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Activity of Ethanolic Extraction of *Malva parviflora* and *liqourice* as Antifungal and Antioxidant in male rats

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Abstract

The current work intended to appraise the efficacy of *Malva parviflora* and liqourice extracts against the hepatic toxicity encouraged by Ochratoxin, this study directed on matured rats male for the duration from July 2017 to October 2017. Forty ripe males Wister rats (measured 190±10 g and elderly 90 days), four identical sets arbitrarily distributed, first one negative control received solitary normal saline, and second positive control received *Asp. niger* with contaminated diet as a single dose(2.5 mg/kg b.w). Third group received *Asp. niger* with contaminated as a lone dose thereafter treated after 2 days with *Malva parviflora* (250 mg /kg b.w.) respectively through stomach orally igniter during 42 days. Fourth group received *Asp. niger* with contaminated as a singular dose after 2 days treated with *liqourice* (250 mg /kg b.w.) with respect through stomach orally igniter within 42 days. Fifth group received *Asp. niger* with contaminated as a singular dose, then 2 days treated with *Malva parviflora* and liqourice together (250,250 mg /kg b.w.) correspondingly within 42 days through stomach tube orally. Males drugged (by injection *ip* of 0.3ml+ 0.1 ml ketamine and xylazine respectively /kg b. w. ip), specimens were gained from heart blood and collected in tubes non-heparinized, separated samples of Blood serum were done for of GOT, GPT estimation. Under our experimental conditions, contaminated with *Asp. niger* resulted evidenced by statistically considerable increase($p>0.05$) in the activities of GOT, GPT in group 2 of just Ochratoxin contrast with the control group, while in the third and fourth groups displayed statistically significant lessening ($p<0.05$) in GOT, GPT level in blood serum of contaminated with *Asp. niger* treated group that treated in contrast with control, histological analysis of group contaminated with *Asp. niger* proved change in the, after the gavage of (*Malva parviflora and liqourice*) The results showed that there is an improvement in liver tissues after treatment with *Malva parviflora and liqourice.*

In present study, we concluded to *Malva parviflora and liqourice* alcoholic dose 250+250 mg/kg b.w. is better than dose 250mg/kg b.w. alone depending on results in recently study. We concluded that the administration of (*Malva parviflora* and liqourice) together act as antifungal and antioxidant for contaminated with *Asp. niger* in males rats.

Keywords: - *Malva parviflora* and liqourice, Males rats, GOT, GPT.

INTRODUCTION

Biotechnological and economical utility in *Asp. niger* had known as well as it is much hired for fabrication organic acids like citric acid and enzymes secrets extracellularly. [1,2] Food and Drug Administration office in United States donated that *A. niger* is Generally Regarded As Safe (GRAS) stature in some industrial production practicability. [3] Abarca *et al* found the treasure trove of *A. niger* strains by making Ochratoxin (OTA). [4] Advanced need employed by food safety prohibition because it had concerted occurrence in different wares as for biotechnological integrity. [5,6,7] hepatotoxicity, robust nephrotoxin of OTA and has carcinogenic merits, furthermore, teratogenic and immunosuppressive ability. Humans and animals assimilated OTA through grains and grain based feed and food because its fettle-over from raw stuff to processed outputs and settled beneath interred food processing employment conditions.[8] These interests developed laterally. Different reports refers that fumonisin B2(FB2−1) produce by *A. niger*.[9] Liqourice is a very well fine herb in conventional Chinese medicine (TCM). In China, it is called “gancao” (meaning “sweet grass”) and has been recorded in the Shennong's Classic of Materia Medica around 2100 BC. In this book, liqourice was supposed to have life-enhancing properties. During the following thousands of years liqourice has been present in most of Chinese traditional prescriptions. It was believed to have the functions of sustaining, mitigating pain, animating spleen and stomach, eliminating phlegm, and soothing coughing [10].

Malva is a widespread tropical and temperate genus of the family Malvaceae [11,12]. Many species of this genus are efficient in cough, ulcers inthe bladders, intestinal infections, colitis, tonsillitis, gastroenteritis, cholesterol and lipid-lowering, anti-hypertensive, antioxidant, analgesics, emollient, pectoralgirdle and arteriosclerosis treatment. In addition, the plants are also used externally as antidandruff, demulcent, softening of tumors and abscess [13]. *Malva parviflora* (known as cheese weed) is growing in waste ground, roadsides and desert plains in Egypt [11].

MATERIALS AND METHODS

Plant material collection:-

The leaves of *Malva parviflora* and liqourice plant were collected during August 2017, from Al-Dewanyia city from market, by researcher.

Preparation of Ethanolic Extracts *(Malva parviflora* and liqourice*)*:-

20gm of *Malva parviflora* and liqourice powdered leaves were taken and extracted with soxhlet apparatus ethanol (70%) Within 24 hours, after that then taking the extract and place it in a petry dishes and put in the oven along 48 hours in (40°C). This extract stays until use at (4°C) [**14**].

Experimental design:-

Forty mature males wistar rat divided to five groups:-

C: Eight mature males rat are gavage normal saline for 42 days.

T1: Eight mature males rat are received with contaminated with *Asp.niger* single dose for 42 days.

T2: Eight mature males rat are received with contaminated with *Asp.niger* single dose and then treated after 2 days with *Malva parviflora* (250 mg/kg b.w.) for 42 days.

T3: Eight mature males rat are received with contaminated with *Asp.niger* one dose and 2 days later treated by *liqourice* (250 mg/kg b.w.) for 42 days.

T4: Eight mature males rat are received with contaminated with *Asp.niger* single dose and then mixed treated after 2 days with *liqourice and Malva parviflora* (250+250 mg /kg b.w.) for 42 days.

Preparation of Serum:-

Tubes with cap contains blood and pliable to clot (within 20 min), tubes were centrifuged at (4000 rpm) for 10 minutes in order to serum separation [**15**]. The serum of each animal separated

subdivided for (6) samples using appendroff tubes (0.5ml) and kept at deep freezer until using for biochemical tests estimation.

Assay of GTP and GOT Biochemically

GTP and GOT are determinate by spectrophotometer (Biochemical test) to GPT and GOT this kits it's from US bio USA.

Microscopic inspection

Liver were betimes fixed and extirpated in neutral buffer solution (10%formalin). Washing of trimmed tissues with tap water followed by graded series of alcohol for dehydration then posteriorly through xylol and paraffin series previously in paraffin entrenched. The blocks of paraffin were incised into sections 5-6 µm which stained with hematoxylin and Eosin for light microscope checking [**16**].

RESULT

Serum GOT concentration

Significant varieties showed in figure (1) between all groups and control. In group T1 with gavage Ochratoxin showed known increase ($p<0.05$) of GOT concentration in serum when comparing it with control, and in groups gavage with single dose of Ochratoxin and continuous gavage *Malva parviflora* and liqourice daily (T2, T3) (250mg/kg B.W.), showed significant lowering ($p>0.05$) in serum concentration of GOT compared with Ochratoxin group and in mixed groups (T4)(250+250 mg/kg B.W.).

GPT concentration in Serum

Figure (2) illustrated significant difference between all experimental groups and control group. In group (T1) with gavage Ochratoxin showed significant raise ($p>0.05$) of GPT concentration in serum as comparing the with the group of control, and in groups gavage with single dose of Ochratoxin and continuous gavage *Malva parviflora* and liqourice daily (T2,T3)(250mg/kg B.W.) is showed significant reduction ($p<0.05$) in GPT concentration contrasted with the Ochratoxin group and in mixed groups(T4)(250+250mg/kg B.W.).

Histopathology profile

The sections obtained from liver of rat were explained of the many histological changes in tissue of liver in all groups of experiments, in control group was explain central vein surrounded by clear radial arrangement and cytoplasm acidophil with hexagonal shape in hepatocyte and liver tissue with permanent nuclei appeared centrally (Fig.3,4). While in Ochratoxin group, hepatic tissue clearly showed spacious necrosis, hepatic cords around the central vein lacks their radial arrangement in liver tissue, bile duct hyper aplasia with congestion. (Fig.5,6).

As for the *Malva parviflora* group was manifested with bionuclatied cells, hepatocyte degenerated, vein with normal control, hepatocyte radially arranged and. (Fig.7, 8).

While the *liqourice* group some cell was showed bionuclatied, hepatocyte degeneration, vein control normally, liver tissue proliferated and hepatocyte with radial arrangement (Fig9, 10).

Finally, in *Malva parviflora* and liqourice group was explained hepatocyte radially arranged, hepatocyte clearly regenerated of which bionuclatid and vacuolated, cytoside tissue of liver and mild dilation. (Fig.11, 12).

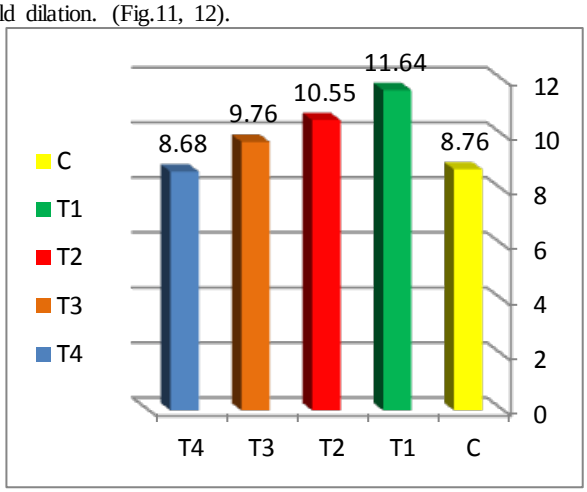

Figure (4-1): Effect of *Malva parviflora* and liqourice treatment on concentration (µMole/ml) in serum GOT of gavaged Ochratoxin in male rats.

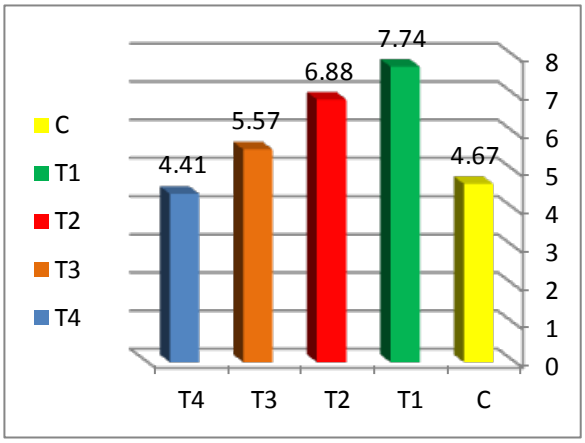

Figure (4-2): Effect of *Malva parviflora* and liqourice treatment on serum GPT concentration (µMole/ml) in mature male rats gavaged Ochratoxin.

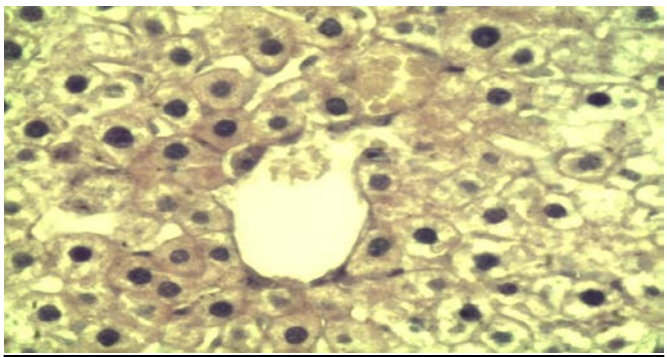

Fig. (3) Section of Liver of rat, Gavage of N.S control group. Central vein surrounded by normal radial arrangement, hexagonal form of hepatocyte with cytoplasm acidophilic, liver tissue with nuclei centrally permanent. (H&E x40)

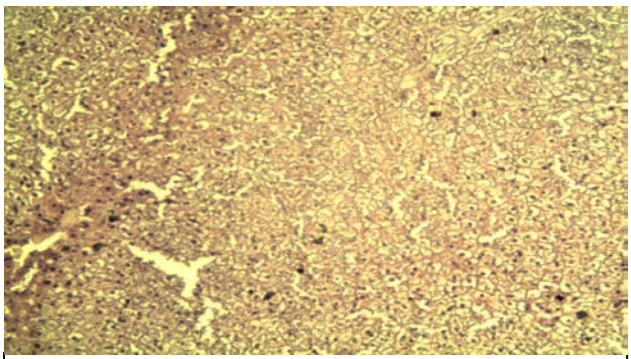

Fig.(4) sec. of Liver of rat , Gavage of OTA (2.5g / kg b.w) once for 42 days. Hepatic tissue with spacious necrosis, hepatic cords around the central vein lacks their radial arrangement.(H&E 10).

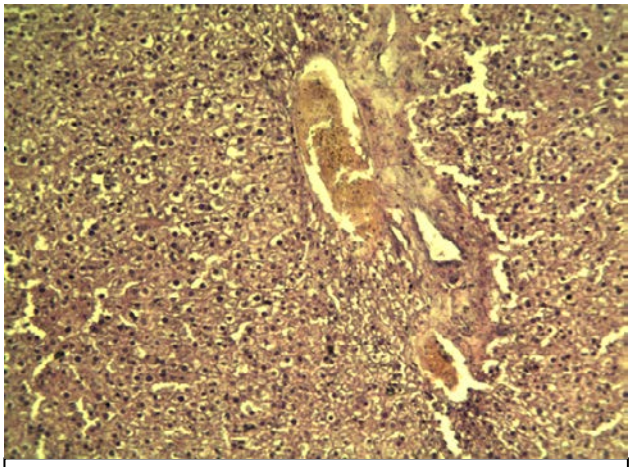

Fig.(5) section of Liver of rat , Gavage of OTA (2.5g / kg b.w) once for 42 days. congestion and hyper aplasia of bile duct in liver tissue.(H&E 10).

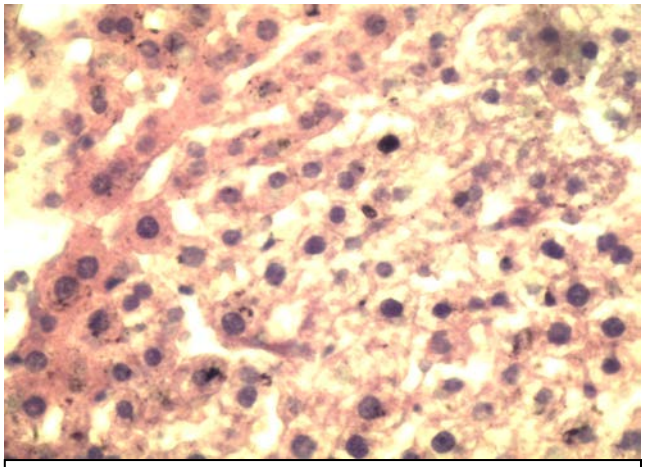

Fig.(6) liver of rat , Gavage of OTA (2.5g / kg b.w.) one time and Gavage for 42 days . some of cell showed bionuclatied, degeneration

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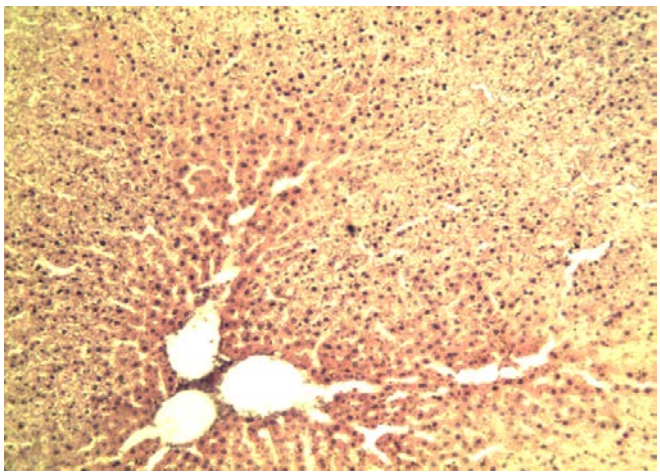

FIG(7) liver of rat , Gavage of OTA (2.5g / kg b.w.) one time and Gavage of *malva*. 250mg/kg b.w.) for 42 days. normal control vein , liver tissue proliferation and hepatocyte with radial arrangement. (H&E x10).

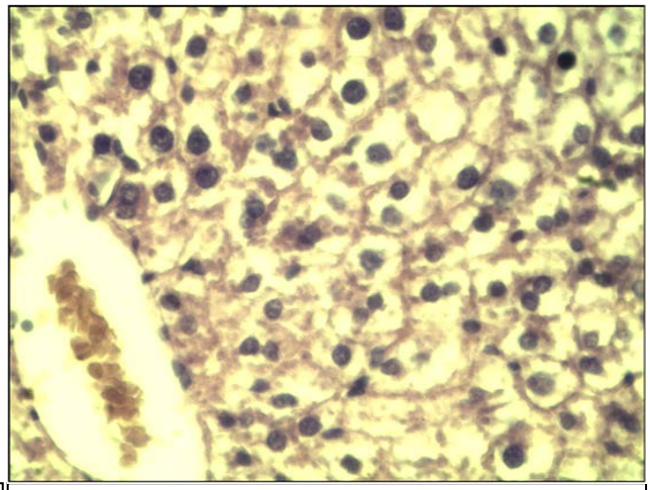

Fig.(8) Liver of rat , Gavage of OTA (2.5g / kg b.w.) one time and Gavage of *malva*. 250mg/kg b.w.) for 42 days . Normal radial arrangement hepatocyte In liver tissue.(H&Ex10)

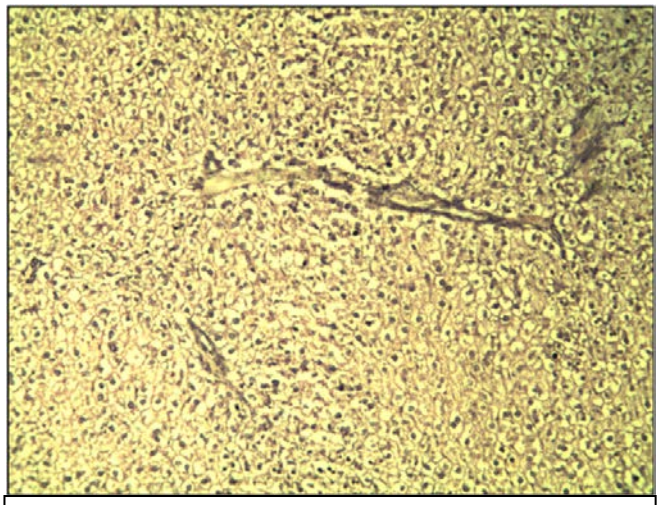

Fig. (9) Liver of rat , Gavage of OTA (2.5g / kg b.w.) one time and Gavage of ***licorice***. 250mg/kg b.w.) for 42 days. Normal control vein, liver tissue proliferation and hepatocyte with radial arrangement.

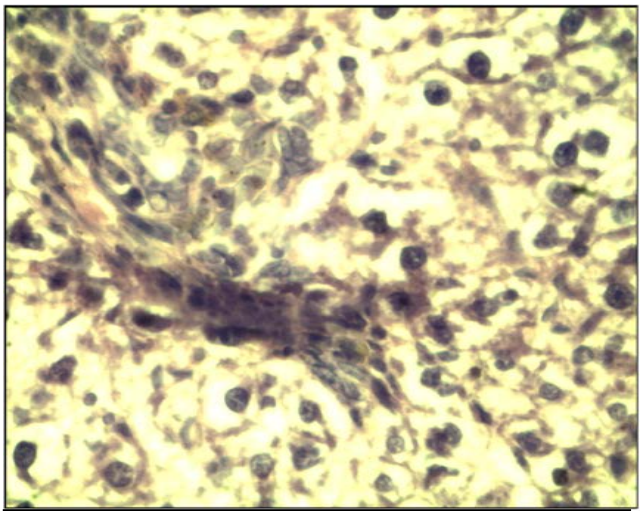

Fig. (10) Liver of rat , Gavage of OTA (2.5g / kg b.w.) one time and Gavage of ***licorice***.250mg/kg b.w.) for 42 days . Normal radial arrangement hepatocyte In liver tissue (H&Ex40)

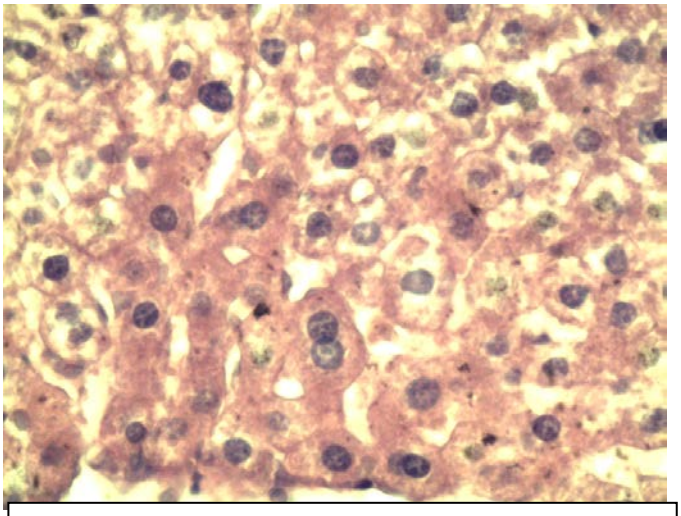

Fig. (11) Liver of rat, Gavage of OTA (2.5g / kg b.w.) one time and Gavage of *malva&linoice* . 250+250mg/kg b.w.) for 42 days .clear regeneration of hepatocyte which showed vacuolated and bionuclatid,

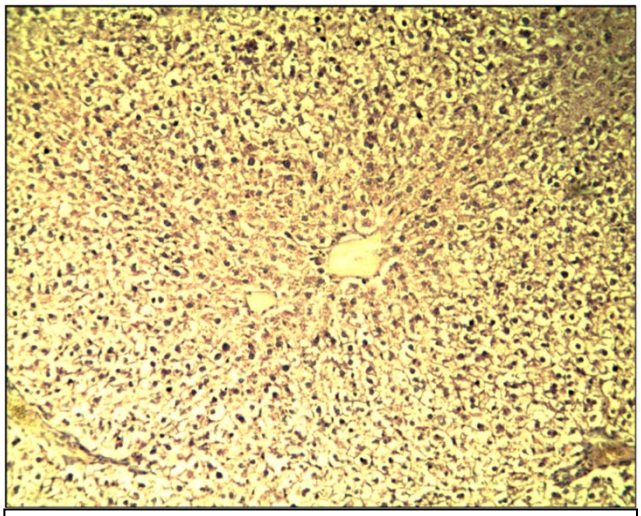

Fig. (12) Liver of rat, Gavage of OTA (2.5g / kg b.w.) one time and Gavage of *malva&linoice.* 250+250mg/kg b.w.) for 42 days . Clear regeneration of hepatocyte which showed vacuolated and

DISCUSSION

The object of this study was to scrutinize the conservative effect of *Malva parviflora and liqourice* on liver toxicity induced by Ochratoxin manifested GOT and GPT levels in blood serum biochemically and histopathological changes. Indeed, our study visibly evince that acute Ochratoxin. In the existent work, we found also that Ochratoxin-induced Liver damage by histological changes in liver of rat were explained of the many histological changes in tissue of liver in all groups of experiments, in control group was explain central vein surrounded by natural radial arrangement and liver tissue appeared with permanent nuclei centrally located and cytoplasm acidophil with hexagonal form of hepatocyte. Whilst in Ochratoxin group was showed hepatocyte highly necrosis, hepatic cords around the central vein lacks their radial arrangement in liver tissue also hyper aplasia of bile duct and congestion. As for the *malva* group explained some cell was showed bionuclatied, hepatocyte degeneration, vein control normally, liver tissue proliferated and hepatocyte with radial arrangement**.**

The *liqourice* group explained certain cells was showed bionuclatied, hepatocyte degeneration, vein control normally, liver tissue proliferated and hepatocyte with radial arrangement. Mixed group of *malva and liqourice* was explained hepatocyte with radial arrangement normally, obvious hepatocyte regeneration appeared vacuolated and bionuclatid, liver tissue cytoside mild dilation and when we discussed of recent results the influence of extract on regeneration of hepatocytes is very good by as antioxidants activity and the extract is richen with the flavonoids, phenols and alkaloid this compound is very active to repair of tissues of liver and regeneration, also exerts antifungals features also perhaps associated with the defense through scavenging ROS leads to oxidative exertion [17, 18]. In Histopathological view plant extract helps Liver ameliorations with biochemical [19]. Plasma proteins falling ratio attributed by Ochratoxin direction scrimpy efficiency of damaged Livers to filter also protein reabsorb [20]. This research refers that protective effective of two extracts towords induced Ochratoxin hepatotoxicity through restoring most GOT and GPT ordinary activities. The casement Liver damage was certained by microscopy examination. This facts are in consent with researchers those earlier mentioned [20]. Plant active compounds such as phenolics, flavonoids and flavonols had nutraceutical effects studies proclaimed that aids of the extract of our plant have been attributed to the, , and compounds [19,20]. Flavonoid location of in membrane, in one hand, and their fetters on membrane fluidity, in other hand, can be accurately interrupts free radicals resulting diffusion created during oxidation of Ochratoxin also through on diminish harmful effects that results [21]. Therefore, the protective role of plants extract might played against Ochratoxin intoxication by cellular GSH pool modulation [20]. According to the above, we proposed that plants did antioxidant effect against harmful species reactived oxygen that results the oxidation of Ochratoxin so could be inhibit damaging of liver.

The fungicidal effect plants extract employed against Chaetomium funicola M002 and Arthrinium sacchari M001, these compounds known as Glabridin (3-(2',4'- dihydroxyphenyl)-8-dimethylpyrano chroman). Others like OEL had broad effective versus filamentous fungi and several bacteria, like heat-stable bacilli include *Bacillus* spp. and *Alicyclobacillus* spp. Furthermore, Glabridin eradicate yeast, filament fungi and toughness adjusting effectivity towards *Candida albicans* in concentration 31.25-250 microg/mL as mentioned by Fatima A. *et al*. [22]. *In vivo* studies reveals that antifungal ability of Glabridin, glabrol and their derivatives against *Candida albicans* and *Mycobacterium smegmatis*. Liqourice Ethanolic extraction comes intact by their phenolic amounts through eliminating of free radicals with hydrogen-liberating, peroxidation of anti-lipid, chelating in metal ion and curtailment capability [23]. The remarkable antioxidant property of flavonoids in Liquorice stronger 100 times than vita E. liquorice flavonoids sweep 20.6% of free radicals with dose 2.58 mg/ml while vita E 11.2% with dose 258 mg/ml so that indicate to the massive ability of flavonoids.[24] found that liquorice flavonoids the most famed antioxidants [24].subsequently , cosmetic combinations that used in skin and hair protection include great concentration of liqourice [25].

Malva parviflora have higher antibacterial as well as antifungal activities against *Bacilluus subtilis, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Aspergillus niger* and *Aspergillus oryzae* than hexane and ethanol extract showing values of growth inhibition zones in the range of 11.67 to 15.58, 10.96 to 14.32, 13.03 to 16.00, 10.38 to 15.37, 10.19 to 14.25 and 10.97 to 16.58mm, respectively. These findings agree with those found in the previous studies [26; 27].

In this present study the results are showed the plants extract activity antifungal and antioxidants by single dose from *Malva* or *Liqourice*, whereas in mixed extract are very good results as antifungal or antioxidants.

CONCLUSION

In the present study concluded the extracted of this plants (*Malva and Liqourice)* are very important as the active compounds on the toxic and as antioxidants in this study singly and mixed. And the effected on OTA is very important because this toxins found in food and in the environment, to this us recommendation to more research on this plants.

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