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CYTOTOXICITY AND SOME IMMUNE CELLS STIMULATION EFFECTS OF LOCAL SUMAC (*RHUS CORARIA*) ON CANCER CELLS AND MICE

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ABSTRACT

The present study was conducted to investigate the *in vitro* cytotoxic effects of sumac extract against two human cancer cell lines, Hep2 (Human larynx epidermoid carcinoma), RD (Rabdomyosarcoma), and one normal cell line Ref (Rat embryonic fibroblast). Dried sumac fruits were extracted in 80% ethanol. The absence of couumarins. The cancer and normal cells were treated with different concentrations of sumac extract ranging from 0.244 µg/ml to 7.81 µg/ml), then incubated for additional 48h at 37̊C. Cancer and normal cell lines elucidate various degree of sensitivity to the cytotoxic effects of sumac extract. The results illustrated that after 48h of exposure, cells showed highest toxicity (80.28%) of the ethanol extract on RD moderate cytotoxicity (52.64%) on Hep2 and slight toxicity (37.158%) on normal cell line (Ref) in a concentration (1.953µg/ml). The study was expanded to include the effects on some immune cells in some organs (liver, spleen, and small intestine). Three groups of mice (six animals each) were ingested sumac extract in different concentrations (0.825, 1.65, and 3.3 mg/kg. of animal body weight).The study showed immune cell (mononuclear and lymphocyte) stimulation effects of the extract in all organs. Microscopically changes in the tissue structure of these organs were observed. Liver showed necrosis increase as the time of exposure and the concentrations increase. Changes in the red pulp and white pulp were observed in spleen, while the small intestine revealed mild atrophy of villi and infiltration of mononuclear cells. These effects were increased as the concentrations of the extract and the time of exposure increased.

KEYWORDS: liver, spleen, and small intestine**.**

INTRODUCTION

Sumac is the common name for a genus *Rhus coriaria* that contain over 250 individual species of flowering plants in the family Anacardiacea.[1] This genus is found in temperate and tropical regions worldwide. It can grow in non-agriculturally variable regions, and various species have been used by indigenous cultures for medicinal and other attributed to these dietary compounds, including antioxidant,[1] anti-inflammatory,[2] and anti-carcinogenic,[3] anti-fibrogenic,[4] hypoglycemic,[5] antiplatelet effect,[6] and reported to have immunomodulatory property.[7]

Alcohol extract from sumac had the highest amount of phenolic compounds represented in flavonoids and tannins and it also exerted the highest percent of growth inhibition in many cell lines,[3, 8, 9] Plants are the essential and integral part in complementary and alternative medicine because they develop the ability for the formation of secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substance which are in turn used to restore health and heal many disease.[10]

Many medicinal plants and pharmaceutical drugs are therapeutic at one does and toxic at another, most reports concerning the toxic effect of herbal medicines are associated with hepatotoxicity although of other toxic effects including kidney, nervous system, blood, cardiovascular and dermatologic effect, mutagenicity and carcinogenicity have also been published in the medical literatures.[11]

This study aimed to evaluate the antitumor activity of ethanol extract of sumac in vitro by using two cancer cell lines Hep2 (Human larynx epidermoid carcinoma), RD (Rabdomyosarcoma) and one normal cell line Ref. (Rat embryonic fibroblast), furthermore to evaluate immunomodulatory effects and histological changes of the administrated extract in experimental animals.

MATERIALS AND METHODS

All the chemicals were obtained from Sigma chemical Co. (USA) and BDH abundance for each of populations (England), and the fruits of the plants were purchased from Irbil local market.

Extraction of Sumac Active Compounds

Active constitutions were extracted according to (Ji, *et al*). 50 gm of sumac fruit powder was extracted twice with 250 ml of 80% ethanol for 1 h at room temperature, then the mixture was filtered through Whatman filter paper No.1 by vacuum pump. The two filtrate of the extract were evaporated at 40Ċ and then freeze dried, the final yield was 3gm (6%) of the extract with a concentration of 0.2 gm/ml. The ethanol extract was kept at refrigerator for further experiments.

Phytochemicals Examinations

The ethanol extract was examined for the presence of phytochemical materials by the following methods:

Tannins: were identified by the method described by Shihata.[13]

Polyphenols: were identified according to Harborne.[14]

Flavonoids: were identified according to Jaffer *et al*.[15]

Resins: were identified according to Shihata.[13]

Alkaloids: were identified according to Stahl.[16]

Couumarins: were identified according to Geissman.[17]

Saponines: were identified according to Shihata.[13]

Cell line Growth and Cytotoxicity Assay

Cell culture: Hep2 (Human Larynx Epidermoid carcinoma), RD(Rhabdomyosarcoma) and Ref(Rat embryo fibroblast) cell lines were obtained from Iraqi center for cancer reserch and medical genetic , Al Mustansiria University. The cells were grown in RPMI-1640 medium containing 10% fetal calf serum and incubated at 37 Ċ to form confluent monolayer.

The attached cells washed firstly with PBS and harvested from the tissue culture flask by treatment with trypsin- versine solution. The cells were counted by trypan-blue (about 95% viability), 200µl of cell suspension were seeded in each well of microtiter plate and incubated in CO2 incubator for 24h at 37 Ċ, then 200µl of different concentrations of sumac extract represented in (7.812, 1.95, 0.976, 0.488, 0.244, and 0.122) µg/ml were prepared in serum free media (SFM) and added to cells, then re-incubate the plates for additional 48h. furthermore, each concentration were replicated in three wells and negative control wells were treated with SFM only.[18] At the end of exposure period, the cells were stained by 100µl of crystal violet solution and incubated at 37 Ċ for 30 mins.[19] The optical density of

each well was read by micro-ELISA reader at 492 nm. The percentage of inhibition rate was calculated according to Delaquis *et al.*[20]

$$\text{Inhibition rate (IR)} = \frac{\text{optical density of control wells} - \text{optical density of test wells}}{\text{optical density of control wells}} \times 100$$

Statistical analysis: The experiments data were analyzed using statistical software SPSS version 16, significant differences between treatments were assessed using student´s T-test and P values ≤ 0.05 were considered significant.

Experimental Animals

Twenty four mice were divided in to four groups, each group contain 6 mice. One of these groups is control group, while the others were ingested a daily dose of 100 µl of three different concentrations of sumac ethanol extract represent (3.3, 1.65, and 0.825) mg/kg of the body weight respectively.

Three mice of each group were sacrificed after 14 days while the other three were sacrificed after 21 days of ingestion. To evaluate the immunological and histopathological effects of the extract on some organs of mice, liver, spleen, and small intestine were taken from sacrificed animals and preserved with 10% formalin, and histological sections were done.

RESULTS AND DISCUSSION

From the obtained results it could be concluded that the major constituent in sumac were flavonoids, tannins, and alkaloids while the minor constituents were polyphenols, resins, alkaloids and saponins. (table 1). This results come in agreement with the results obtained by Abou- Hashem and Amy [21, 22] who reported the presence of all these compounds in 80% ethanolic extract of sumac.

Table 1: The qualitative tests of the main constitutes of the ethanolic extract of sumac. +++ high amount, ++ moderate amount, and + slight amount.

Constituents	Amount In The Extract
Flavonoids	+++
Polyphenols	+
Tannins	++
Resins	+
Alkaloids	+
Couumarins	-
Saponins	+

To investigate the effects of sumac extract on the growth of two cell lines (Hep2 and RD) and one normal cell line (Ref), the cells cultures exposed to six different concentrations of the ethanol extract (0.244, 0.488, 0.978, 1.953, 3.9, and 7.81) µg/ml. The results showed a significant differences ($p<0.05$) in percentages of inhibition rate (IR) on Hep2 cell line as compared to control, depending on the concentration of the extract, the IR was 18.75% at a concentration 0.244 µg/ml than increases to 52.64% at a concentration 1.953 µg/ml, than it decreased at higher concentrations of the extract (fig. 1).

The same pattern of inhibition rate was revealed in the growth of RD cell line, the results illustrated that IR was 45.3% at a concentration of 0.244 µg/ml and increased significantly ($p<0.05$) to 80.28% at a concentration of 1.953 µg/ml, and the inhibition rate decreases by increasing concentration of the extract (fig. 2). From these results it is clear that the effects of the active compound obvious in low concentrations.

Cultured normal cell line (Ref) showed a significant differences ($p<0.05$) in percentage of inhibition rate, the highest IR was from 66.67% at low concentration 0.244 µg/ml, and decreased significantly as the concentration of the extract increased, and the lowest IR was 1.64% at a concentration 7.81 µg/ml the normal cell line (fig 3). The results were closely related to the results obtained by Jeong- Chae *et al*. [23] that is the addition of 50 µg/ml of extract increase thymocyte viability by 79.4% - 84.3%. It is also proved by [24] that some flavonoids are cytotoxic at a higher concentration toward human normal cells.

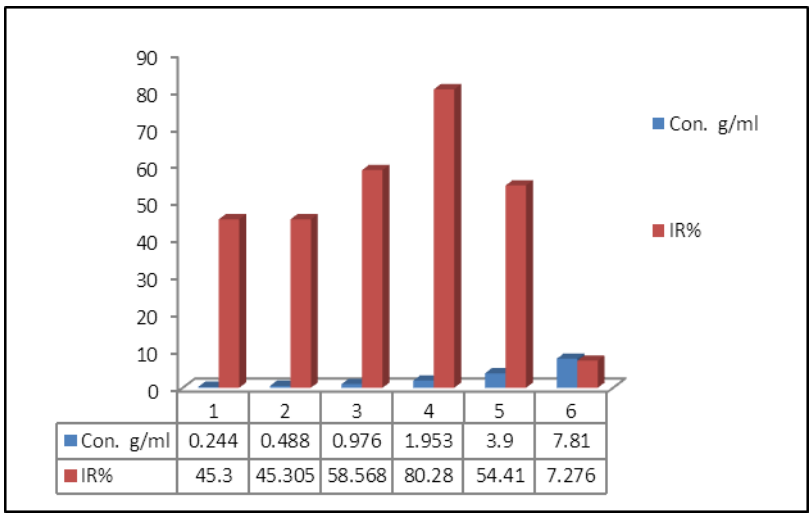
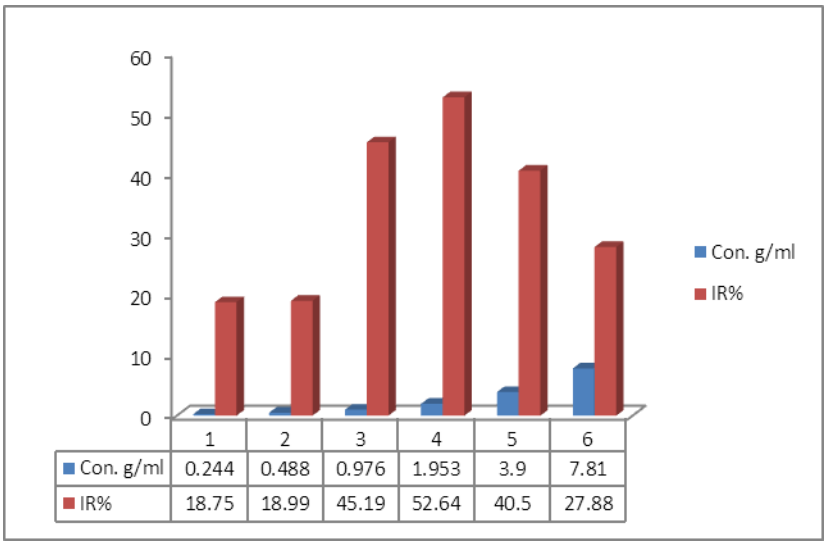
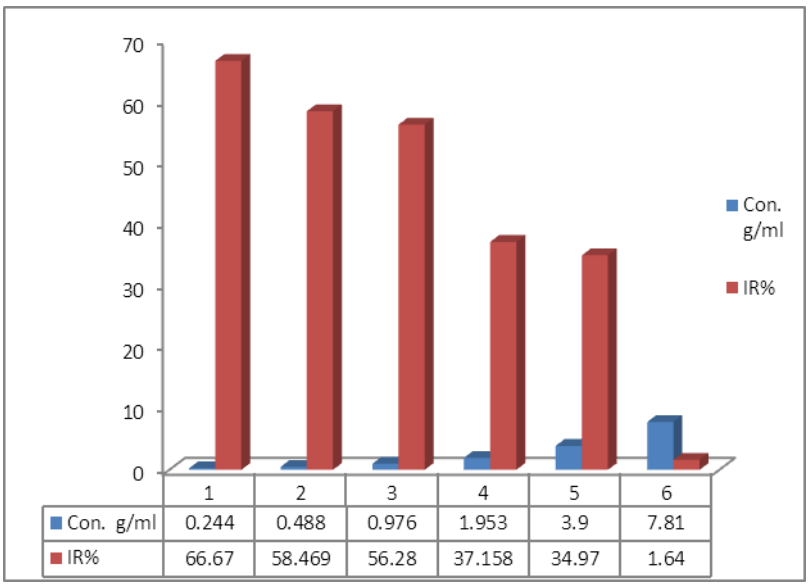


Fig. 1: Percentage of cytotoxicity represented by inhibition rate (IR) in RD cell line treated with different concentrations of sumac ethanolic extract after 48h of exposure.



	1	2	3	4	5	6
Con. g/ml	0.244	0.488	0.976	1.953	3.9	7.81
IR%	18.75	18.99	45.19	52.64	40.5	27.88

Fig. 2: Percentage of cytotoxicity represented by inhibition rate (IR) in Hep2 cell line treated with different concentrations of sumac ethanolic extract after 48h of exposure.



	1	2	3	4	5	6
Con. g/ml	0.244	0.488	0.976	1.953	3.9	7.81
IR%	66.67	58.469	56.28	37.158	34.97	1.64

Fig. 3: Percentage of cytotoxicity represented by inhibition rate (IR) in REF cell line treated with different concentrations of sumac ethanolic extract after 48h of exposure.

From the results obtained, it can be concluded that the strongest cytotoxicity of the extract was at a concentration 1.953 µg/ml, since it is effective against Hep2 and RD cell line and less effective against normal cell line Ref.

These results come in agreement with the previously improved results which used the *Rhus spp*. extract as antitumor and antioxidant.[7, 25, 23, 26, 27]

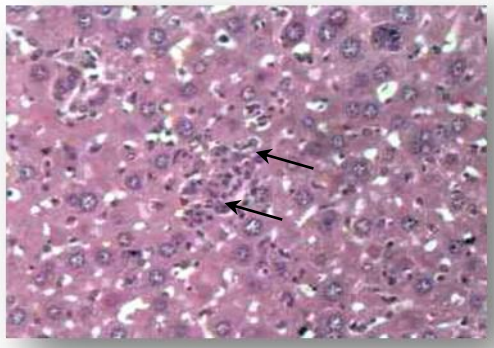
The addition of the ethanolic extract of sumac to the cultured cell line promote cell cycle arrest at either the G0/G1 or G2/M phase rather than causing cell damage directly,[25] and

eventually induced apoptosis in cell line.[12] It was believed that the induction of apoptosis is related to the inhibition of the activity of signal transduction molecules involving in the cell cycle, since certain antioxidants, such as flavonoids, have been reported to exert inhibitory effects on P13- kinase,[12] protein kinase C, protein tyrosine kinase, and some transcriptional factors. Furthermore, such inhibition has, in turn, been shown to arrest cell growth and induce cell death in several carcinoma cell lines.[28, 29] The doses studied *in vivo* were (200, 100, and 50) mg/kg of body weight and the animals were observed for maximum of 21 days. No mortality occurred during the time of study. the result agree with the results of [30] in using a dose up to 3g/kg of ethanol extract in mice and no mortality effects occurred.

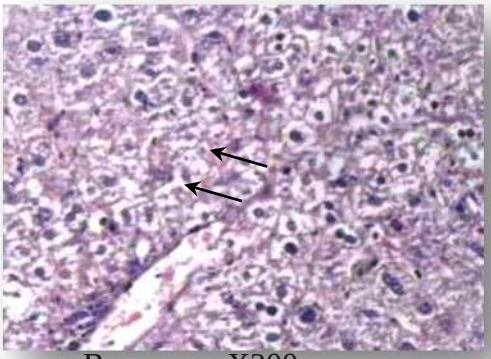
**after 14 days of ingestion in mice, liver showed dispersed foci of necrosis with inflammatory cells infiltration mononuclear cells and showed kupffer cells hyperplasia. This effect increased as the concentration of the ingested extract increased until it shows congestion and mild hydropic degeneration. (fig. 4:A, B, C, D) as compared with control (fig.4E).

In 50 mg/ml, the spleen showed widening of white pulp and follicular hyperplasia with reduction of red pulp (fig. 5A). This effects increase as the dose of the ingested extract increased. At 100 mg/kg spleen shows slight necrosis of parenchymal tissue. (fig. 5B) and at a dose 200 mg/kg, it shows follicular hyperplasia were seen with the widening of the white pulp and necrosis of the red pulp (fig. 5C) as compared with the control (fig. 5D).

The intestine showed normal looking appearance after 14 days of ingestion with mild inflammatory cells infiltration at a dose 50 mg/kg (fig. 6A), and at 100 mg/kg, mild atrophy of villi were observed and slight inflammatory cells infiltration (fig. 6B). While at 200 mg/kg, un normal looking of the intestine was detected with mild atrophy of villi and infiltrations of mononuclear cells were seen (fig. 6C) as compared with control (fig. 6D).



A X200



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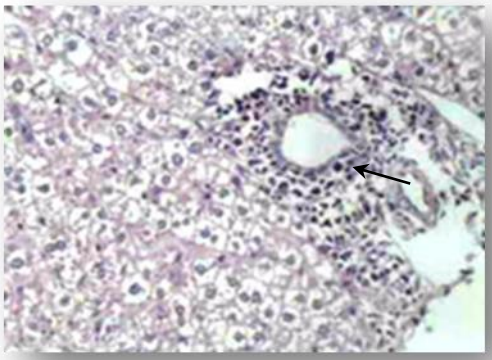
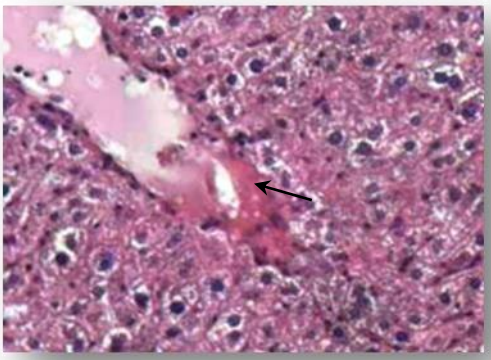
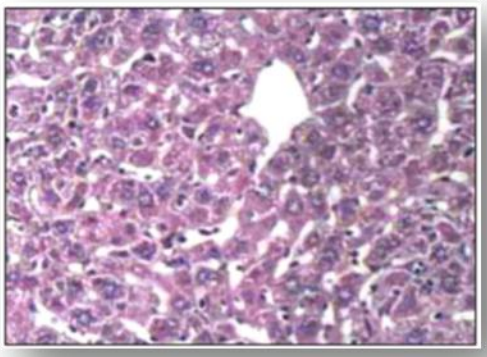


C X200

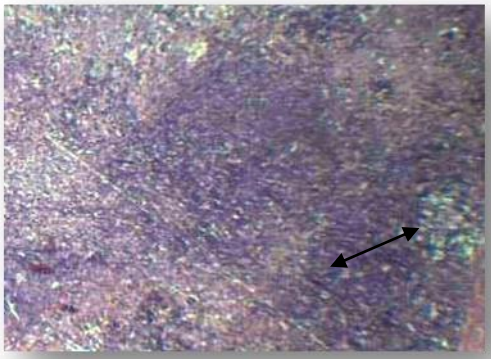


D X200

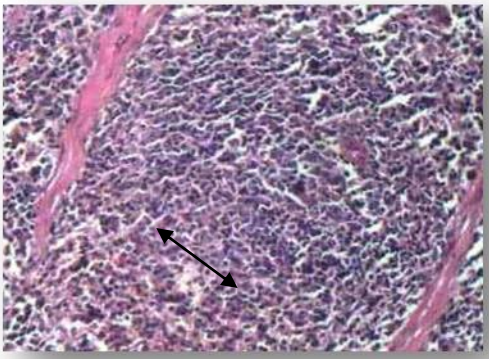


E X200

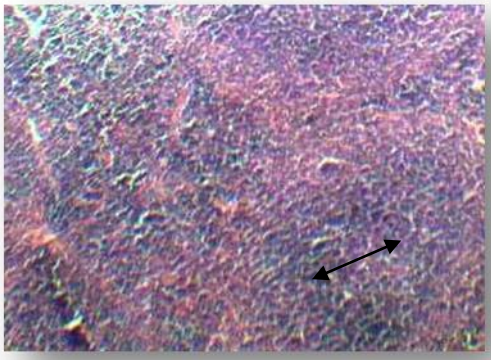
Fig. 4: Shows the effects of sumac ethanolic extract on liver after 14 days of ingestion as compared with the control. A: at a dose of 50 mg/kg. B and C: 100 mg/kg. D: 200 mg/kg. E: control.



A X100



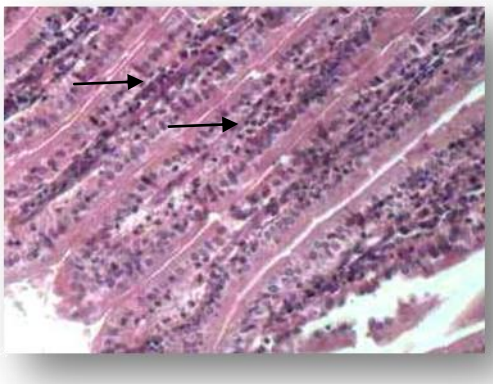
B X100



C X100

D X100

Fig. 5: Shows the effects of sumac ethanolic extract on spleen after 14 days of ingestion as compared with the control. A: at a dose of 50 mg/kg . B : 100 mg/kg. C: 200 mg/kg. D: control.



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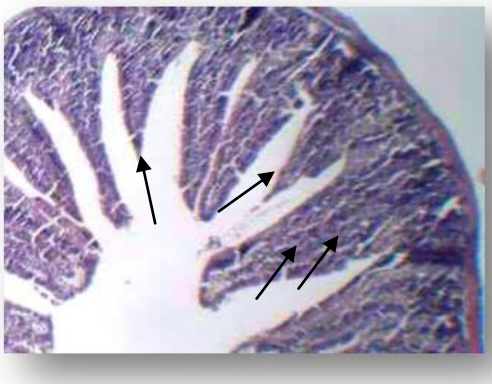

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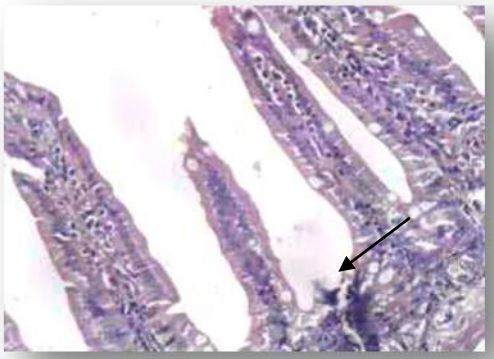

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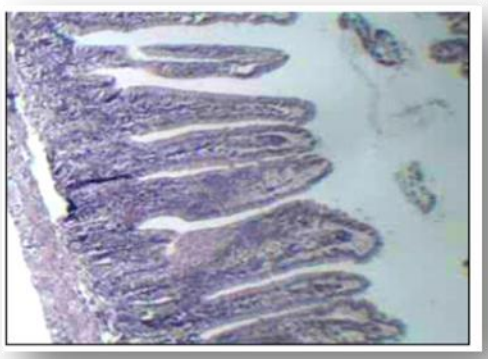
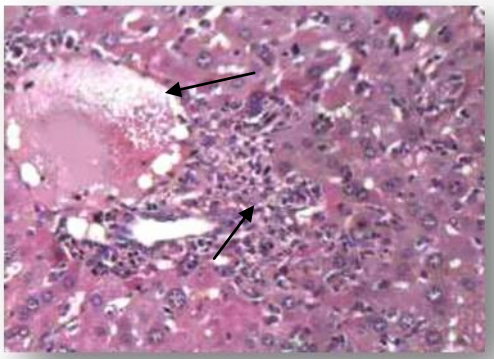


D X200

Fig. 6: Shows the effects of sumac ethanolic extract on intestine after 14 days of ingestion as compared with the control. A: at a dose of 50 mg/kg, B: 100 mg/kg. C: 200 mg/kg. D: control.

When the time of ingestion expand to 21 days dramatic changes were observed. The liver showed congestion, prominent inflammation, immune cells infiltration, mild degenerative changes and necrosis at all doses used (fig. 7A, B, C) as compared with the control (fig. 7C). For the same period, spleen showed diffused hyperplasia of parenchymal cells with mild necrotic cells at a dose 50 mg/kg (fig. 8A). In addition a lymphoid tissue atrophy were seen at a dose 100mg/ml. (fig.8B), while at a dose of 200 mg/kg a follicular hyperplasia and widening of the white pulp of the spleen were observed (fig. 8C) as compared with the control (fig. 8D).

The intestine showed un normal looking appearance at a dose 50 mg/kg, while prominent atrophy of villi with mild inflammatory cells infiltration were detected at a dose 100 mg/kg (fig. 9B), And at a dose 200 mg/kg the same effects were detected in addition to infiltration of mononuclear cells in tissue (fig. 9C) as compared with the control (fig. 9D).



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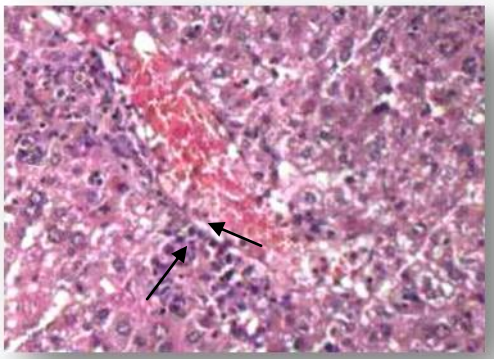

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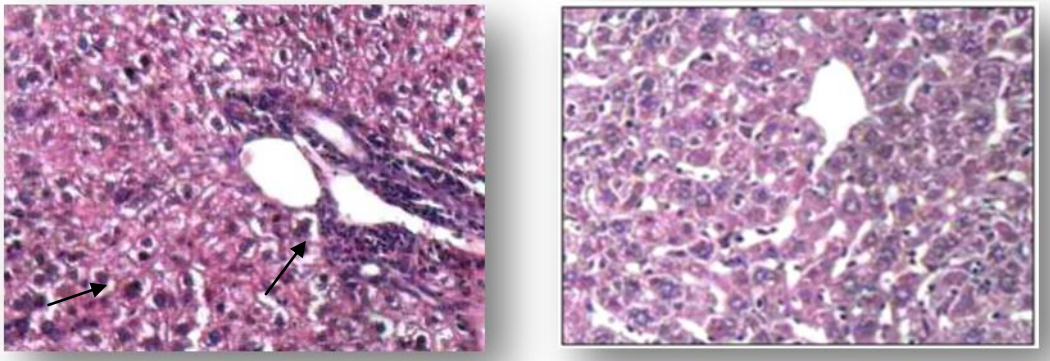
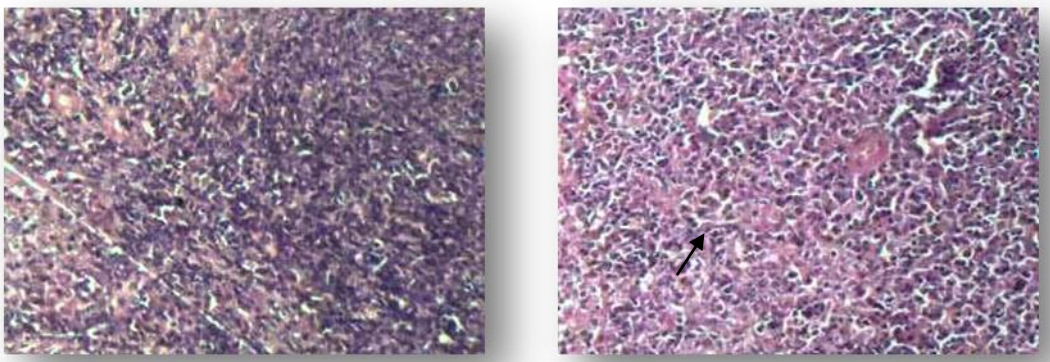
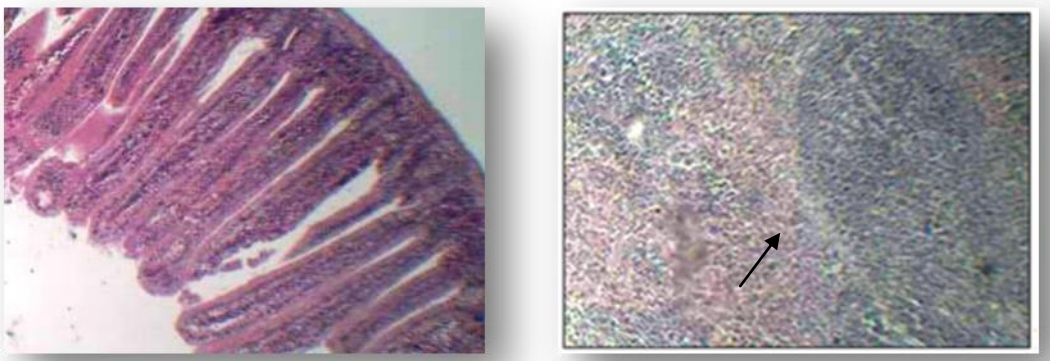


C X200 D X200

Fig. 7: Shows the effects of sumac ethanolic extract on liver after 21 days of ingestion, as compared with the control. A: at a dose of 50 mg/kg. B: 100 mg/kg. C: 200 mg/kg. D: control.



A X200 B X200



C X200 D X200

Fig. 8: Shows the effects of sumac ethanolic extract on spleen after 21 days of ingestion, as compared with the control. A: at a dose of 50 mg/kg. B: 100 mg/kg. C: 200 mg/kg. D: control.

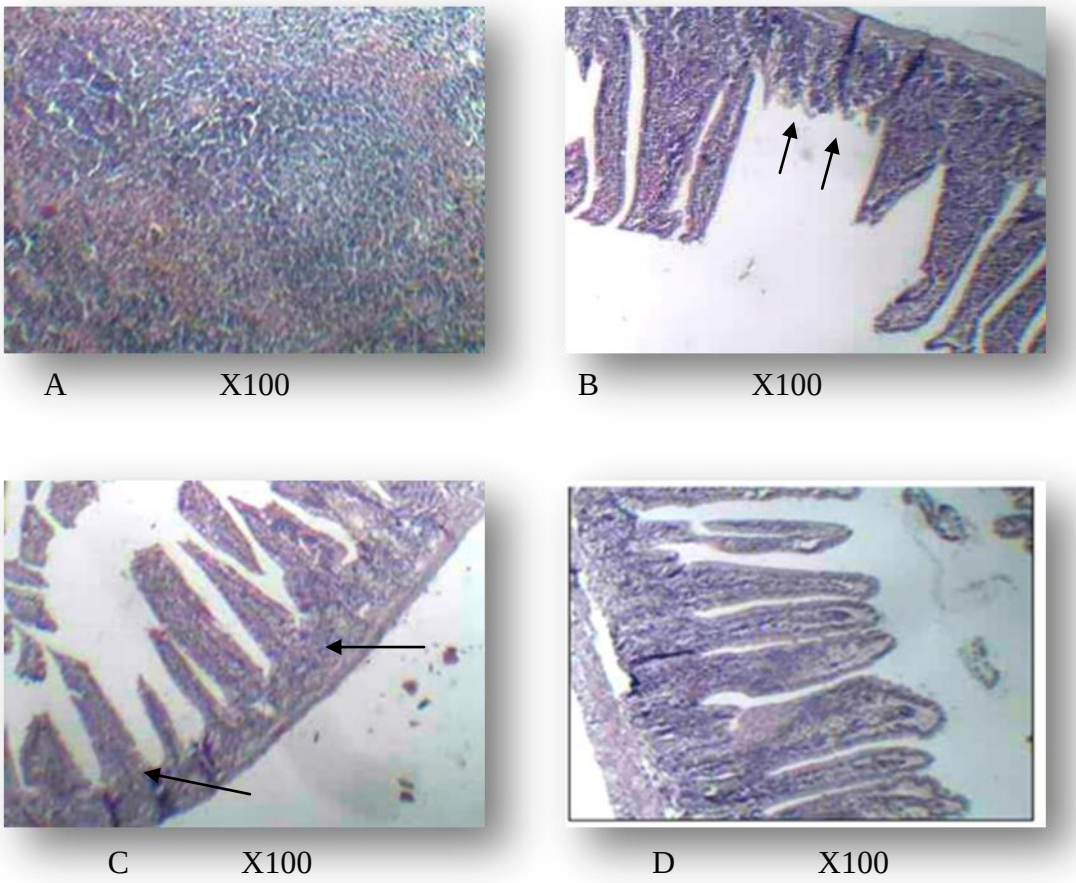


Fig. 9: Shows the effects of sumac ethanolic extract on intestine after 21 days of ingestion, as compared with the control. A: at a dose of 50 mg/kg. B: 100 mg/kg. C: 200 mg/kg. D: control.

The present study indicates large effects on liver supposing that the liver is the major site of detoxification in the body for all drugs/ toxins. Therefore, it is an important organ in any toxicological study.[31] Interestingly, many medicinal plants have been found to be toxic to the liver suggestive of a possible toxic effect of sumac at high doses. My results come in agreements with the results of Pamukcu *et al*. [32] who reported gross abnormalities in liver and other organs including intestine, stomach, kidney, urinary bladder, lung, and brain studied by light microscope.

It is well known that the susceptibility of the animals of feeding plant material is dependent on the type of the active constituents and concentrations in the amount added to the diet as well as on the rate of their metabolic conversion in the liver to metabolites and consequent excretion,[33] since that the crud extract was used in feeding animals and it is reported to contain flavonoids, tannins, and polyphenols.

Results reported by 29 indicate toxicity of some flavonoids at high doses in experiment in dogs. Other results obtained by [34] showed that flavonoids have toxic effects on the spleen, pancreas and heart of male albino rats. [35] elucidate that animals treated with one type of flavonoids(quercetin) were able to induce focal areas of dysplasia in 22% of normal mice and perturb the growth program of normal colonic epithelial cells by enhancing the apoptotic process, and in another study it exhibit intestinal tumors with intussusception and obstruction.[36] Other results suggest that dietary flavonoids may have the potential for producing intestinal injury.[37] So the use of these compounds in specific dose is recommended.

Tannins were reported to have toxic effects when absorbed from small intestine. The *in vivo* study did not support results obtained *in vitro* because mice fed ethanolic extract exhibit a gross histological changes. One of the reasons for this is that for *in vitro* study, compounds (extract) were directly put in contact with the cancer cells; in the animal study, compounds were delivered through the diet and were absorbed and metabolized or modified in different ways inside the body.[8] the same explanation demonstrated by,[37] for *in vivo* study, he mentioned the limitation of bioviolability of the extract following digestive and absorption, or the fact that hepatic first pass metabolism of bioactive constituents may limit the exposure at target organs, in addition many medicinal herbs and pharmaceutical drugs are therapeutic at one dose and toxic at another.[11]

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