*IOSR Journal Of Pharmacy*
*(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219*
*www.iosrphr.org Volume 5, Issue 1 (January 2015), PP. 48-53*

# Evaluation of antioxidant activities of *Cyperusrotundus* (Ethanolic extract and purified flavonoid) Against Tetrachloride-Induced Hepatic Damage in Mice

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**Abstract:** The presence of significant amount of phenolic compounds in the flavonoid purified extract and ethanol extract *Cyperusrotundus* may account for its high antioxidant activity and exhibit concentration.
In this study an interaction was carried out between one dose of $CCl_4$ (3.2 mg/Kg) and the two doses (150 and 300 mg/kg) of ethanolic extract and purified*Cyperusrotundus* and one dose of vitamin C (180 mg/kg) were evaluated in mice (*in vivo*) given injection for 14 days inducting biochemical function SGOT,SGPT and SALP) in liver homogenate, liver function enzymes and histopathological changes of liver.
The results showed that tetrachloride carbon declared obvious devastating effects presented by increasing significantly ($P<0.05$) in concentrations of enzymes SGOT and SGPT and ALP in the blood serum of mice and all of the flavonoids purified and ethanol extract *Cyperusrotundus* has decreasing significantly ($P<0.05$) from the increasing in the concentrations of enzymes levels after 14 days of feeding compared to the treatment of carbon tetrachloride. It was noted that there is an increase of uneven concentrations of enzymes in blood compared to the control group indicating that both purified flavonoid and ethanol extract *Cyperusrotundus* in providing production hepatic damage through these effects or made it within the normal level and repair the damage. If induced liver increased the values of effectiveness of the enzymes of blood in mice are increased after given these substances.

**Key words:**Antioxidant activity, *Cyperusrotundus*, liver function enzymes, Tetrachloride-induced hapatic damage

## I. Introduction

*Cyperusrotundus rhizome*is one of the worst weed plants in the world, although*C.rotundus*causes serious problems in more crops in more countries than any other weed [1].Previous phytochemical studies on *C.rotundus*revealed the presence of alkaloids, flavonoids, tannins, starch, glycosides and furochromones, and many novel sesquiterpenoids [2].

In Asian countries,*C.rotundus* (Cyperaceae) is a traditional herbal medicine used the treatment of stomach and bowel disorders, and inflammatory diseases, analgesic, stomach disorders and to relieve diarrhea[3].There are in Iraq 13 to 14 species distributed on the provinces of the country and the most important and common species in the province of Baghdad is Saad normal and the so-called purple who lives in the land wet sand and in the aspects of irrigation canals and river banks, a herbaceous plant Muammar based smooth free of papillae and capillaries.

The ratio of AST to ALT is sometimes useful in differentiating between causes of liver damage.Elevated AST levels are not specific for liver damage and AST has also been used as a cardiac marker [4, 5].ALP is also present in bone and placental tissue [5].But that the concentration in the liver, placenta and bone is usually high and that damage or injury of disease in these tissues leads to the liberation of this enzyme into the blood as is the case in some of the bone disease or liver [6].The aim of this study is to evaluating the physicochemical characteristics of the ethanolic extract and purified flavonoid and study the effect in mice compared to that caused by carbon tetrachloride as a hepatotoxic model.

## II. Materials and Methods

The tubers of *C. rotundus*Rhizomes from local Baghdad market and farms Basrah has been diagnosed by the College of Science / Baghdad University. cleaned it after that Broke and grinded it by using Electric grinder. The extract has been prepared according to the method used by Ozaki *et al* [7] *with some modification* .In this method used 50 gram of powder tubers of therhizomes of *C. rotundus* with 350 ml of petroleum ether solvent in the flask of extraction and the extraction conducted by using Soxhlet extractor at the temperature 50°C for 6 hours.After that took the second material after removing the oily material from it and put it in the volumetric flask and added to it a certain size up to 350 ml of the ethanol solvent in concentration 70% and left it at room temperature for 48 hours, the solution have been filtered through a filter paper (Whatman No.1) and

evaporated to dryness under vacuum at 40°C., the dried extract (which called ethanol extracted) and stored at 4 °C.

**Gel-filtration Sephadex LH-20 column :**

Sephadex LH-20 gel was preparedby weight 40 gram mixed with ethanol 99.9% solution. According to Al-Jumaily*et al*.[7], the flavonoid compound was separated from ethanol extracted had been preceded using glass column (1.75 x 49) cm filled with Sephadex LH-20 . Five ml of ethanol extracted of the rhizoms of *C.rotunds* was subjected to column and eluted with ethanol solution, and the flow rate regulated to be 60 ml/min. The elusions had been collected in large tubes for each of mobile phase used and numbered as fractions; all fractions have been tested by ferric chloride solution 1%. Fractions containing flavonoid compound were pooled and concentrated to the required volume.

Forty –five mice (25-30 gm) of about six weeks old were obtained from the national center for drug control and research and Infertility Treatment / Al-Nahrain University and bred in the animal house of Biotechnology Researches Center / Al-Nahrain University were used in this study. They were randomly selected and kept in nine groups of 5 mice per group. Each group was kept in a separate cage. All animals were fed with commercially formulated mice feed and tap water *ad libitum* that supplied by the center. Their cages were cleaned daily; food and water have been changed daily. The animals were allowed to acclimatize for 2 weeks.

**Treatment schedule of animals**

Forty-five mice have been used to study the possible antioxidant effect of different injection of flavonoid purified extract and ethanolic extract compared to CCl4-induced liver and blood damage allocated [9] as follows:

**Group One**– five mice treated with daily oral injectiondiet anddranktap water for 14 days. The animals were killed by anesthetic ether on the day 15. The group served as control .

**Group Two**- five mice treatedthisgroupcarbontetrachloride(3.2 mg /kg) the firstdayandtheeighth. The animals were killed by anesthetic ether on the day 15 The group served as positive control.

**Group Three** - five mice had been treated with oral daily injection of flavonoid purified extract 150mg/kg/day for 14 day and treated with CCl4 first and eight day ; the animals were killed by anesthetic ether on the day 15.

**GroupFour**- five mice had been treated with oral daily injection of flavonoid purified extract 300mg/kg/day for 14 day and treated with CCl4 first and eight day ; the animals were killed by anesthetic ether on the day 15.

**Group Five** - five mice had been treated with oral daily injection of ethanolic extract 150mg/kg/day for 14 day and treated with CCl4 first and eight day ; the animals were killed by anesthetic ether on the day 15

**Group Six** - five mice had been treated with oral daily injection of ethanolic extract 300mg/kg/day for 14 day and treated with CCl4 first and eight day ; the animals were killed by anesthetic ether on the day 15.

**Group Seven**- five mice had been treated with oral daily injection of with vitamin C180mg/Kg/day for 14 day and treated with CCl4 first and eight day; the animals were killed by anesthetic ether on the day 15.

**Group Eight** - five mice had been treated with oral daily injection of flavonoid purified extract 300mg/kg/day for 14 day; the animals were killed by anesthetic ether on the day 15.

**Group Nine** - five mice had been treated with oral daily injection of ethanolicextract 300mg/kg/day for 14 day; the animals were killed by anesthetic ether on the day 15.

**Samples collection**

**Preparations of Post-mortum Serum Samples:**

After sacrifice the animals by anesthetic ether, blood was collected from the animals by intracardiac puncture using insulin syringe. The clot was dispersed with glass rod and then centrifuged at 3000 xg for 15 minute. The serum was used for the estimation of serum glutamic oxaloacetic transaminase (**SGOT**) , serum glutamic pyruvic transaminase(**SGPT**) and serum alkaline phosphatase(**ALP**) as parameters of liver function tests, Provan, [10] The blood obtained from each mice ranging from 0.7-1 ml.

**Determination of Serum Aspartate Aminotransferase enzyme activity (AST) (SGOT):**

Serum aspartate aminotransferase (AST) (EC 2.6.1.1)that formerly known as serum glutamate oxaloacetate aminotransferase ( SGOT) have been determined according to the method of Reitman and Frankle[11].

**Determination of Serum Alanine transaminase (ALT) (SGPT):**

Serum alanine transaminase (ALT) (Ec .2.6.1.2) that formerly known as serum glutamic pyruvic transaminase( SGPT) was determined according to the method of Reitman and Frankel [11].

**Determination of Serum Alkaline Phosphatase (ALP):**

Serum alkaline phosphatase activity have been determined according to the method of Kind and King[12] using ready-made kit . Results were analyzed statistically using completely randomized design (CRD) within the Statistical Analysis System- SAS [13]. The least significant difference-(LSD) test as used to comparative significant between means in this study[14].

## III. Results and Discussion

Results presented in Figure (1) and Table (1) shows that the serum GOT levels were significantly increased ($p < 0.05$) in Carbon tetrachloride –treated mice(78.25 U/L), as compared to control mice (45.75 U/L) . Mice were treated with 150mg/kg of purify flavonoid (with $CCl_4$) showed a significant ($p<0.05$) increase in the serum activity level of serum GOT(77U/L) (group three) as compared to control mice(45.75 U/L), but it is significantly ($p<0.05$)less than $CCl_4$-treated group.Mice were treated with 300 mg/kg of purify flavonoid (with $CCl_4$) showed a significant ($p<0.05$) decrease in the serum activity level of serum GOT(29 U/L) (group four) as compared to control group, but it is significantly ($p<0.05$) it is less than CCl4-treated group. Mice treated with 150mg/kg ofethanolic extract (with $CCl_4$) showed a significant ($p<0.05$) increase in the serum activity level of serum GOT(49U/L) (group five) as compared to control mice(45.75 U/L) (group one) , but significantly ($p<0.05$)less than $CCl_4$-treated group.Mice treated with 300 mg/kg ofethanolic extract (with $CCl_4$) showed a significant ($p<0.05$) increase in the serum activity level of serum GOT(59U/L) (group Six) as compared to control mice(45.75 U/L) (group one) , but significantly ($p<0.05$) it is less than $CCl_4$-treated group.

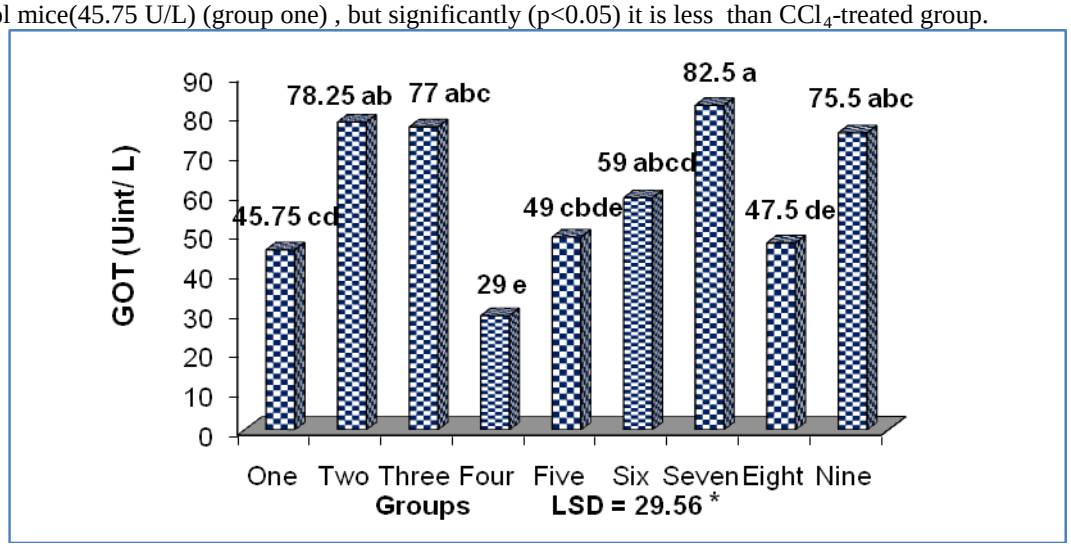


**Figure 1: The effect of different dose of purified flavonoid and ethanolic extract *C. rotundus*rhizomes on the activity of serum GOT**

Different letters represent significant ($P \leq 0.05$) different between means of column. Vales are expressed as the mean $\pm$ SE

**Table 1: Effect of purified flavonoid and ethanolic extract *C. rotundus* rhizomes on serum GOT, GPT, and Alkaline phosphate (U/L)in control ,$CCL_4$ treated mice at different concentration . (mean ± SE)**

| Group | Mean ± SE | | |
|---|---|---|---|
| | GOT (U/L) | GPT (U/L) | ALP (U/L) |
| One | 45.75 ± 16.48 | 9.00 ± 1.91 | 119.94 ± 19.20 |
| Two | 78.25 ± 3.70 | 20.50 ± 7.39 | 137.79 ± 15.96 |
| Three | 77.00 ± 12.00 | 18.25 ± 4.15 | 159.86 ± 10.71 |
| Four | 29.00 ± 3.55 | 19.75 ± 5.20 | 140.87 ± 22.65 |
| Five | 49.00 ± 5.61 | 15.00 ± 4.14 | 64.91 ± 11.13 |
| Six | 59.00 ± 9.03 | 10.25 ± 2.01 | 171.19 ± 35.92 |
| Seven | 82.50 ± 3.75 | 32.50 ± 17.68 | 199.56 ± 12.18 |
| Eight | 47.50 ± 15.19 | 16.25 ± 1.88 | 214.05 ± 60.29 |
| Nine | 75.50 ± 11.58 | 11.50 ± 2.66 | 151.66 ± 9.66 |
| LSD value | 29.566 * | 20.461 * | 77.355 * |
| * (P≤0.05). | | | |

*P value significant

Mice were treated with 180mg/kg of vitamin C (with $CCl_4$) showed a significant ($p<0.05$) increase in the serum activity level of serum GOT(82.5U/L) (group seven) as compared to control mice(45.75), but significantly at ($p<0.05$) more than $CCl_4$-treated group.

Mice were treated with 300 mg/kg of purified flavonoid (group eight) showed a significant ($p<0.05$)increase in the serum activity level of serum GOT(47.5U/L) as compared to control group, but significantly ($p<0.05$) less than CCl4-treated group. Mice were treated with 300 mg/kg ofethanolic extract (group nine) showed a significant ($p<0.05$) increase in the serum activity level of serum GOT(75.5 U/L) as compared to control mice(45.75 U/L) (group one) , but it is significant at ($p<0.05$)less than $CCl_4$-treated group.

The result showed that tetrachloride carbon declared the effect presneted by significantly increased at ($P<0.05$) in concentration of enzyme (SGOT) in blood serum of mice and 300mg/kg purified flavonoid with $CCl_4$ (group Four) has decreased significantly at ($P<0.05$) from increase in the concentration of enzymes level compared to the treatment of carbon tetrachloride,also the group eight (purified flavonoid 300mg/kg ) has decreased significantly at ($P<0.05$) level in concentration of enzyme (SGOT) in blood serum of mice. From this it is concluded that the purified flavonoid (300mg/kg with $CCl_4$) was one of the best treatments to reduce the impact of $CCl_4$.

**The effect of pure flavonoid and ethanolic extract on serum Alanine Transaminase (SGPT)**

Carbon tetrachloride –treated mice (group two) showed a significant increase at ($p<0.05$) level the serum activity GPT (20.5 U/L) as compared to control mice (9 U/L) (group one). (Figure 2).

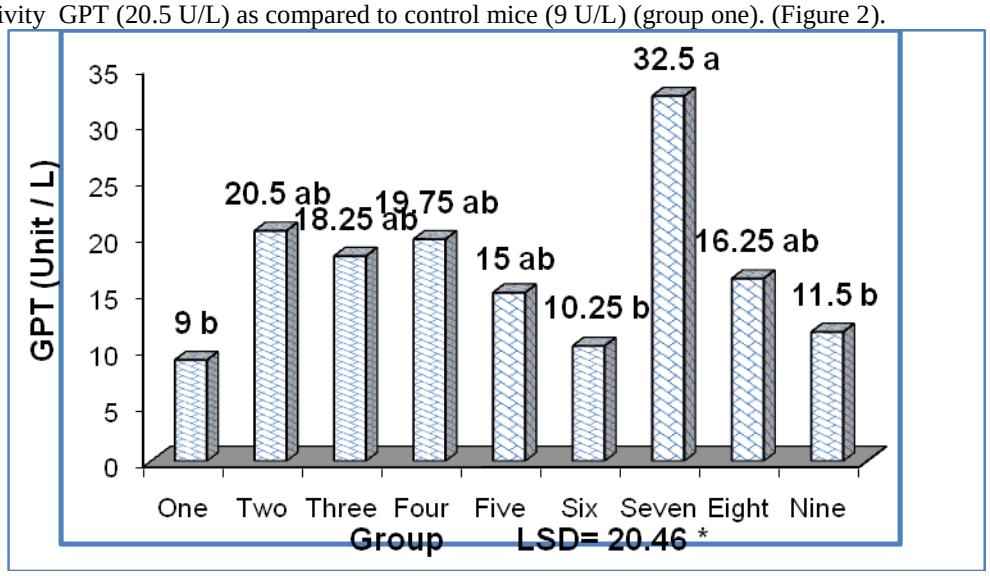


**Figure 2: The effect of different dose of purified flavonoid and ethanolic extract *C. rotundus* rhizomes on the activity of serum GPT.**

Different letters represent significant (P≤ 0.05) different between means of column.

Mice were treated with 150mg/kg of purified flavonoid (with $CCl_4$) showed a significant ($p<0.05$) increase in the serum activity level of GPT(18.25 U/L) (group three) as compared to control mice(9 U/L)

(group one) , but significantly ($p<0.05$)less than $CCl_4$-treated group.Also there was a significant ($p<0.05$) increase in the serum GPT in mice which were treated with300 mg/kg of purified flavonoid (with $CCl_4$) (group four) (19.75U/L) as compared to control group, but significantly ($p<0.05$) less than CCl4-treated group. Mice were treated with 150mg/kg ofethanolic extract (with $CCl_4$) showed a significant ($p<0.05$) increase in the serum activity level of GPT(15U/L) (group five) as compared to control mice (group one) , but significantly ($p<0.05$)less than $CCl_4$-treated group.Mice were treated with 300 mg/kg ofethanolic extract (with $CCl_4$) showed a significant ($p<0.05$) increase in the serum activity level of GPT(10.25 U/L) (group Six) as compared to control group , but significantly ($p<0.05$)less than $CCl_4$-treated group.Mice were treated with 180mg/kg of vitamin C (with $CCl_4$) showed a significant ($p<0.05$) increase in the serum activity level of GPT (32.5 U/L) (group seven) as compared to control mice (group one) , but significantly ($p<0.05$) more than $CCl_4$-treated group.Mice were treated with 300 mg/kg of purified flavonoid showed a significant ($p<0.05$) increase in the serum activity level of GPT(16.25 U/L) (group eight) as compared to control group, but significantly ($p<0.05$) less than CCl4-treated group.Mice were treated with 300 mg/kg ofethanolic extract showed a significant ($p<0.05$) increase in the serum activity level of GPT(11.5 U/L) (group nine) as compared to control mice(45.75 U/L) (group one) , but significantly ($p<0.05$)less than $CCl_4$-treated group.

The result showed that the effect of tetrachloride carbon was clear and increased significantly at ($P<0.05$) level in concentration of enzyme (SGPT) in blood serum of mice and 300mg/kg ethanolic extract with $CCl_4$ (group Six) has decreased significantly at ($P<0.05$) level from increase in the concentration of enzymes level compared to the treatment of carbon tetrachloride. Futhermore, group Nine (ethanolic extract 300mg/kg ) has a decreasing significantly at ($P<0.05$) level in concentration of enzyme (SGPT) in blood serum of mice.

It can be concluded that the ethanolic extract (300mg/kg with $CCl_4$) was one of the best treatment to reduce the impact of $CCl_4$ .The *C.rotundus* treatment proved to be effectiveactivity of *C.rotundus* due to the presence of phenolicgroups). Dominic *et al* .(2012) have selectedtetrachloridecarbonintheir study.,Being one of thetoxic substancesthat has theeffects ofimmunesuppressingandshatteringof the liverhas provedthese effects. Hence, their research has includedthe possibility of identifyingmaterialnatural plantandpurified one for useas a treatmentto reduce thetoxic materials to the liver.

**The effect of purified flavonoids and ethanolic extract on serum Alkaline phosphatase (ALP)**

Mice were treated with carbon-tetrachloride (group two ) showed a significant ($p<0.05$) increase in the serum activity level of ALP (137.79 U/L) as compared to control mice (119.94 U/L) (group one). ( Figure 2). Mice were treated with 150 and 300 mg/kg of purified flavonoid (with $CCl_4$) showed a significant ($p<0.05$) increase in the serum activity level of ALP (group three and four) as compared to control group, but it showed significantly at ($p<0.05$) more than $CCl_4$-treated group.

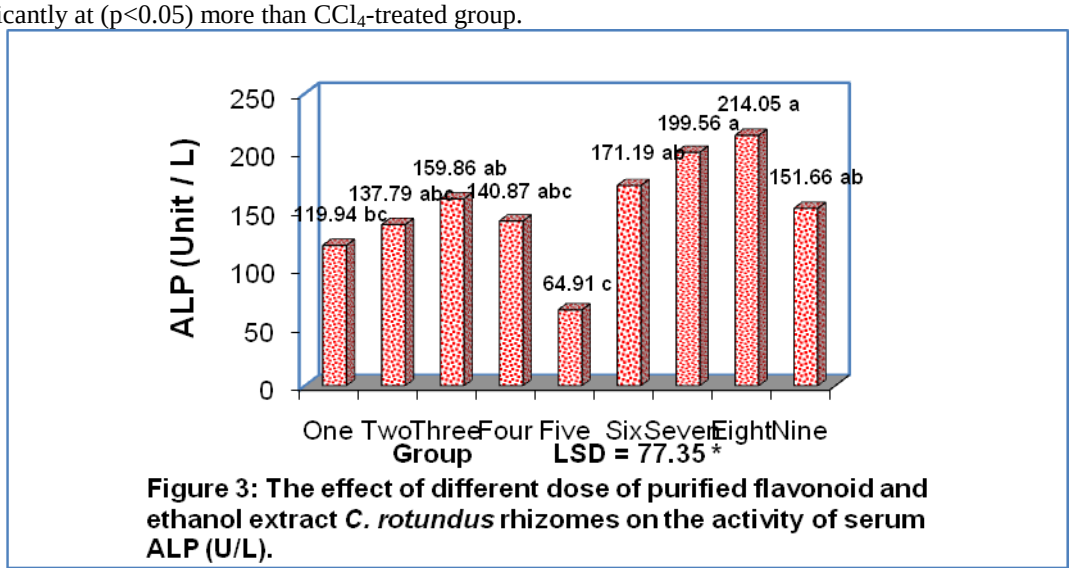


**Figure 3: The effect of different dose of purified flavonoid and ethanol extract *C. rotundus* rhizomes on the activity of serum ALP (U/L).**

Different letters represent significant ($P\leq 0.05$) different between means of column.

Moreover, the best effective group was (Group five) ethanolic extract (with $CCl_4$) (64.91U/L) for two weeks when compared to the $CCl_4$ treated group and control group decrease in the serum activity level of ALP. Mice treated with 300 mg/kg ofethanolic extract (with $CCl_4$) showed a significant ($p<0.05$) increase in the serum activity level of serum ALP (171.19 U/L) (group Six) at ($P<0.05$) level as compared to control group , but significantly more than $CCl_4$-treated group at ($P<0.05$) level.Mice were treated with 180mg/kg of vitamin C (with $CCl_4$) showed a significant increase in the serum activity level of ALP(199.56 U/L) (group seven) at ($P<0.05$) as compared to control mice (119.94 U/L ) (group one) , but significantly more than $CCl_4$-treated

group at (P<0.05) level.Mice were treated with 300 mg/kg of purify flavonoid showed a significant increase in the serum activity level of ALP (214.05 U/L) (group eight) at (P<0.05) level, as compared to control group, but significantly (p<0.05) more than CCl4-treated group. Mice were treated with 300 mg/kg ofethanolic extract showed a significant (p<0.05) increase in the serum activity level of ALP (151.66 U/L) (group nine) as compared to control mice (45.75 U/L) (group one) , but significantly (p<0.05) more than $CCl_4$-treated group.
The results showed that the effect of tetrachloride carbon declared was presented by the significantly increase at (P<0.05) level in concentration of enzyme (ALP) in blood serum of mice. From previous results, it was found that the best treatment is the fifth group. It was noted that the showed rest of the groups the increase in the serum activity level of ALP which indicates the damage was due to carbon tetrachloride and the rest of the extracts did not address the damage.
The serum activity of alkaline phosphatase (ALP) that is present in the lining membrane of the hepatocytes . Therefore the measurement of the activities of serum marker enzymes like ALP could make assessment of liver function [16]. This can be explained by stress likewise it is noted that in studies related to stress oxidative increase in ALP activity [17, 18].

After treatment of mice with *C.rotundus*, ethanolic extract and purify flavonoid activities SGOT,SGPT and ALP were normalized to their control value result an agreement with the findings that propolis induced reduction increase of SGOT and SGPT in serum of mice [19].

It was observed that high rates of enzymes and liver function (SGOT, SGPT and ALP) in the serum of mice treated with carbon tetrachloride and this is a clear indication of the extent of damage to the liver cells, which previously signaled his [20]. On the contrary, it was noted by the resultsprovided that no damage occurredin the work ofthe liverwhen thedosage of *C.rotundus*was given to the mice. There was no impacton themetabolic processesof the liver.Theseantioxidantshave the ability toprotect themembranesfromfree radicals andinhibitinflammationthat works onmakingand editingtheseroots [21].Our study demonstrated that ethanolicextract and flavonoid compounds of the *C. rotundus* could protect the liver tissues against CCl4-induced oxidative stress probably by increasing antioxidativedefense activities.

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