l)	18.33 ± 11.33	24.13 ± 10.34
AST (u/l)	25.66 ± 71.13	26.12 ± 5.09
ALP (u/l)	116.54 ± 71.13	110 ± 39.75

Values are expressed as mean ± S.E.M
N = No. of animal per group
* Significant different from control p<0.05
ALT = Alanine transferase
AST = Aspartate transaminase
ALP = Alkaline Phosphatase

Hamegonorrhea insignificantly (P>0.05) altered liver enzymes.

Table 6. Effect of Hamegonorrhea on body electrolytes

Parameters	Control	Test (1000 mg/kg)
Calcium (mg/dl)	6.08 ± 0.52	7.75 ± 0.38
Sodium (Mequiv/L)	70.45 ± 6.81	70.75 ± 4.15
Potassium (Mequiv/l)	9.35 ± 1.87	10.39 ± 1.46

Values are expressed as mean ± S.E.M
N = No. of animal per group = 5
* Significant different from control p<0.05

There is no significant difference in the electrolytes concentration compare to control.

Effect of hamegonorrhea on histology of internal organs

Gross examination of isolated tissues revealed that there were no significant or detectable pathological abnormalities in all the tissue examined. Signs of pathology were not observed on microscopic examination of the internal organs (i.e kidney, liver, lungs, spleen, stomach and heart) of animals treated with Hamegonorrhea.

HISTOPATHOLOGICAL RESULTS

See figures 1-6 below

DISCUSSION AND CONCLUSION

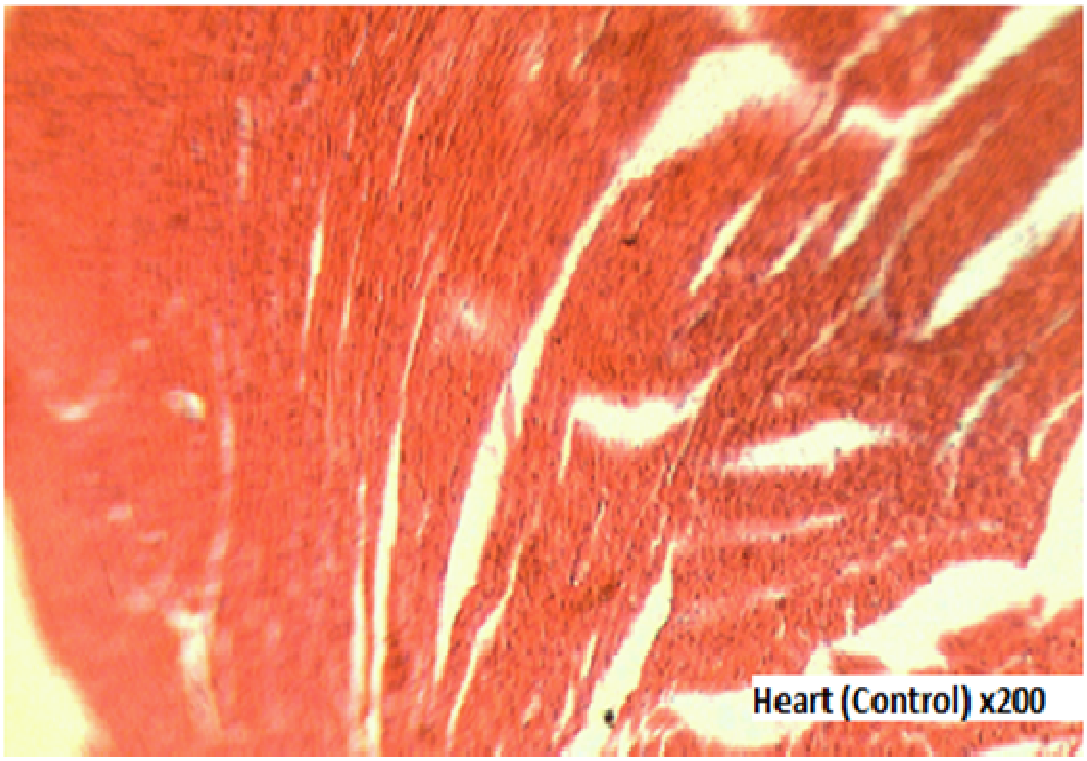
Hamegonorrhea did not produce mortality or significantly altered the behavioral pattern of rats as compared with the control group. Weight gain in treated rats were not significantly different from the control group.

A subacute toxicity study was evaluated because the Nigeria traditional medical practitioners claims that the preparation sold in the local market is safe for chronic treatment of gonorrhea. Some of the constituents of the preparation have been reported to have anti-bacterial activity. For instance, *Gossypium Hirsitum* and *Linum usitatissiumun* are known to be effective against a wide rage of pathogenic organisms, especially gonococci species, (Blumenthal *et al*., 2000, Montamat *et al*., 1982; Abe et al., 2004).

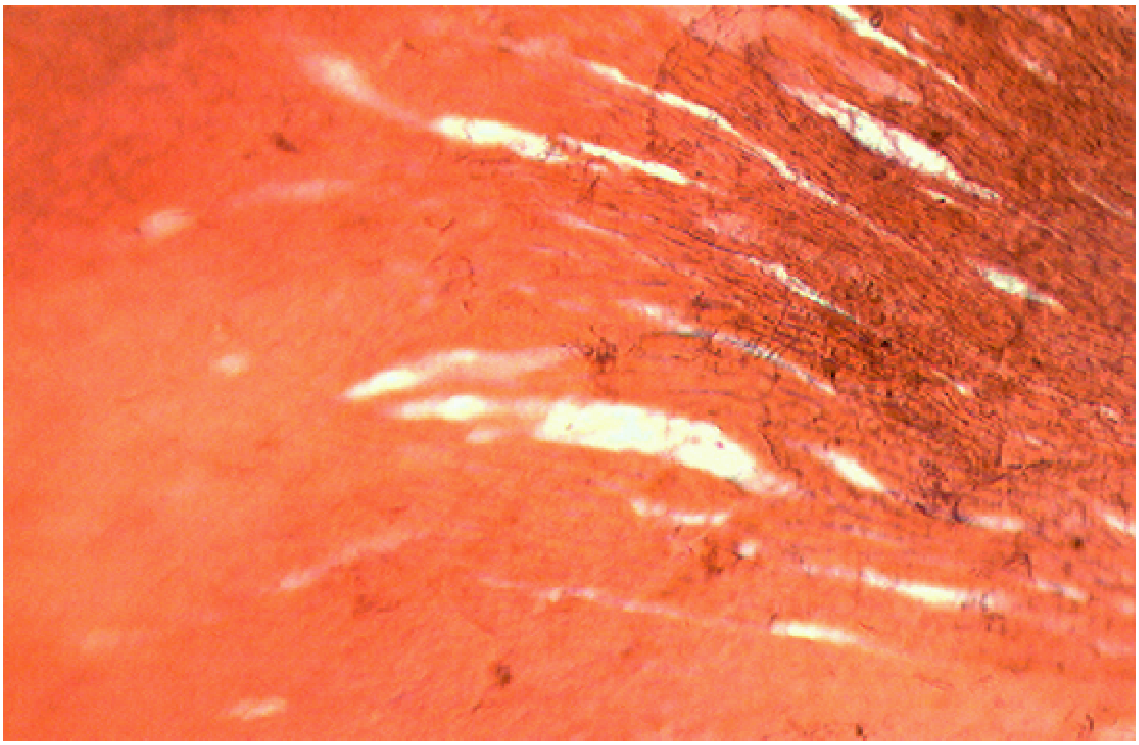
The averagely prescribed daily dose of hamegonorrhea is 500 mg three times a day. Acute toxicity test dose of 5000 mg/Kg (single dose) of the preparation did not produce any toxic effect on the rats used for this study. This implies that a daily dose of 1500 mg (500 mg X 3) is unlikely to produce toxic effects. The preparation is often prescribed for two weeks, but in chronic situations, patients may be advised to continue treatment for another two weeks, making a total of four weeks (28 days). This may result in the accumulation of the extracts if it is not properly disposed from the body. The good safety profile of the preparation was further supported by the results obtained from the sub-acute toxicity study, which revealed that a daily dose of 1000 mg/Kg of the preparation for 28 days did not significantly alter the hematological, biochemical, and histological parameters of the rats used for this study. The cumulative dose of 28,000 mg/Kg was administered to a single rat in the group during the 28 days test period. At this very high dose, the internal organs of the animal were preserved, showing that the animal body system is able to dispose the preparation efficiently, with out compromising the integrity of the organs (liver, kidney, spleen, stomach, heart and lung).

Liver integrity is often determined by measuring the blood levels of ALT, AST, and ALP. Two of these enzymes (AST and ALT) are most often associated with hepatocellular damage, of the two (AST and ALT), only ALT is specific for liver function, because of its presence in noticeable amount in hepatocytes. AST is found in hepatocytes, myocardium, skeletal muscle, brain and kidney (Witthawaskul *et al*., 2003). Its presence in this other cells and tissues makes it not specific for liver

Figure 1. Effect of Hamegonorrhea on heart cells

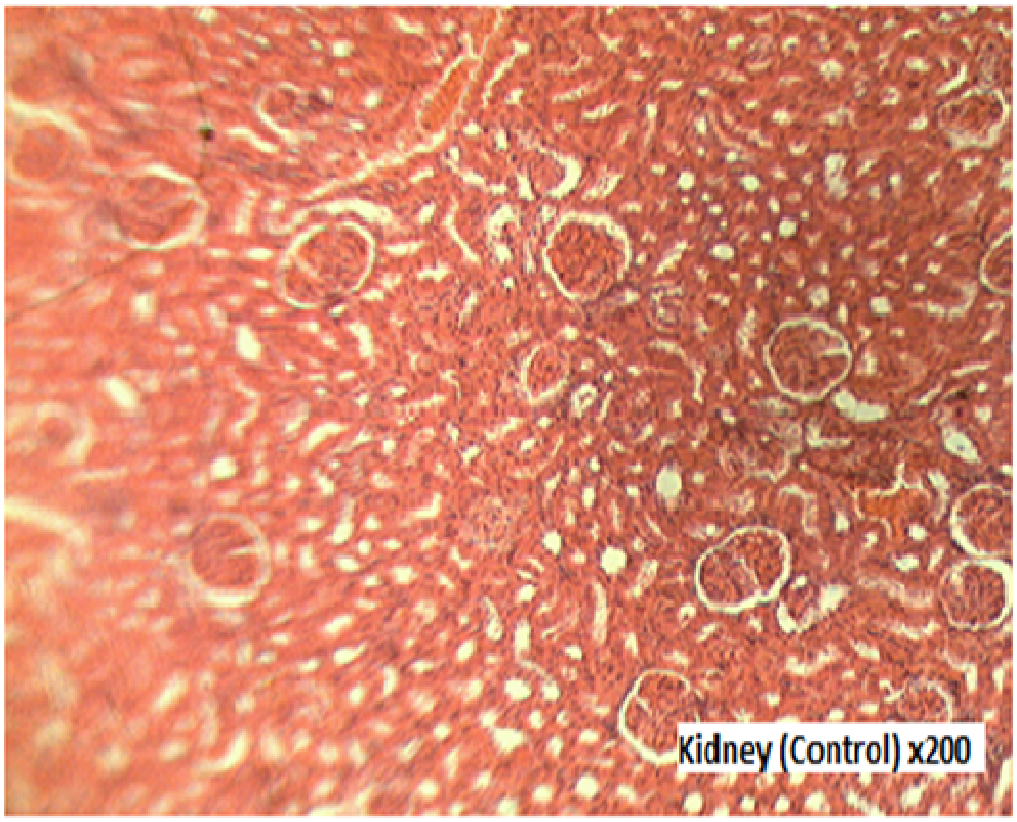


Control group showing normal cells

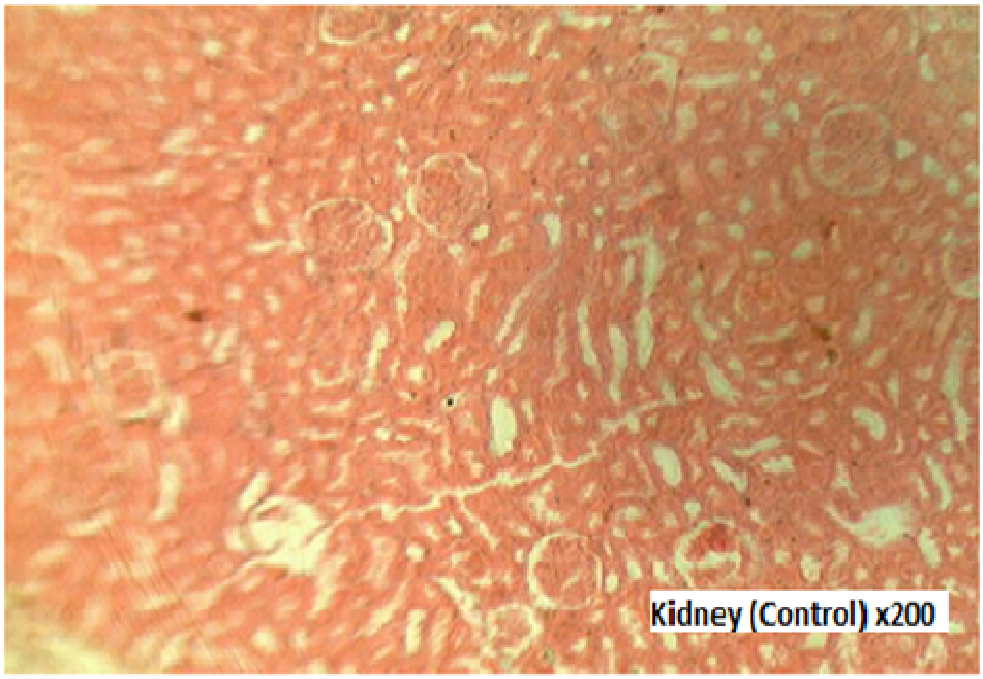


Heart cells of rat treated with Hamegonorrhea (X 200)

Figure 2. Effect of Hamegonorrhea on kidney cells

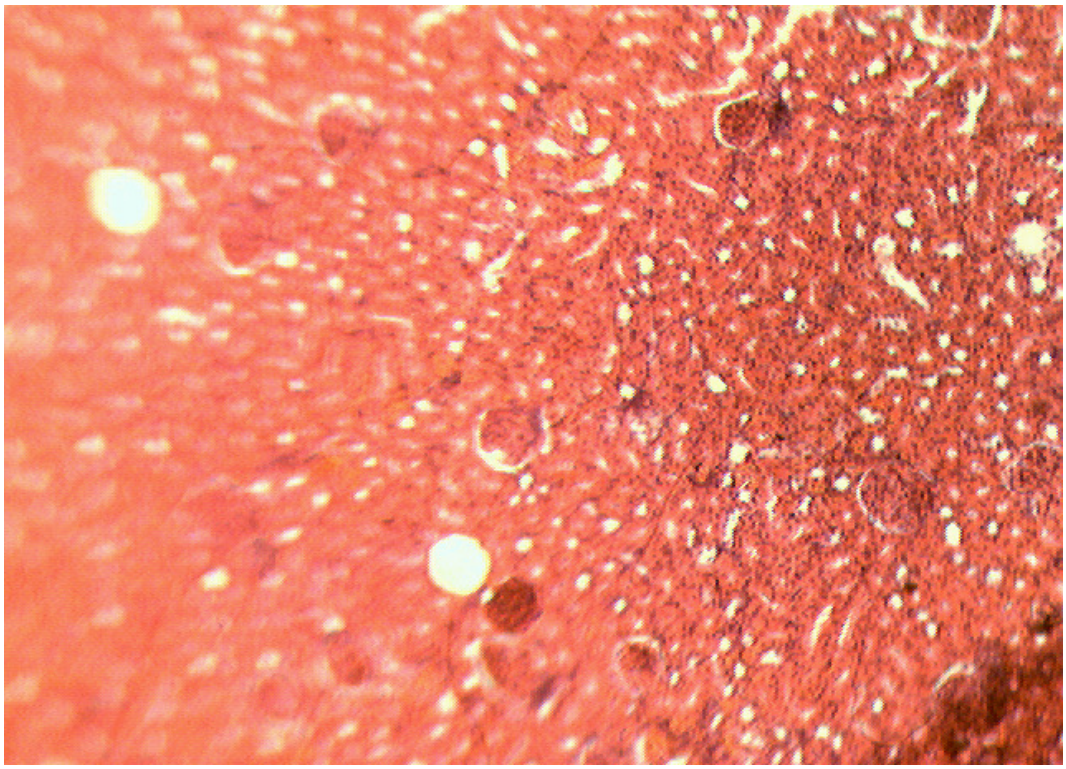


Control group showing normal cells

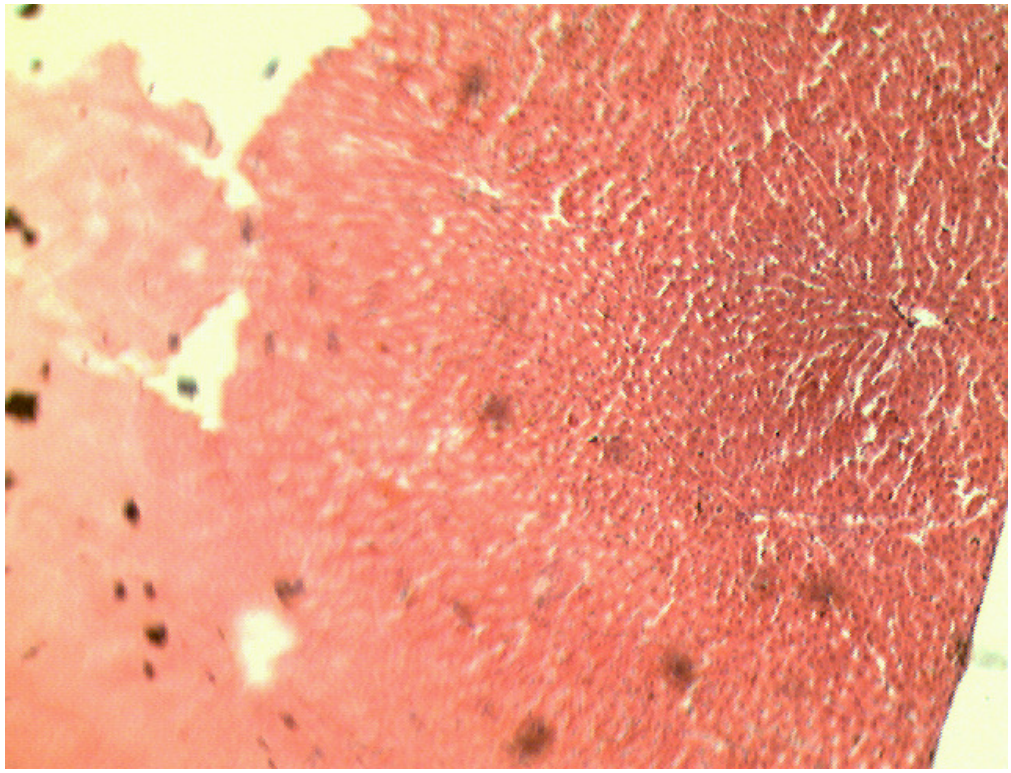


Kidney cells of rat treated with Hamegonorrhea

Figure 3. Effect of Hamegonorrhea on liver cells

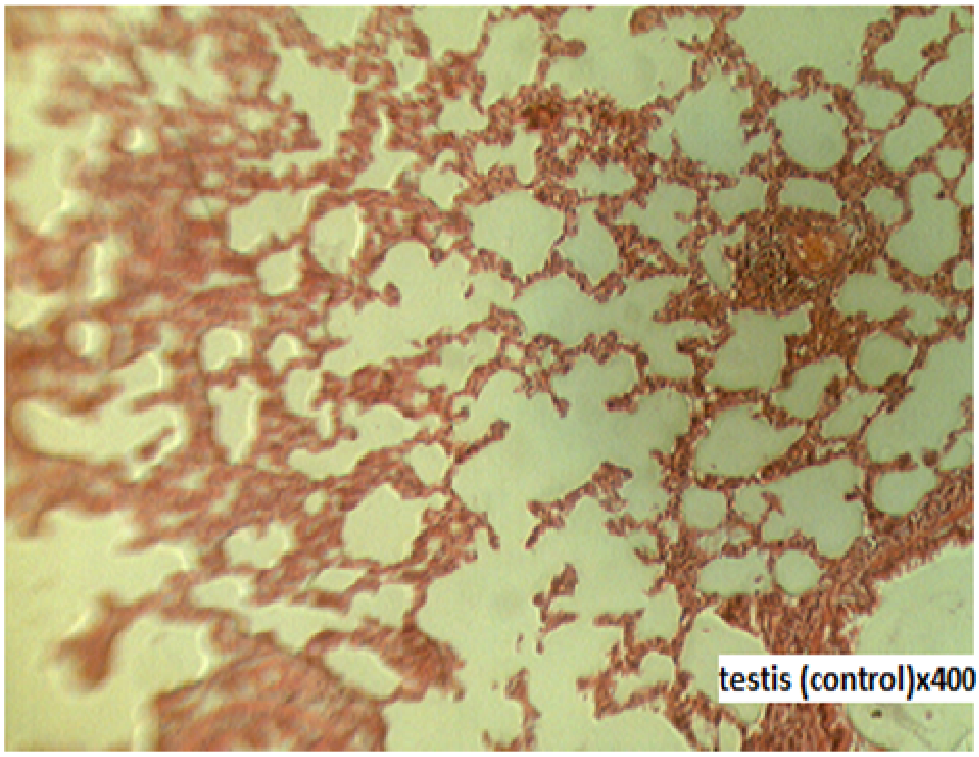


Control group showing normal cells (X 200)

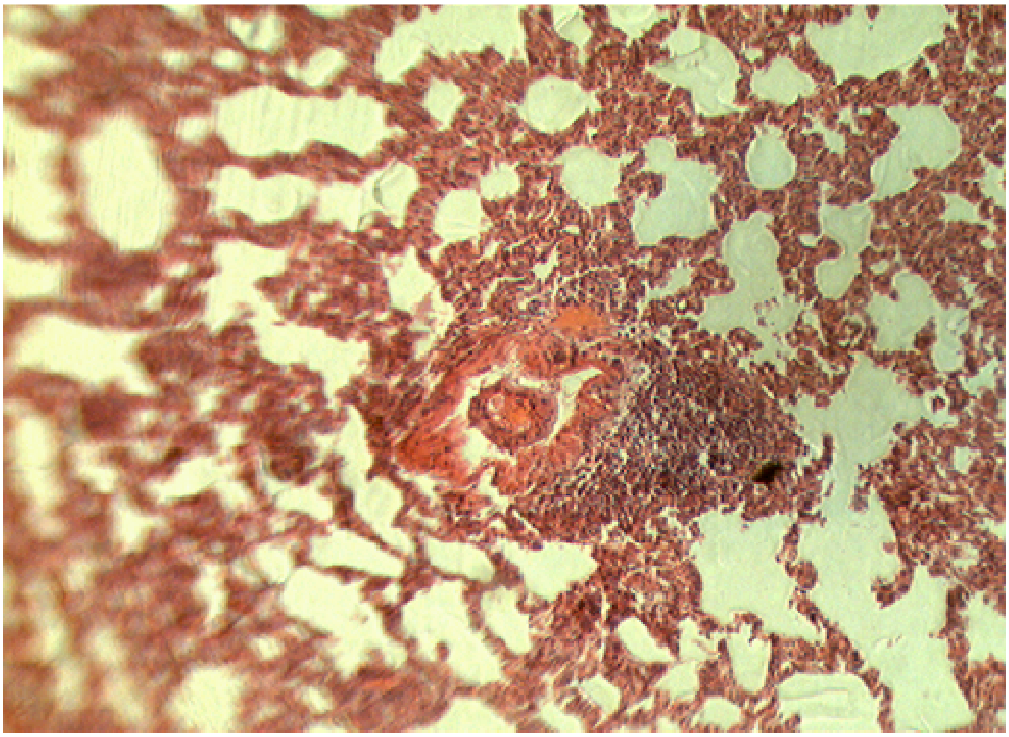


Liver cells of rat treated with Hamegonorrhea (X 200)

Figure 4. Effect of Hamegonorrhea on lungs

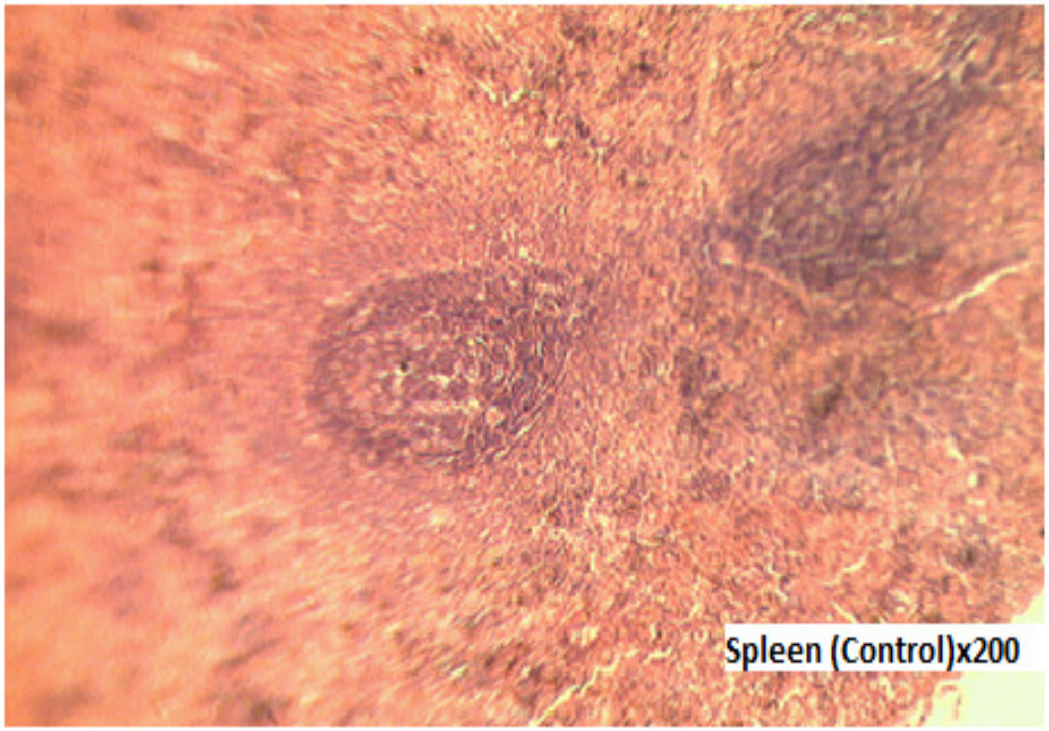


Control group showing normal cells

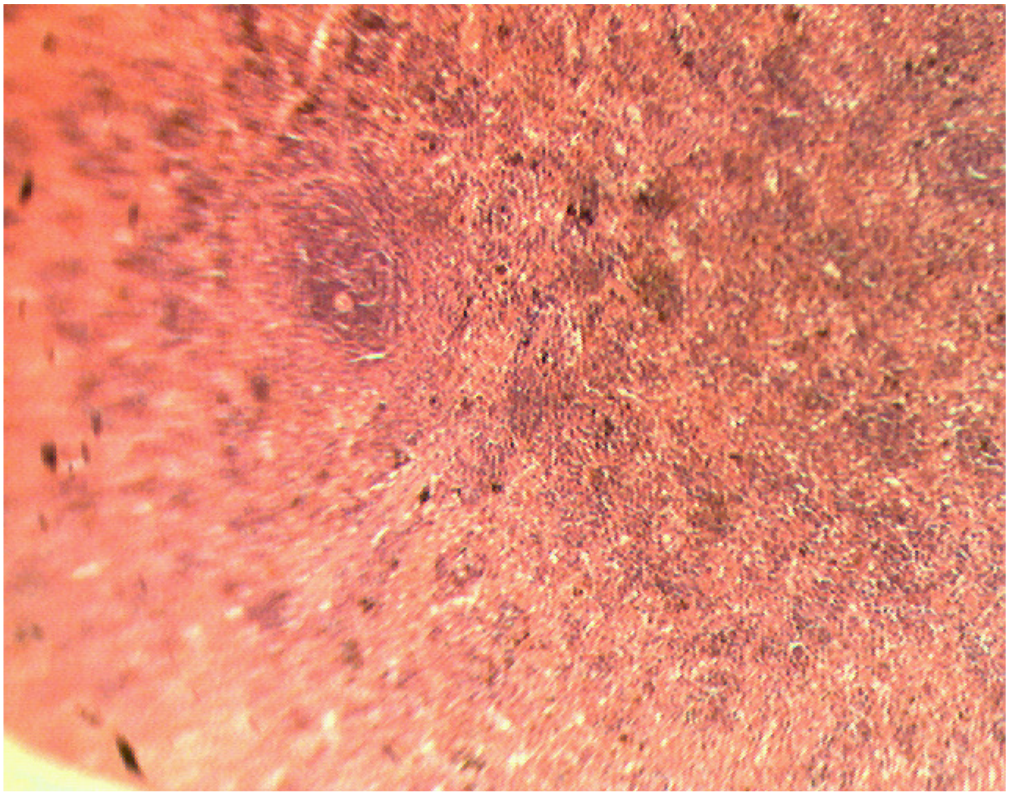


Lungs of rat treated with Hamegonorrhea (X 400)

Figure 5. Effect of Hamegonorrhea on spleen

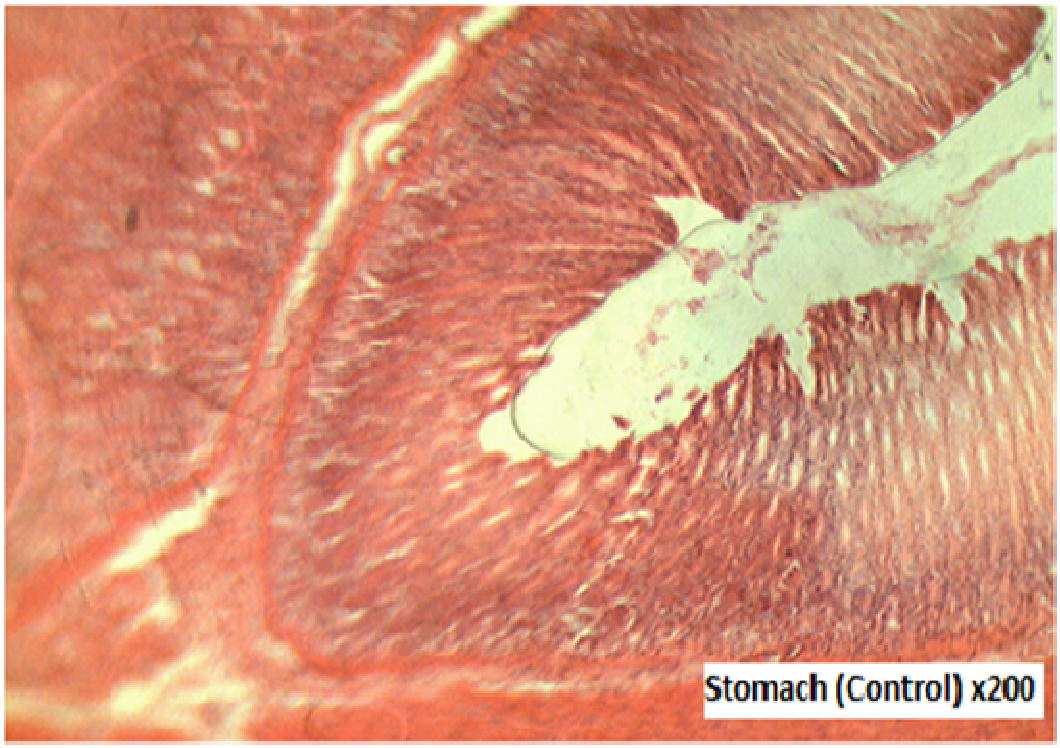


Control group showing normal cells

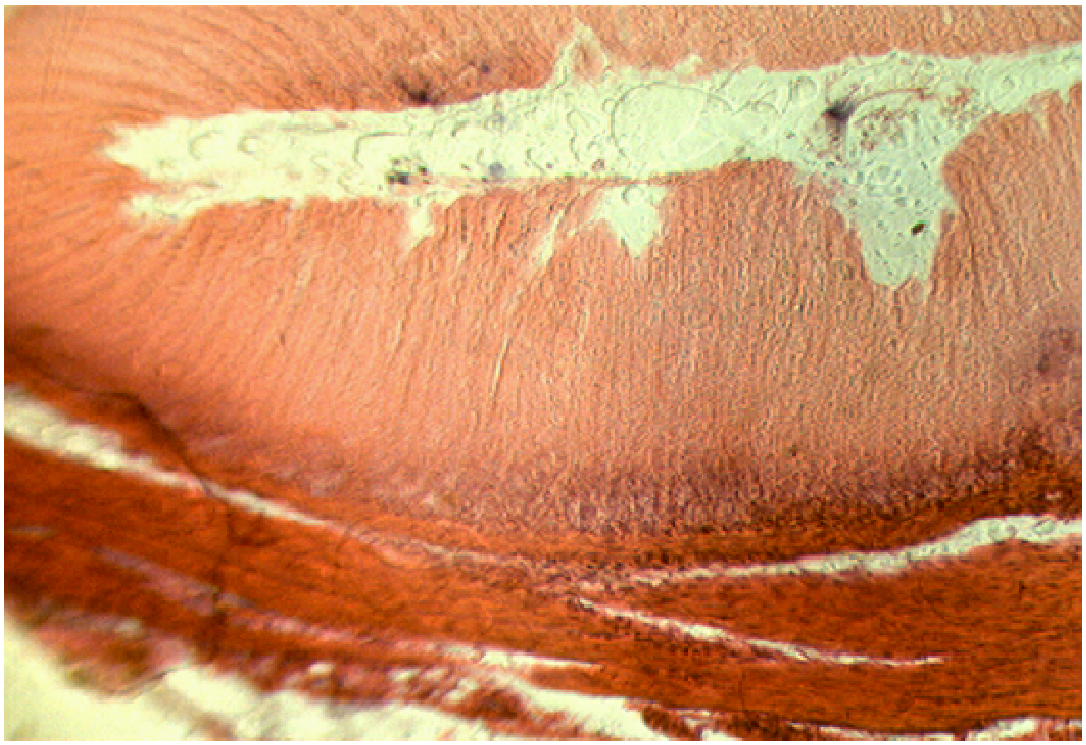


Spleen of rat treated with Hamegonorrhea (X 200)

Figure 6. Effect of Hamegonorrhea on stomach



Control group showing normal cells



Stomach cells of rat treated with Hamegonorrhea (X 200)

function, but in liver disease, there is a rise in serum levels of AST and ALT, followed by a fall in parallel (Sacher and McPherson, 1991). In the present study both AST and ALT levels were insignificantly increased in the test, showing that the preparation did not have significant effect on liver cell integrity. The serum levels of ALP was also not greatly affected, ALP is most often measured to indicate bile duct obstruction. High levels of ALP exist in cells that are rapidly dividing or are otherwise metabolically active. These cells include the epithelium of the biliary tract and liver, osteoblasts laying down new bone, granulocytes of circulating blood, intestinal epithelium, proximal tubules of the kidney, placenta, and lactating mammary glands. ALP levels reach spectacular heights in primary biliary cirrhosis, in conditions of disorganized hepatic architecture (cirrhosis), and in diseases characterized by inflammation, regeneration, and obstruction of intrahepatic bile ductules (Sacher and McPherson, 1991). In this study, ALP levels were insignificantly increased in test group. Showing that the test extract did not have any noticeable effect on the Liver cells and other body cell metabolic activities.

The preparation, hamegonorrhea did not alter the plasma levels of urea, albumin, cholesterol, triglyceride, creatinine and total protein. Implying that hamegonorrhea will not encourage arterochlerosis and cardiovascular diseases. The unaltered creatinine levels revealed that the kidney integrity was not compromised. This was also supported by histology of the kidney, and the fact that the plasma levels of sodium, potassium and calcium were not significantly affected.

From the results obtained from this study, hamegonnorrhea has good safety profile and its used may not pose any health risk in patients. The effect of the preparation on microorganisms should be assayed to ascertain its usefulness in the management of bacterial infection. One is hopeful that it does have the claimed antibacterial activity as reported for some of the herbal components of the preparation.

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