



Global Advanced Research Journal of Environmental Science and Toxicology (ISSN: 2315-5140) Vol. 3(4) pp. 057-064, November 2014
 Available online <http://garj.org/garjest/index.htm>
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Full Length Research Paper

Effect of *Rhazya stricta dense* leaf extract on the liver and kidney tissue structure of albino mice

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Abstract

Rhazya stricta plant is considered an important medical species used as a herbal drug in traditional medicine, and have always played a major role in the treatment of human and animal diseases. Over 100 alkaloids have been isolated, characterized and identified from *R. stricta* leaves, stems, roots. The aim of this research was a histological study to investigate the effect of different doses of *Rhazya stricta* extract administrated orally to albino mice for 15, 30 and 45 days on the hepatic and renal tissues and cells of these experimental animals. The tissues were examined under three types of stain, Haematoxylin and eosin, Masson trichrome staining and the immunohistochemistry method. The results indicated the safety of administering the extract up to 30 days in spite of the little changes that were observed in the hepatic tissues of the treated animals. But extending the administration period up to 45 days caused serious variations in the tissue structure, hepatocytes got swollen with cytoplasmic vacuolization and granulation, accompanied by presence of thick collagen fibers and little activity of the specific hepatocyte antigen (OCHIE5).

Keywords: *Rhazya stricta*- traditional medicine histological structure- liver – kidney- albino mice

INTRODUCTION

The plant species *Rhazya stricta dense* locally known as Harmal is a member of the family *Apocynacea* and is widely distributed throughout the world, and in Saudi Arabia. It is a glabrous erect shrub with dense erected braches (Western, 1984) and is widely used in traditional medicine as curative for rheumatism, sore throat, syphilis, diabetes, helminthiasis inflammatory conditions, fever and other diseases (Ageel et al., 1987; Ali et al., 2002; Ali et al., 1998). The leaves of the plant contain alkaloids like akuammidine and rhazimine (Bashir et al., 1994). The *R.*

stricta leaves have been shown to contain flavonoides, glycolides, triterpenes, trannis, volatile bases and probably other substances (Ahmed et al., 1983; Al-Yahya et al., 1990).

On the other hand extensive work have been carried out on the adverse effect of *R. Stricta* leaf extract. In Saudi Arabia, Adam (1998) dosed sheep with powdered *R.stricta* leaves in water and found that the higher doses produced body weight depression, bloating, diarrhea, dyspnoea and weakness of the hind limbs and

histologically there were entero-hepatonephroty, pulmonary congestion, haemorrhage and emphysema in addition he found an increase in the concentration of the enzymes aspartate transaminase (AST) and lactate dehydrogenase (LDH) in blood plasma of the liver and kidney, accompanied by increase in bilirubin and urea concentration and reduction in the net protein rate and the albumin, and calcium leucocytes .

When *R. stricta* extract was given to dogs at dose of 80mg/kg produced intense salivation and vigor, followed by respiratory failure, convulsions and death within 15 minutes (Siddique and Bukhari, 1972).

Administration of *R. stricta* aqueous leaf extract didn't affect the liver enzyme activities or kidney functions (Baeshin et al., 2009).

This histological sections of the liver of mice given *R. stricta* leaf extract at rates up to 200 mg/kg caused acute tissue irritation, fatty liver and acute blood congestion in the blood vessels (AL-Hazmy et al., 2000; Al-Saggaf, 2004). The oral administration of *R. stricta* extract at doses of 10 and 20 mg/kg for 3 days to rats gave significant increase in the metabolizing process of the cytochrome P450 (El-Kadi et al., 2003). The rats fed the leaf extract of *Pauwolfia vomitoria* which belongs to the same family of *R. stricta* were subjected to hepatocellular scattering and renal function (Eteng et al., 2009). Nobre et al. (1994) found that horses fed on seeds of *Crotalaria* suffered from liver fibrosis. The toxic dose (up to 120 µg/L) of the extract of *Oncorhynchus mykiss* plant stimulates division of cells and nuclei of the liver and kidney and disorganization of the unhomogeneous chromatin, nuclear multiplication and change of the rough endoplasmic reticulum to smooth bodies and increase in number of mitochondria.

The aim of this research is to investigate and examine the hepatic and renal histologist structure alterations if any that may be caused by administration of *Rhazya stricta* leaf extract to albino mice, and to try to determine the optimum period for the administration of this leaf extract.

MATERIALS AND METHODS

Materials

Leaf extract

Leaf samples of *R. stricta dense* were collected from Al-Khomrah area south Jeddah along the coastal road during spring season (Jan – April) where the leaves are in fully green color. The leaves were thoroughly washed with water, dried by means of hot air, and then ground to very fine powder with a blender. The powder was then put in distilled water (9g:40ml) and boiled and filtered using bacterial filter. The filtrate was boiled again, cooled to room temperature and again filtered and put in a glass

tube inside the fridge at 10C°. The extract was diluted with distilled water according the weight of each rat .The dose of 2.36 gm /kg was demonstrated orally by the use of the stomach feeding tube (Wasfi et al., 1994).

The animals

A total number of 80 adult male albino mice were used in this research. The mice were in good health, approximately having the same weight (25 ±5 gm) and 45 days in age. They were collected from king Fahad Center for Medical Research in King Abdulaziz University.

They were housed in groups of 3 animals per cage at a temperature of 18-20C° under a 12h dark–light cycle . They were fed standard pelleted diet (grain soils with plant fibers, vitamin A amino acids, salts of ca, P, Fe ,K) and clean drinking water. The animals were left for one week before start of treatments.

The animals were grouped into four groups each one is ten mice, the first one is control (G1) which was treated orally with distilled water, the second (G2) was treated orally using the stomach tube with *Rhazya stricta* extract at dose of 2.36 gm/kg for 15 days, the third group (G3) was administered the plant extract at the same dose for 30 days, and the fourth group was orally administered the leaf extract of at the dose of 2.36 gm/Kg for 45 days.

At the end of each treatment the animals were anaesthetized with diethyl ether, fixed on the dissection board and small parts and small parts of the liver and kidney (each sample is 3mm³) were taken for sectioning.

Preparation of tissues

Tissue samples were fixed in equivalent formaldehyde solution (10%), then washed with tap water for 12 hours . The samples were then dehydrated by being passed through increasing levels of ethanol from 30% up to 100%. Xylene was then applied for 30-40 minutes to clear the samples from ethanol .

Paraffin infiltration

The tissues were subjected to paraffin infiltration using melted paraffin wax inside the oven at 60C° for one hour.

Embedding

Molting wax was poured inside embedding moulds, and the tissue samples were transferred from the oven inside these moulds which were then cooled to room temperature .

Sectioning

The embedding moulds with the samples were fixed on the specimen holder, and rotary microtome (Lieca) was used to provide sample sections 5 µm in thickness The

paraffin was removed from the sections by putting in water at 24C°. The sections were picked out and put on a hot plate at 30C° to get rid of the extra water. Deparaffinization was carried out by immersing the tissues in xylene for 8 minutes.

The tissue sections were immersed in hematoxylin for one minute then in alcoholic eosin 1% , for the examination of the arrangement of the hepatic and renal cells, their sizes, and the presence or absence of vacuolization or granulation.

The tissue sections were also stained using Masson trichrome staining, for the detection of the structure of Gilson's capsule, and the collagen fibers. Also the tissue specimens were prepared using the technique immunohistochemistry which detects certain antigens that are found associated with their antibody, and which are added to the tissue under reasonable conditions. The sites of these antibodies in the tissue are recognized by the use of labeled secondary antibodies which can directly attach to the primary antibodies (AL-Khateeb, 2001).

The (OCHIES) was used for detection of the hepatic specific antigen, and CD34(QBEnd/10) for the kidney sections. Light microscope was used for tissue examinations.

RESULTS

Histological studies of (hepatic and renal tissue) using haematoxylin –eosin stain.

The Liver

Mice fed *Rhazya stricta* extract for 15 days

The treated animal hepatocytes show normal arrangement and the nuclei in the center of homogeneous cytoplasm are circular. Some cells show some cytoplasmic vacuolization specially in the portal area, and cytoplasmic granulation, and nuclear margination behind the nuclear wall of some cells. Also the normal structure of sinusoids which contain kupffer cells can be observed, and branch of the portal vein and the portal hepatic vein can also be seen.

Mice fed *Rhazya stricta* extract for 30 days

After orally administering the animals with the plant extract for 30 days the normal arrangement of the hepatocytes is observed, and in the portal area the hepatocytes can be seen with homogeneous cytoplasm and circular nuclei, but some of it in showing cytoplasmic vacuolization with normal nuclei, with the presence of nuclear margination behind the nuclear wall. Kupffer cells became large in size and heavily stained, and the

structure of the branch of the portal vein, and the branch of the bile duct is normal.

Mice fed *Rhazya stricta* extract for 45 days

After being fed the plant extract for 45 days, the examined sections show swollen hepatocytes, which led to narrowing of the sinusoids, and also cytoplasmic vacuolization and cytoplasmic granulation can be observed (Slide 1).

The Kidney

Mice fed *Rhazya stricta* extract for 15 days

As for renal histological structure at this feeding level, the tissue structure of part of the kidney cortex, medulla, glomerulus and medullary rays appear normal and also Bowman's capsule surrounding the glomerulus can be seen in the normal tissue of the cortex, and the proximal and distal tubules and the tubule endothelial cells are observed. The normal histological structure of part of the medullary region is clear together with the thick and thin limbs of Henle Loop, and also the collecting tubule.

Mice fed *Rhazya stricta* extract for 30 days

A nearly normal histological structure of the cortex region is seen, and this is observed in the glomerulus, and the proximal and distal tubules and also in the macula densa. The semi normal structure of the medullary with the thick limb of Henle Loop carrying a number of collecting tubules is observed.

Mice fed *Rhazya stricta* extract for 45 days

Feeding the animals the plant extract for 45 days created dilation of the endothelial cells of the proximal and the distal tubules with hypertrophy of the glomerulus which causes narrowness of the renal distance.

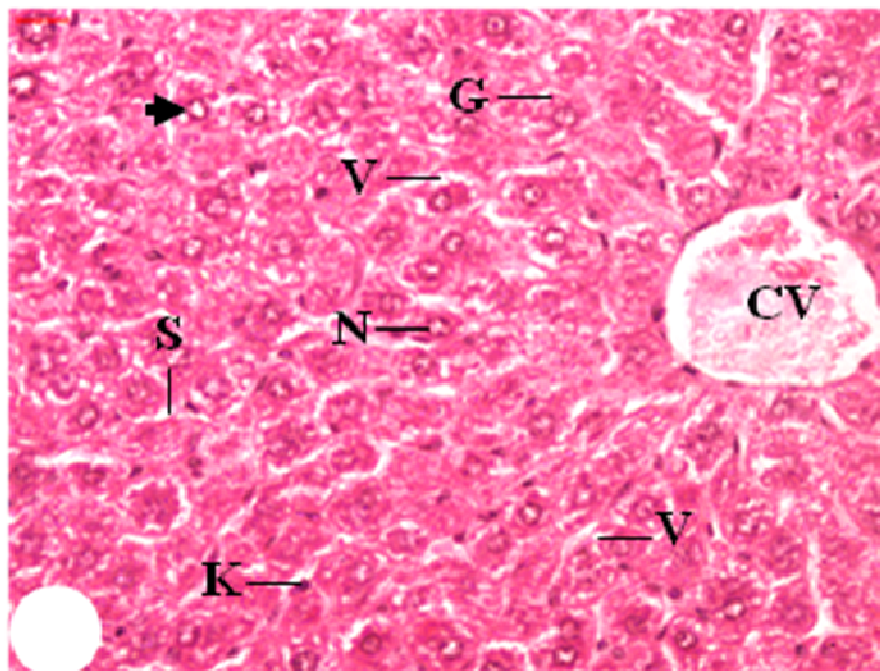
The damage of some tubular endothelial cells is observed in the medullary region and some collecting tubules can be seen with epithelial cells with empty looking cytoplasm Slide (2).

Histological studies on connective tissues of hepatic and renal tissues using Masson trichrome stain

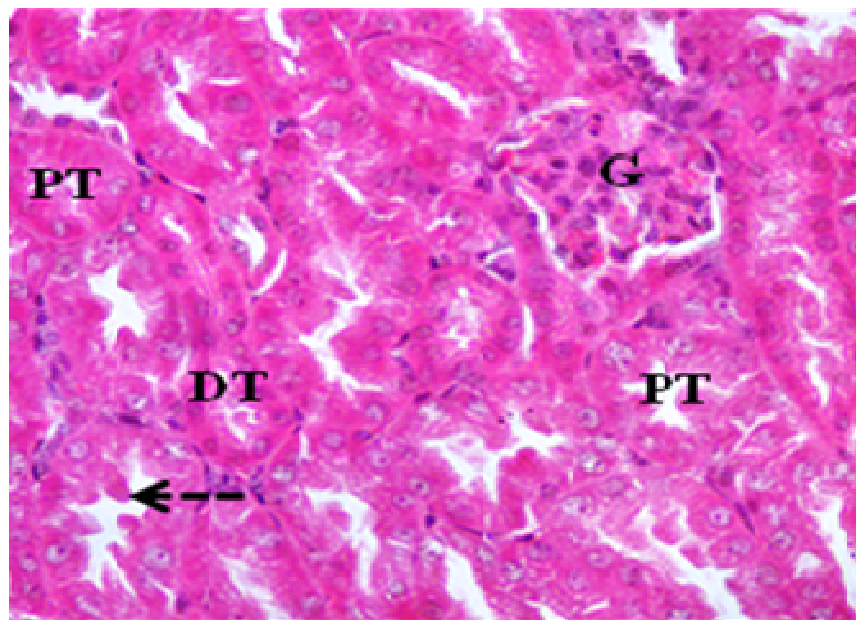
The Liver

Mice fed with *Rhazya stricta* leaf extract for 15 days

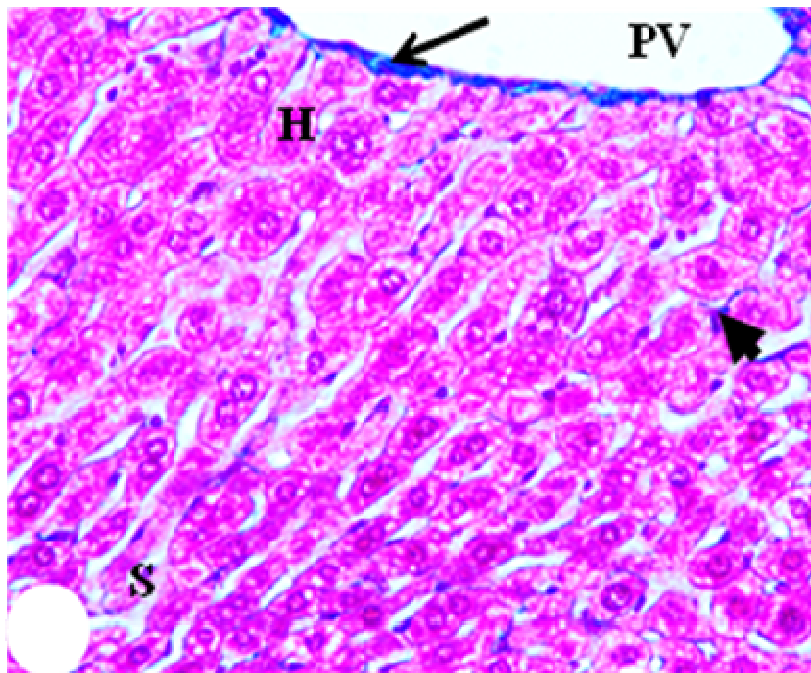
After being administered the plant extract for 15 days, collagen fibers at the wall of the central vein and collagen fibers at the sinusoidal wall were observed in the liver tissues. Also the collagen fibers at the wall of the portal vein was observed in the cells.



Slide (1): Section of mice liver administered *R. stricta* leaf extract for 45 days. Small swollen in hepatocytes resulted in narrowing of sinusoids (S). There is vacuolization (V), cytoplasmic granulation (CG), and circular nuclei(N) some of it suffer nuclear margination (arrow head), Kupffer cells (K), and central vein (CV).



Slide (2): Section of mice kidney administered *R. stricta* leaf extract for 45 days. It shows dilation of proximal tubule endothelial cells (dotted arrow) (PT) and the distal tubular cells (DT) in addition to glomerular enlargement (G).



Slide (3): Section of mice liver administered *R. stricta* leaf extract for 45 days . It shows the collagen fibers at the wall (arrow) of the portal vein (PV) with presence of some fibers (H) between hepatic sinusoids(S)

Mice fed plant extract for 30 days

The examination of the hepatic tissue of animals fed the plant extract for 30 days showed the presence of collagen fibers at the wall of the central vein and also collagen fibers at the sinusoidal wall, together with dense collagen fibers at the wall of the portal vein.

Mice fed plant extract for 45 days

Feeding the animal the plant extract for 45 days resulted in the presence of thick collagen fibers around the central vein between the hepatocytes and the hepatic sinusoids, and also at the wall of the portal vein Slide (3).

The Kidney

Mice fed the plant extract for 15 days

As for the renal histological sections at this level of extract feeding the collagen fibers are observed in the basement membrane of the proximal tubules and in the branch borders.

Mice fed the plant extract for 30 days

For the kidney, the collagen fibers appear in the glomerulus and in the basement membrane of the

Bowman's capsule, and also in the basement membrane of the proximal tubule and in the branch borders . The collagen fibers in the basement membrane of the renal tubules are illustrated also.

Mice fed the plant extract for 45 days

The collagen fibers are observed in the basement membrane of the proximal tubules and the brush border, and that of the Bowman's slide, and that of the renal tubules Slide (4).

Histological studies on hepatic and renal tissues by immunohistochemistry

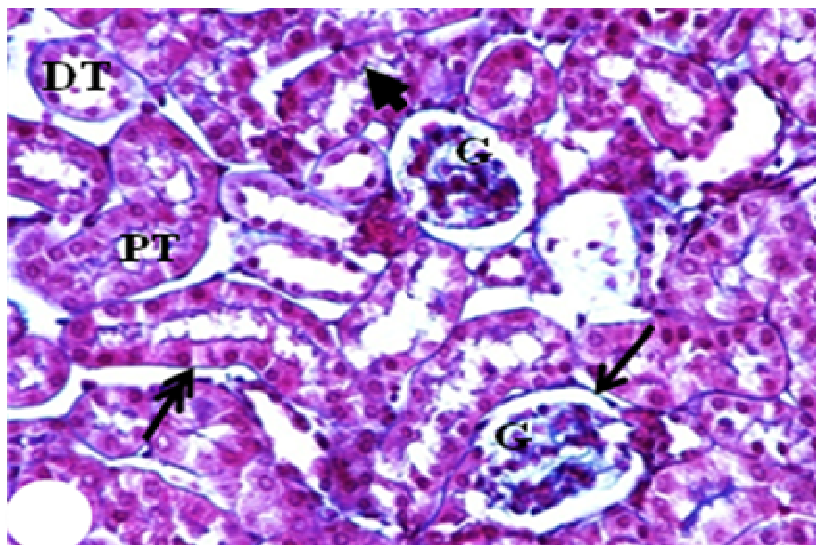
The Liver

Feeding mice *Rhazya stricta* extract for 15 days

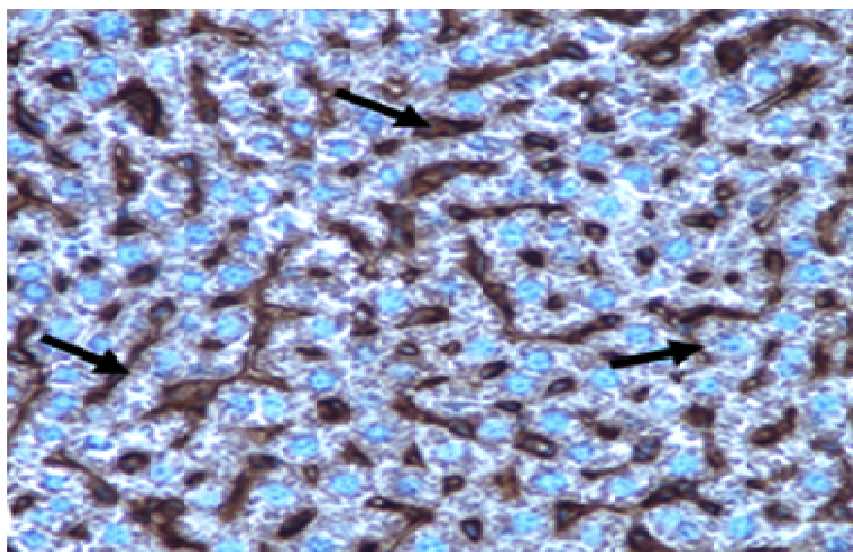
At this feeding level strong activity of the hepatocyte specific antigen (OCHIE5) was noticed inside the endothelial cells of the hepatic sinusoids while only little activity was observed of the antigen in a form of small granules inside the hepatocytes.

Mice fed the plant extract for 30 days

Only little activity of the hepatocyte specific antigen



Slide (4): Section of mice kidney administered *R. stricta* leaf extract for 45 days. It shows the collagen fibers in the basement membrane of the portal tubule(PT) (dotted arrow), the brush borders(arrow head) containing some collagen fibers, and collagen fibers in the basement membrane of Bowman's capsule(arrow).



Slide (5): Section of mice liver administered *R. stricta* leaf extract for 30 days. It shows only little activity of the hepatocyte specific antigen (OCHIE5) inside the sinusoidal endothelial cells (arrows).

(OCHIES) was observed inside the endothelial cells of the hepatic sinusoids, and also little activity of the antigen in the form of small granules was seen inside the hepatocytes.

Mice fed the plant extract for 45 days

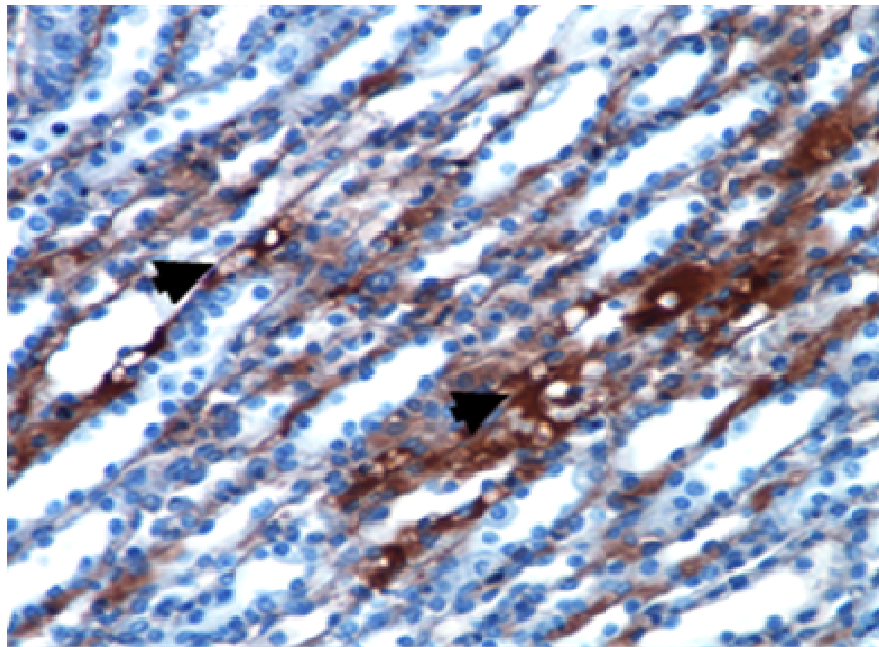
Only minute activity of the hepatocyte specific antigen (OCHIES) was observed inside the endothelial cells of

the hepatic sinusoids, with minute activating of the antigen in the form of small granules inside the hepatocytes (Slide 5).

The Kidney

Mice fed the plant extract for 15 days

A strong activity of the endothelial cell marker (CD34) can



Slide (6): Section of mice kidney administered *R. stricta* leaf extract for 45 days. It shows activity of the blood capillaries endothelial cells marker (CD34) similar to the intertubular renal blood capillaries endothelial cells (arrows)

be seen in the renal intertubular blood capillaries from the medullary region. And the same strong activity of this marker antigen is seen in the renal tissues.

Mice fed the plant extract for 30 days

As for the renal histological sections of the animals fed the plant extract for 30 days, a similar trend of activity as the control was shown by the endothelial cell marker (CD34) antigen in the endothelial cells of the glomerular capillaries and the renal intertubular blood capillaries.

Mice fed the plant extract for 45 days

Also a similar trend of the activity of the endothelial cell marker (CD34) antigen as the control group was seen on the endothelial cells of the glomerular capillaries and in the renal intertubular blood capillaries after administration of the plant extract for 45 days (Slide 6).

DISCUSSION

It can be said that the oral administration of *R. stricta* leaf extract to mice for a period up to 30 days gave no substantial alterations in the hepatic and renal histological structure, but some cells show some cytoplasmic vacuolization specially in the portal region of the liver and cytoplasmic granulation, and nuclear

margination behind the nuclear wall of some cells after administering the leaf extract for period up to 15 and 30 days. But there were substantial alterations in the hepatic and renal histological structure when the animals were fed the plant extract for up to 45 days. There were swollen hepatocytes which led to narrowing of the sinusoids with cytoplasmic vacuolization and granulation, presence of thick collagen fibers around the central vein, and between the hepatocytes and the hepatic sinusoids, and also at the wall of the portal vein.

And as regards the antigen, only minute activity of the hepatocyte specific antigen (OCHIE5) was observed inside the endothelial cells of the hepatic sinusoids.

As for the effects of *R. stricta* leaf extract on the renal histological structure, no substantial alterations have been observed for administration of the extract for a period up to 30 days. But taking the leaf extract up to 45 days created dilation of the endothelial cells of the proximal and distal tubules, glomerulus hypertrophy, necroses of some tubular endothelial cells in the medullary region, and some endothelial cells of some collecting tubules are with empty looking cytoplasm.

These results may agree with the results found by Adam (1998) who administered sheep with the leaf powder of *R. stricta* and found that only the higher doses have affected the liver and kidney tissue and induced histological changes as entro-hepatonephrobathy, and pulmonary congestion, haemorrhage and emphysema. And also Al-Hazmy et al., 2000, and AL-Saggaf, 2004

found that administration of *R. stricta* leaf extract didn't affect the histology of the treated animals, but only at the higher doses up to 200mg/kg where it caused acute tissue - and hepatic ----, and acute blood congestion in the blood vessels.

Safety in the use of the extracts of other medicinal plant species on hepatic and renal histological structure is also recorded in other studies. Hasseib et al., 1993 found that the administration of the water extract of the plant *Ambrosta maritime* (Compositae) at high rates to rats for five weeks didn't cause histopathological changes, and the effects were included only in the blood capillaries of the liver tissues. Consequently Cristiani et al. (2005) recorded that the administration of the alcoholic extract of the plant *Wedelia paludosa* (Asteraceae) didn't cause any changes in the hepatic enzymes and activity, and Mehmet et al. (2006) didn't notice any histopathological changes in the liver sections that were subjected to chronic and acute treatment with the extracts of the two plants *Artemisia herba alba* (Asteraceae) and *Teucrium polium* (Lamiaceae), while Cavalcanti et al. (2006) found the safety of the water extract of the plant *Achillea mellifolium* (Asteraceae).

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