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DETECTION OF THE ORGANS PROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *CYPERUS ROTUNDUS* ON ALBINO MALE MICE DAMAGED BY METHOTREXATE DRUG.

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Abstract

The objective of this study was to consider as an explorer for *in vivo* studies on the production of some secondary metabolites from local medical plants named *Cyperus rotundus*. The plant *Cyperus rotundus* (Family Cyperaceae) is indicated both as a functional food and as a drug. *Cyperus rotundus* Linn. commonly known as 'Nagarmotha' is a pestiferous perennial weed with dark green glabrous culms, arising from underground tubers. A number of pharmacological and biological activities including antidiabetic, antidiarrhoeal, cytoprotective, antimutagenic, antioxidant, antimalarial, Antiinflammatory activities have been reported for this plant. The phytochemical investigation of *Cyperus rotundus* have revealed the presence of polyphenol, flavonol glycoside, alkaloid, saponins, sesquiterpenoids and essential oil. Different organs were used in this study such as intestine, kidney, spleen and testes for this experiment. Each organ response was recorded and Histopathological section declared the ability of plant extract to counteract the organs damaged by methotrexate (MTX) drug. These results clearly well-known the protective potency organ of *C. rotundus*, which may explanation for some of the medical claims ascribed to this plant.

Key word: secondary metabolites, herbal remedies, perennial plants, drugs, antioxidant.

Introduction

Medical plants, means the using of active sub substances for treatment of disease (Ibrahim *et al.*, 2019). Medicines derived from plants are widely famous due to their safety, easy availability and low cost. Medicinal herbs are more significant to the health of individual and community. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Maeh *et al.*, 2019). Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds and many more (Thanabhorn *et al.*, 2005). These natural compounds formed the foundations of modern prescription drugs as it is know today (Mandal *et al.*, 2005). *Cyperus rotundus* a species of sedges (*Cyperus rotundus*) is a perennial plant and one of the most invasive weeds known, having spread tropical, subtropical and temperate regions (Al-Ezzy *et al.*, 2018).

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Cyperus rotundus is widely distributed Native to India, purple nuts edge has been reported from more countries, regions and localities than any other weed in the world (Hsu *et al.*, 2006). According to the Ayurveda, *C. rotundus* rhizomes are considered astringent, diaphoretic, diuretic, analgesic, antispasmodic, aromatic, carminative, antitussive, emmenagogue, litholytic, sedative, stimulant, stomachic, vermifuge, tonic and antibacterial (Mattila *et al.*, 2007*)*. It is also useful for dietary management of psychotic diseases and metabolic disorder. They are used in treatment of Nausea and vomiting, dyspepsia, colic, flatulence, diarrhoea, dysentery, intestinal parasites, fever, malaria, cough, bronchitis, renal and vesical calculi, urinary tenesmus, skin diseases, wounds, food poisoning, indigestion, nausea, infertility, cervical cancer and menstrual disorders and the aromatic oils are made of perfumes and splash (Serrano *et al.*, 2009). This study aimed to evaluate the organs protective activity of Cyperus methanolic extract on mice damaged by methotrexate (MTX).

Materials and Methods

Cyperus rotundus collection and *identification*

During the period of September 2018 *C. rotundus* plant is collected from local market which previously acknowledged by National herbarium of Iraq.

Preparation and dose of *C. rotundus*

C. rotundus assessed in the present study was supplied from the local market of Baghdad, Iraq. At first, the fresh *C. rotundus* leaves were cleaned, peeled, sliced and sun dried for seven days. After that the dried leaves were ground to powder by using an electric blender apparatus then 60 gm of plant soaked in 300 ml of 90% ethanol, then the flask was incubated for 5 days at room temperature with continuous shaking at 140 rpm. The crude extract was filtered by using 0.22µm filter unit then concentrated in a rotary evaporator to obtain dried portion. Dried crude extract dissolved in DMSO separately to prepare the final concentration of 200 mg/ml (AlChalab *et al.*, 2017).

Assessment of histological Effects

The plant histological activity were estimated in albino male mice administrated methotrexate (MTX) drug through evaluating the histopathological examinations of different mice organs represented in (kidney, intestine, testis and spleen) to determine the ability of plant extract to counteract the damage caused by drug (MTX) at a dose of 40 mg\kg.

Experimental Design

Albino male mice aged 6-8 weeks and weighted 23-25 gm were purchased from Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq. Four animals were housed per cage with *ad libitum* access to water and food pellets. The animals distributed to four groups each groups contain (4 mice), the details of each groups were:

• Group I: a single daily dose (0.1 ml) of distilled water was intraperetonially injected to mice for 7 days (Control I).

• Group II: a single daily dose (0.1 ml) of methotrexate was intraperetonially injected to mice for 7 days.

• Group III: a single daily dose (0.1 ml) of *Cyperus rotundus* ethanolic extract was intraperetonially injected to mice for 7 days.

• Group IV: in this group,An the plant extract interac MTX drug in which mice were administered with (0.1 ml) of MTX drug from (1-2) days and with *Cyperus rotundus* ethanolic extract from (4-7) days Then, at day 8, the mice were scarified.

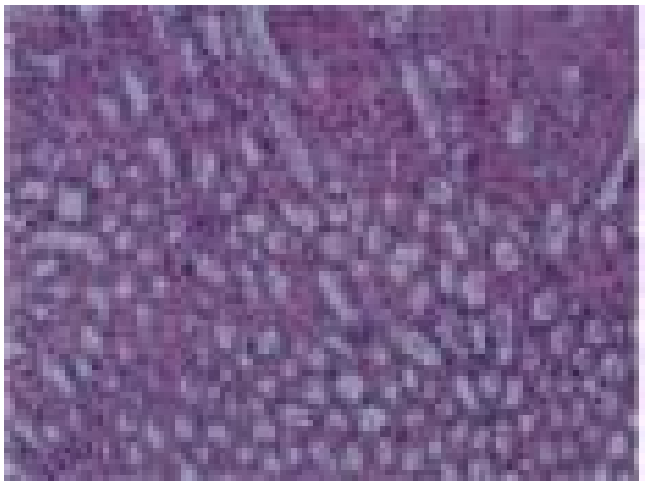

Fig. 1: Section showing normal appearance of kidney in control groups (200×; H and E)

All the tested organs were fixed in 10% formalin for 24h., followed by gradual series of dehydration alcohol 30-100% for 5 min. the organs cleared in two changes of xylene before embedded in paraffin wax for sectioning. 5µm thickness of Cross sections of samples and finally stained with hematoxylin (Harison) and eosin. All changes in tissues examined under light microscope in comparison with control group (Ibrahim *et al.*, 2017).

Results and Discussion

Treated mice with MTX caused necrosis together with mild inflammatory cell infiltrate and fatty changes in kidney tissue (Fig. 2) while the results indicated the ability of the plant to counteract these adverse effect in mice and made its appearance looks like normal (Fig. 4).

Also, the results of treated animals with MTX for intestine matches with the results if kidney in which the drug caused necrosis and degeneration of nephrocytes

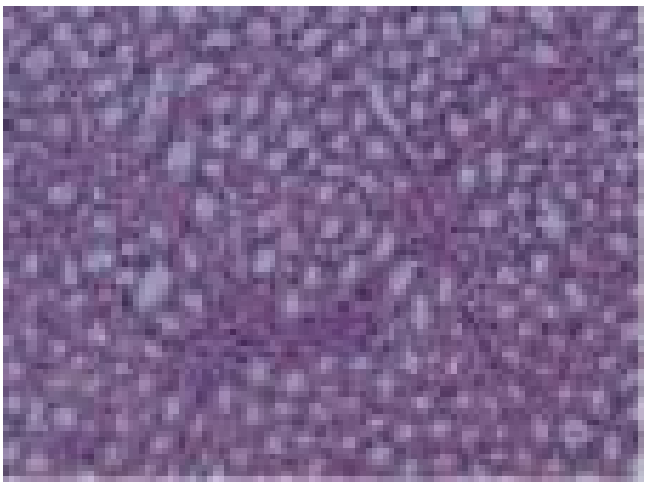

Fig. 2: Necrosis is present together with mild inflammatory cell infiltrate (mononuclear cells) and fatty changes in kidney tissue of mice treated with methotrexate drug (200×; H and E).

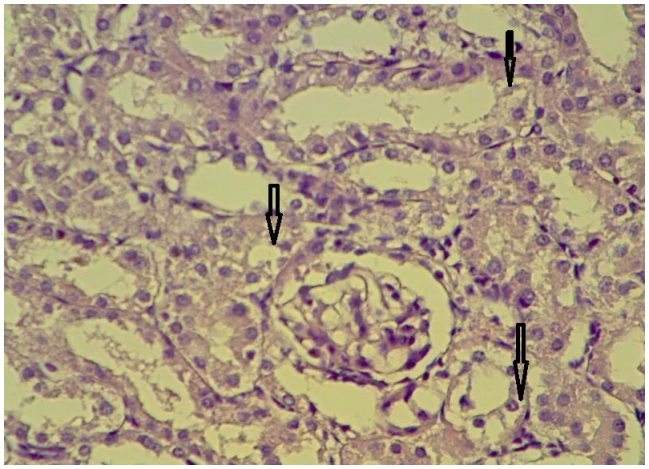

Fig. 3: Section showing degenerative changes of epithelial cells of renal tubules in mice treated with plant extract (proximal and distal convoluted tubules. (X40) (H&E).

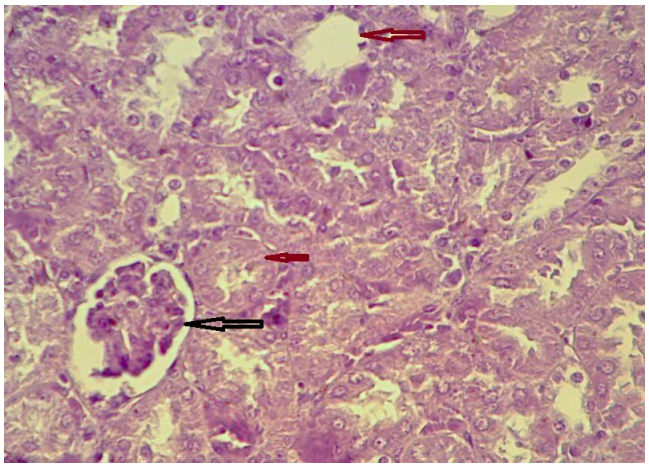

Fig. 4: Section showing look like normal histological structure appearance of parenchyma of glomeruli and renal tubules after interaction the plant with drug (Proximal and distal convoluted tubules) (X40) (H & E).

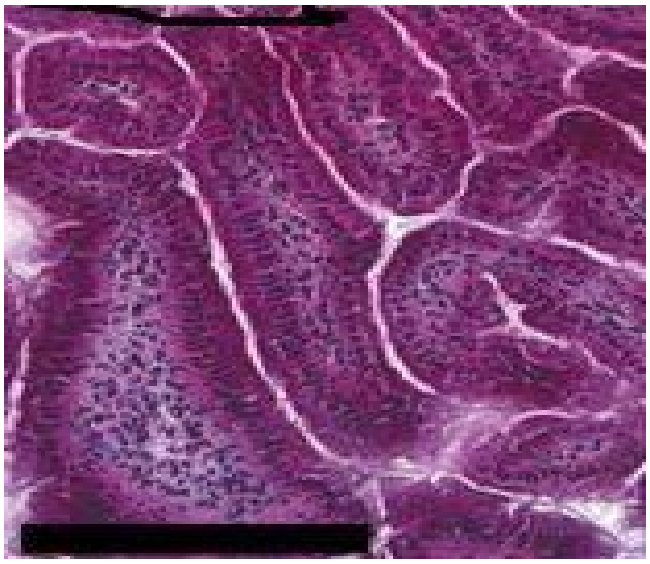

Fig. 5: Section showing normal appearance of intestine in control groups (X40) (H & E).

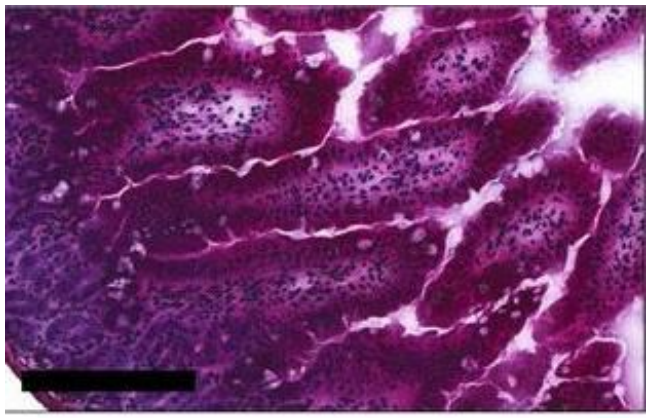

Fig. 6: Section of intestine tissue in mouse treated with methotrexate showing slight necrosis and degeneration of nephrocytes are observed (200×; H and E).

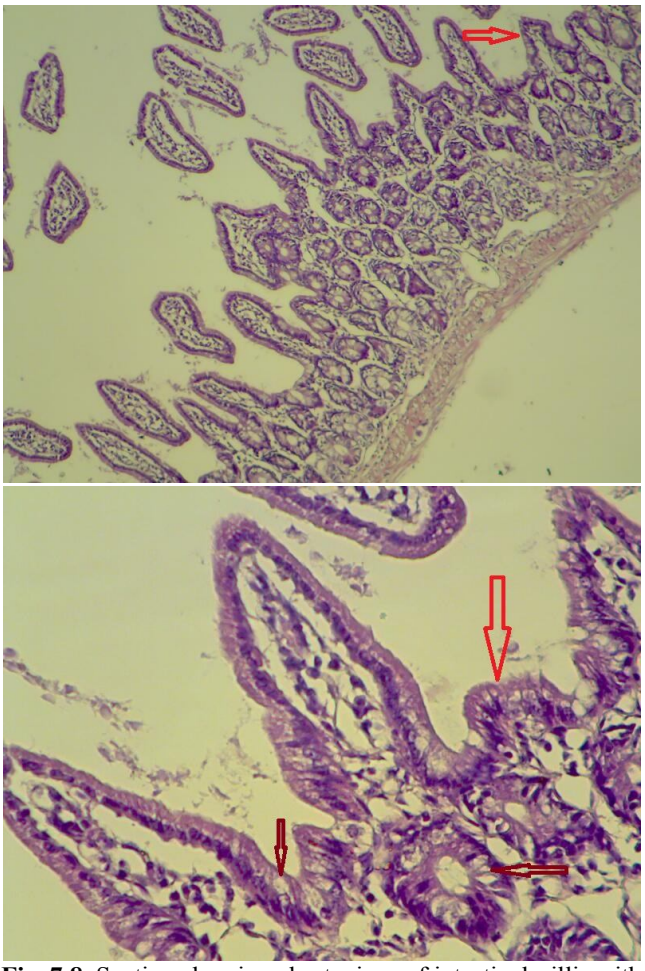

Fig. 7,8: Section showing shortening of intestinal villi with reduction in numbers of goblet cells in mouse treated with plant extract.(x10)(X40)(H & E).

(Fig. 6) while treated mice with plant alone or in interaction with drug caused reduction in numbers of goblet cells in mouse treated with plant extract (Fig. 7, 8) and return the Section look like normal histological structure of intestinal villi with increment in numbers of goblet cells (Fig. 9, 10).

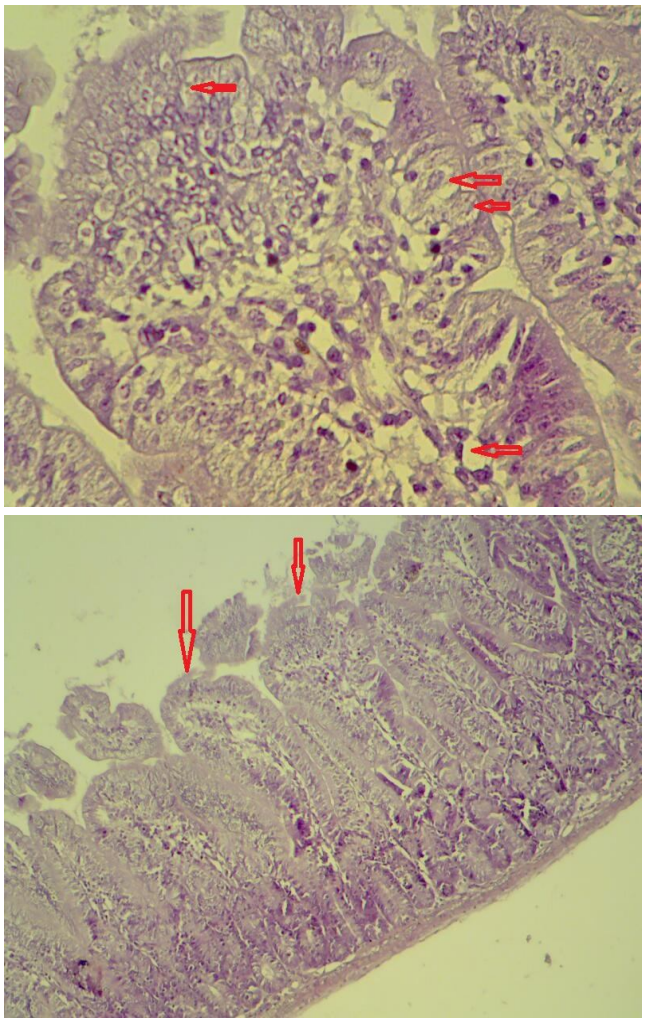

Fig. 9,10: Section showing look like normal histological structure appearance of intestinal villi with increment in numbers of goblet cells in interaction group. (X10)(X40)(H & E).

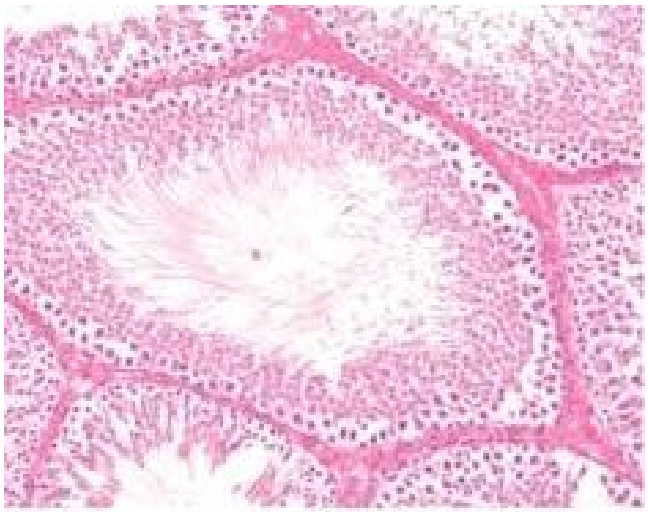

Fig. 11: Section of testis tissue in control negative mice showing normal seminiferous tubules, spermatids and spermatogenic cells at different stages of development (400×; H and E)

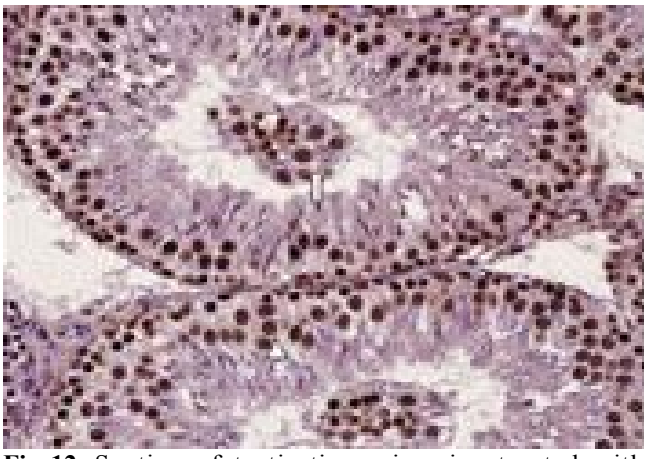

Fig. 12: Section of testis tissue in mice treated with methotrexate drug, widespread of apoptotic germinal cells in seminiferous tubule germinal epithelium and in the lumen (200×; H and E).

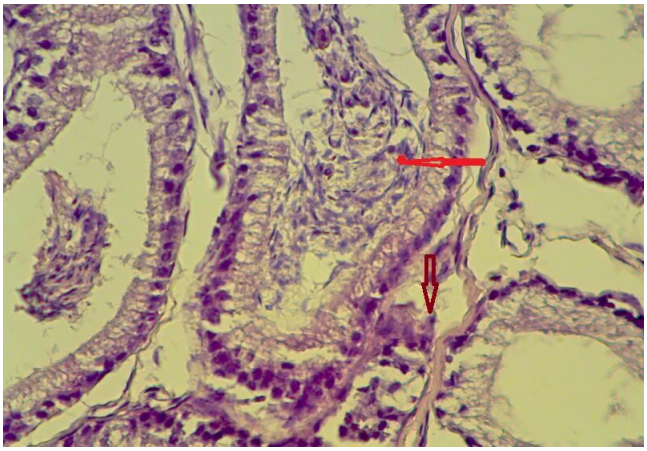

Fig. 13: Section showing normal histological structure of testis which consist of seminephrous tubules containing sperms, with presence of interstitial cells in plant treated group (Leydig cells) (X40)(H & E).

Results of testes histology showing widespread of apoptotic germinal cells in seminiferous tubule germinal epithelium and in the lumen in mice treated with MTX but when plant extract gave to animals (Fig. 12), these effect absent and the histological appearance look like normal when treated animals with plant alone or in interaction groups (Fig. 13, 14).

The results of spleen section showed the infiltration of Polymorphonucleur cells, degeneration of lymphocytes D and necrosis of the tissue Megakaryocytes (Fig. 16) in compared to section showing widening of white pulp with reduction of red pulp in plant treated group (Fig. 16) and increment widening of white pulp (hyperplasia) with reduction of red pulp and presence of numerous numbers of megakaryocyte cells in interaction group (Fig. 18).

A variety of organs disorders are caused by high levels of drugs throughout producing pro-oxidants/reactive oxygen species (ROS), which have the capacity to

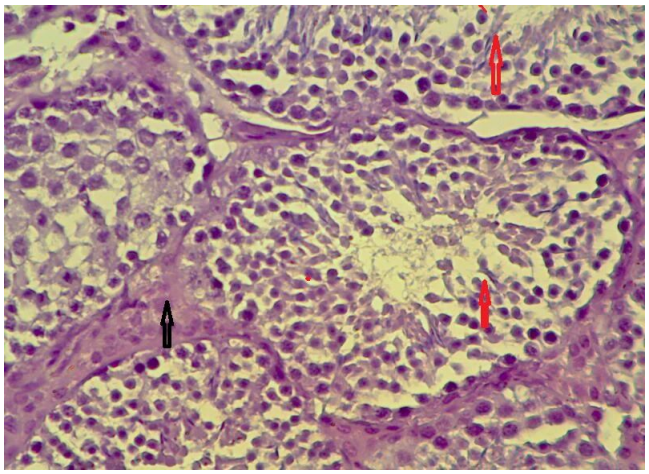

Fig. 14: Section showing look like normal histological structure with normal development of spermatogoni cells and presence of sperms inside the lumen of tubules with hyperplasia of leydig cells in interaction group.(X40)(H & E).

stimulate cellular damage in a multiplicity behavior like affecting the cellular biomolecules, such as lipids, DNA and proteins (Meena *et al.*, 2010). Drug MTX is comparable in structure to dihydrofolate (FH2) and its mechanism is a competitive inhibitor of DHFR resulting in tetrahydrofolate (FH4), essential for DNA synthesis, is then not created. The mitosis of cancerous cells interferes with this by inhibiting the de novo synthetic pathways for purines, pyrimidines, formation of polyamines and transmethylation of DNA, RNA, phospholipids and proteins (Durate *et al.*, 2005). From ancient time, plant scavenging free radicals and down regulate inflammatory mediator synthesis and release (Al-

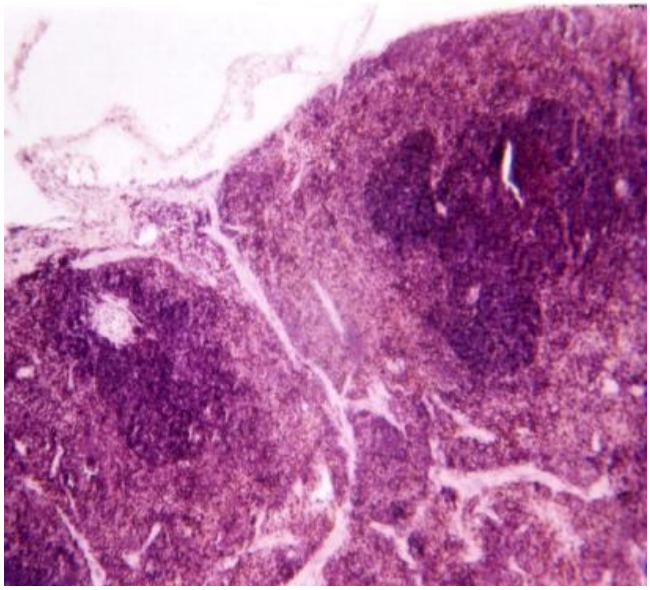

Fig. 15: Section of spleen tissue in control negative mice. A cross section in spleen showing white, W, red R palps and scattered (200×; H and E)

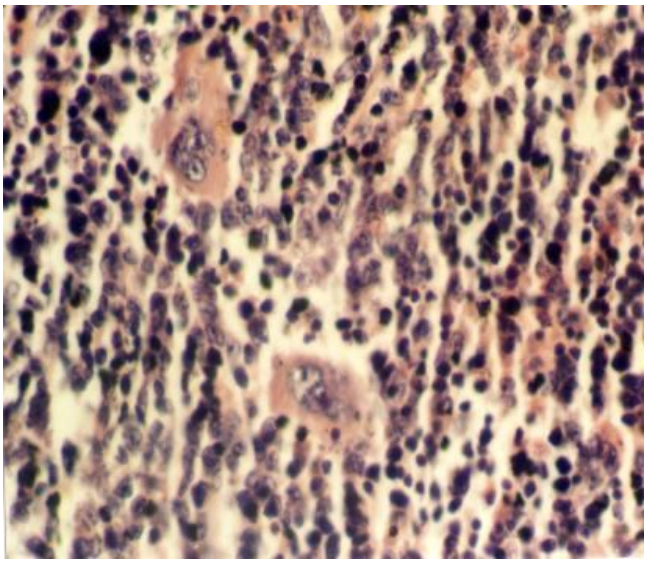

Fig. 16: Transverse section of the red pulp area in the spleen treated with methotrexate showing the infiltration of Polymorphonucleur cells, degeneration of lymphocytes D and necrosis of the tissue Megakaryocytes (200×; H and E).

Ezzy *et al.*, 2019). Some of the most important bioactive phytochemical constituents presented in cyperus are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds and many more and the more effect has been regarded to flavonoids although by a possible synergistic or antagonistic effect of flavonoids with other compounds in the extracts cannot be barred. The activity of *C. rotundus* in scavenge free radicals *in vivo* and look after mitochondrial, endoplasmatic reticulum and plasma membranes from damage induced by free radicals attributed to flavonoid (Chaulya *et al.*, 2011). All these previous findings cleared the mechanism by which phenolic compounds mainly flavonoid was due to their antioxidant properties (Yazdanparast *et al.*, 2007). Phenolic compounds because of their redox properties

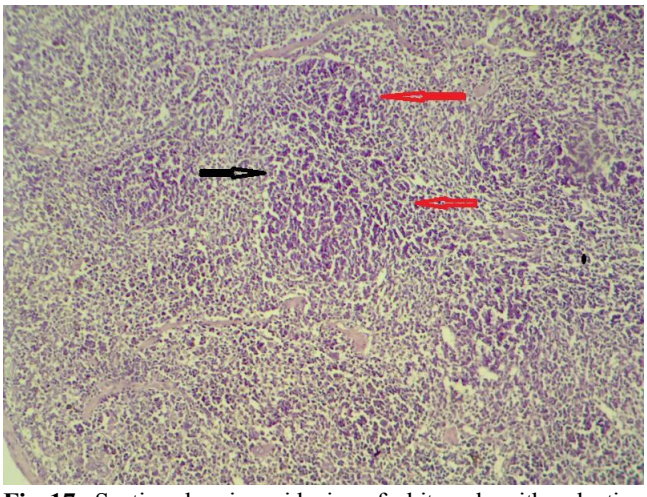

Fig. 17: Section showing widening of white pulp with reduction of red pulp in plant treated group.(X10) (H & E).

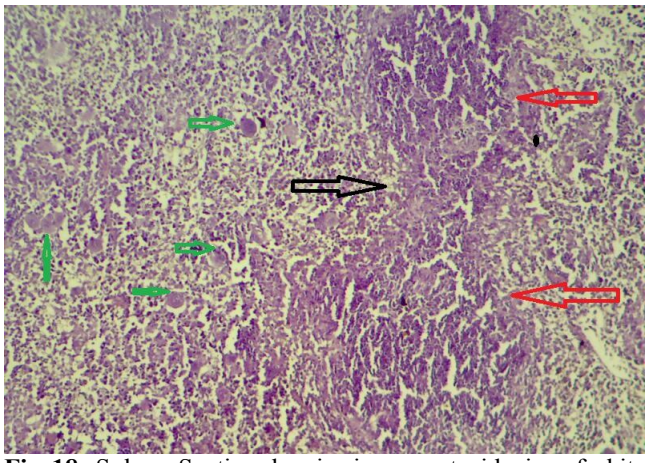

Fig. 18: Spleen: Section showing increment widening of white pulp (hyperplasia) with reduction of red pulp and presence of numerous numbers of megakaryocyte cells in interaction group.

which permit them to be active as reducing agents, hydrogen donors and singlet oxygen quenchers contribute directly to their antioxidant action (Oladipupo *et al.*, 2009). Previous studies also showed that *Cyperus rotundus* inhibits the generation of superoxide radicals (Ranjani *et al.*, 2012), in addition to that, recent evidence reported that GSH-PX and GST play a major role in the abolition of H_2O_2 and lipid per oxidative stress in rats. Thus, inhibition this enzymes may results in the accumulation of the H_2O_2 with subsequent oxidation of lipids (Kilani *et al.*, 2008). Finally, the inhibition of oxidants and protection of the cell membrane through restoration of lactate dehydrogenase (LDH) endorsed by flavonoids which are the major component in the volatile oil of *Cyperus rotundus* (Ibraheem *et al.*, 2018).

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