

# Phytochemical Screening and *In Vivo* Hepatoprotective Activity of the *Cordia Myxa* L Fruit Extracts Against Paracetamol-Induced Hepatotoxicity in Rats

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## ABSTRACT

This study aimed to test several *Cordia myxa* fruit extracts to study their protective activity in vivo after inducing hepatic injury due to oxidative stress caused by paracetamol at a concentration of 500 mg/kg. The phenolic compounds were extracted from the fruits of the *Cordia myxa* fruit using five solvents to obtain the extracts of crude methanolic root extract (CME), hexane (HxE), chloroform (ChE), ethyl acetate (EAE), and aqueous extract (AE). The results showed that the extracts contain different types of active compounds, including phenols, flavonoids, alkaloids, saponins, tannins, and glycosides. The amounts of phenols and flavonoids were estimated in these extracts and were found that the alcoholic extract contains the highest amount of total phenols  $24.86 \pm 0.93$  mg / g Gallic acid equivalent (GAE) from the extract weight. However, the flavonoids were  $2.76 \pm 0.10$  mg / g Rutin equivalent of the alcoholic extract weight, followed by the rest of the extracts. Further, the methanolic extract and the aqueous extract of *Cordia myxa* fruits at a concentration of  $0.22 \pm 0.01$  mg/ml showed activity in free radical scavenging DPPH by 50%. The total antioxidant capacity (TAC) was  $0.9 \pm 23.65$  mg / g of the alcoholic extract. The aqueous and alcoholic extract was selected for giving a forced oral administration for the experimental animals concurrently with paracetamol. It was observed that there was an increase in the level of liver enzymes ALP, AST, ALT in the serum of male rats, as well as in the level of malondialdehyde MDA and a decrease in the level of catalase activity in the group dosed with paracetamol at a concentration of 500 mg/kg compared to the negative control group. The daily dosing of paracetamol combined with the alcoholic and aqueous extracts enabled to reduce the level of serum ALP, AST, and ALT liver enzymes, as well as a decrease in the level of MDA and an increase in the level of catalase enzyme activity, especially the group dosed with alcoholic extract at a concentration of 300 mg/kg close to the level of the normal negative control group. These results were supported by a histopathological study which showed that the hepatocytes retained their features and the infiltration and central necrosis were reduced after treatment with the alcoholic and aqueous extracts, especially at the concentration of 200 and 300 mg/kg body weight of the alcoholic extract compared to the tissue treated with the aqueous extract at the same concentration.

**Keywords:** *Cordia myxa*, Liver, Antioxidant, Paracetamol, Medical Plants

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## INTRODUCTION

The term Medical Plants refers to a variety of plants rich in active compounds with physiological effects on organisms that can be used to develop and manufacture medicines using the whole plant or parts of it (Jamshidi-Kia *et al.*, 2018). However, the *Cordia myxa* L. plant is one of the subtropical medicinal plants belonging to the genus *Cordia*, which is cultivated in central and southern Iraq in which its fruits are of high nutritional and medicinal value (Al Mayahi and Fayadh, 2015). *Cordia myxa* L plant is used as a pain reliever and anti-inflammatory, microbes, parasites, and insects, where (Al-Snai, 2019) indicated that the leaves extracts of *Cordia myxa* plant had inhibitory activity against three bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. In addition to their activity in treating trypanosomiasis (Gupta *et al.*, 2017). Furthermore, leaf extracts are also used externally to treat burns, wounds, and Glossina bites, and internally in treating stomach pain, coughing, and chest problems, in addition to being a diuretic. On the other hand, the sticky gum of the fruit pulp is useful in treating sore throats

(Shwaish and Imarah, 2016), while fresh and dried plant fruit extracts have strong antioxidant efficacy. They may be responsible for preventing in-vitro fatty liver fibrosis induced by carbon tetrachloride and acetamide (Afzal *et al.* 2007). For example, many metabolic disorders are associated with high hepatotoxicity that damages its cells (Singh *et al.*, 2019), where an overdose of some drugs such as paracetamol APAP, a commonly used antipyretic and analgesic drug, is oxidative stress and inflammatory liver damage (Viswanathan *et al.*, 2019). This study aims to extract the flavonoids from the fruits of the *Cordia myxa* plant by using different solvents and to investigate their content of effective chemical. In addition to measuring the antioxidant activity of fruit extracts in vitro and studying its protective role against paracetamol-induced hepatotoxicity in vivo by studying some indicators of oxidative stress, in addition to studying the changes that occur in the liver tissue after induction with paracetamol.

## MATERIAL AND METHODS OF WORK

- Collecting the plant samples:

The plant fruits were collected in mid-July 2019 from a farm in the Jadriya district in Baghdad. The fruits were washed first with tap water to get rid of the impurities, then with distilled water and dried with a paper towel. Then, the fruit was cut in half, the seeds were discarded and then left to dry under the shade at air temperature for two weeks. The dried fruits were collected in sealed glass bottles and kept in the refrigerator at 4 ° C until the extraction was carried out.

- Plant classification:

The plant was classified in the Advanced Plants Classification Laboratory of the Department of Life Sciences - College of Education for Pure Sciences, Ibn Al-Haytham, University of Baghdad, by Dr. Areej Abdul-Sattar Farman Al-Rawi, a specialist in plant classification.

- Extraction

Polar and non-polar solvents were used mainly to extract the phenolic compounds, according to the method of (Markham, 1982) to obtain five extracts, which are methanol crude extract, hexane extract, chloroform extract, ethyl acetate extract, and aqueous extract.

• **Qualitative detection of some effective chemical and food compounds:**

- Mayer's test

A drop or two of Meyer's reagent was added to the side of the test tube to a specific volume of the extract dissolved in a 1% HCl solution. The formation of a white precipitate indicates the presence of alkaloids as a positive result (Shrestha *et al.*, 2015).

- Wagner's test

A drop or two of Walkner's reagent was added on the side of the test tube to a specific volume of extract dissolved in a 1% HCl solution. The formation of a reddish-yellow precipitate indicates the presence of alkaloids (Shrestha *et al.*, 2015).

- The Saponin test:

(0.5) g of extracts was dissolved with 5 mL of distilled water in a test tube and shake vigorously for 30 seconds. The formation of a stable foam even after 30 minutes indicates the presence of saponin (Shrestha *et al.*, 2015).

- Keller Killani Test:

The dried extracts were mixed separately with 1 milliliter of glacial acetic acid, then two drops of a 2% solution of ferric chloride FeCl<sub>3</sub> were added to it. Then, 2 mL of concentrated sulfuric acid H<sub>2</sub>SO<sub>4</sub> were put in another test tube, and the two solutions were mixed carefully. The formation of a brown ring (reddish-violet) indicates the presence of glycosides (Shrestha *et al.*, 2015).

- Ferric chloride test:

A few drops of 5% ferric chloride solution was added with 1 mL of each extract. The appearance of a greenish-black precipitate indicates the presence of phenols (Singh *et al.*, 2019).

- Alkaline reagent test:

A few drops of 20% NaOH was added to 1 mL of extract. The transformation of the extract to a yellow color and the disappearance of the color upon the addition of drops

of HCl indicates the presence of flavonoids (Nathaniel *et al.*, 2019).

- Lead acetate test:

A specific volume of each extract was added with an equal volume of 10% lead acetate solution. The formation of a white precipitate indicates the presence of tannins (Singh *et al.*, 2019a).

- Estimation of total phenols

The total phenol concentration in each extract was estimated according to Folin's method using Folin ciocalteu (Kairupan *et al.*, 2019).

- Estimation of total flavonoids:

Flavonoids were estimated according to the method described by (Alobaidy *et al.*, 2017).

• Measurement of the antioxidant activity of fruit extracts in vitro:

- The DPPH ( $\alpha$ ,  $\alpha$ -diphenyl- $\beta$ -picrylhydrazyl) radical inhibition potency test:

The ability of *C. myxa* fruit extracts against scavenging free radical DPPH was measured according to (Narzary *et al.*, 2016).

- Total antioxidant capacity (TAC) test:

The total antioxidant capacity of *C. myxa* fruit extracts was determined by the phosphomolybdate method described by (Abdel-Aleem *et al.*, 2019).

• Experimental Animals:

Male albino rats were obtained from the animal house at the Biotechnology Research Center / Al-Nahrain University. The experiment was carried out in the same place using 48 rats, their age at the beginning of the experiment ranged between 3-4 months, and their weight was about 200  $\pm$  25 g.

• Experimental Design:

The laboratory animals (male rats) were divided randomly into three groups namely A, B, and C.

1. Group A:

- Six animals were given 100 mg/kg of body weight aqueous extract with paracetamol at a concentration of 500 mg/kg.
- Six animals were given 200 mg/kg of body weight aqueous extract with paracetamol at a concentration of 500 mg/kg.
- Six animals were given 300 mg/kg of body weight aqueous extract with paracetamol at a concentration of 500 mg/kg.

2. Group B :

- Six animals were given 100 mg/kg of body weight an alcoholic extract with paracetamol at a concentration of 500 mg/kg.
- Six animals were given 200 mg/kg of body weight an alcoholic extract with paracetamol at a concentration of 500 mg/kg.
- Six animals were given 300 mg/kg of body weight an alcoholic extract with paracetamol at a concentration of 500 mg/kg.

3. Control Group C:

- Negative control group C- : It includes six animals that were given distilled water.

- Positive control group C+ : It includes six animals that were given paracetamol at a concentration of 500 mg/kg of body weight.

- **Preparation of serum samples**

The blood samples were obtained, after anesthetizing the animals with chloroform, by a direct drawing of blood from the heart (heart puncture), 3 ml of blood were taken and placed in the Gel tube and allowed to clot and then the serum was separated by centrifugation for 10 minutes at 3000 rpm. The isolated serum was stored at -18 ° C (Abdullah and AL-Mahdawi, 2018) to conduct the following tests on serum: ALT, AST, ALP, MDA, and CAT.

- **Estimation of Liver Enzymes Activity**

The ready-made kit produced by the British Randox Company was used to measure the activity of liver enzymes in serum, which include: Alkaline phosphatase enzyme (ALP), Aspartate transaminase enzyme (AST), Alanine transaminase enzyme (ALT) and was measured according to the manufacturer's instructions.

- **Testing the indicators of oxidative stress for the liver:**

- Measuring the Malondialdehyde (MDA):

MDA was determined in serum using the ready-to-use diagnostic kit for lipid oxidation test from the US company, Bio Vision, according to the company's instructions.

- Measuring the activity of catalase (CAT):

The activity of the catalase enzyme was determined using the kit manufactured by the American company Bio-Assay Systems and the estimation was carried out according to the manufacturer's instructions.

- **Histopathological Study:**

The method used by (Al-Mukhtar *et al.*, 1982) was followed to prepare the histological sections. Then, the liver tissue of the treated rats was examined by optical

microscopy and diagnosed in the College of Veterinary Medicine, Anatomy and Histology Department - University of Baghdad.

## RESULTS AND DISCUSSION

- **Qualitative detection of some effective chemical compounds:**

Table 1 showed the results of the qualitative detection of some active compounds in the extracts of the *C. myxa* fruit (CME, HxE, ChE, EAE, AE), which shows that all the extracts contain phenolic compounds that are characterized by their ability to act as an antioxidant. As well as, their function as hormone modulators or as an Immune Enhancer, in addition to its ability to inhibit some enzymes that act on inflammatory disorders (Okwu and Iroabuchi, 2009). Table 1 also showed the presence of tannins and flavonoids, which are characterized by their ability to act as antioxidants, inflammations, and anti-bacterial, with the possibility of using them as a treatment for diabetes (Jarial *et al.*, 2018). Whereas, methanol, chloroform, and hexane extracts contained alkaloids with distinct pharmacological properties, some acting as antiparasitic and others as a stimulant to the central nervous system (Othman *et al.*, 2019). As for the saponins classified with glycosides within the terpenoids, it was inferred in three extracts, which are alcoholic, hexane, and aqueous, other than chloroform and ethyl acetate extracts. The results of this study are consistent with the study of (Shwaish and Al-Imarah, 2017) and (Aberoumand 2011), which indicated that the *C. myxa* fruits contain active compounds, the most important of which are phenols, flavonoids, tannins, alkaloids, saponins, and glycosides.

Table 1. The qualitative detection of active chemical compounds

| Compounds  | test             | Extracts |     |     |     |     |
|------------|------------------|----------|-----|-----|-----|-----|
|            |                  | AE       | EAE | ChE | HxE | CME |
| Phenols    | Ferric-chloride  | +++      | +++ | ++  | +   | ++  |
| Flavonoids | Alkaline reagent | ++       | ++  | +   | +   | ++  |
| Tannins    | Lead acetate     | +        | +   | +   | +   | ++  |
| Alkaloids  | Wagner's         | -        | -   | ++  | +   | +++ |
|            | Mayer's          | -        | -   | +   | -   | ++  |
| Saponins   | Saponin          | +        | -   | -   | +++ | ++  |
| Glycosides | Keller Killani   | ++       | ++  | +   | +   | +++ |

\*+ = Positive detection. ++ = Strong positive detection. +++ = Very strong positive detection. - = negative detection.

- **Determination of total phenols of *C. myxa* fruit extracts:**

The total amount of phenols was estimated in mg / g Gallic acid equivalent after obtaining the standard curve for Gallic acid as shown in Figure 1. The results of Table 2 showed that CME extract obtained the highest amount of phenols as it was estimated by  $24.86 \pm 0.93$  mg / g Gallic acid from the extract. Followed by AE extract by  $21.21 \pm$

0.55 mg / g Gallic acid, then ChE and EAE at concentrations estimated by  $18.2 \pm 1.06$ ,  $15.46 \pm 1.19$  mg / g Gallic acid, respectively. Whereas, HxE extract was the lowest amount, which was estimated by  $13.81 \pm 0.68$  mg / g Gallic acid. The results obtained were twice that of (Afzal, 2009). These differences are attributed to the difference in the extraction method and the environment in which the plant grows. Naturally, the Iraqi environment differs from other environments in terms of

climate, plant nutrition conditions, and environmental conditions that surrounded the model during the growth stages. This difference is observed even within the country itself and this is confirmed by the study of (Abdlrahmaan and Yaseen 2017) in the difference in concentration of some active compounds of *Heliotropium lasiocarpium* L due to the environmental difference inside Iraq.

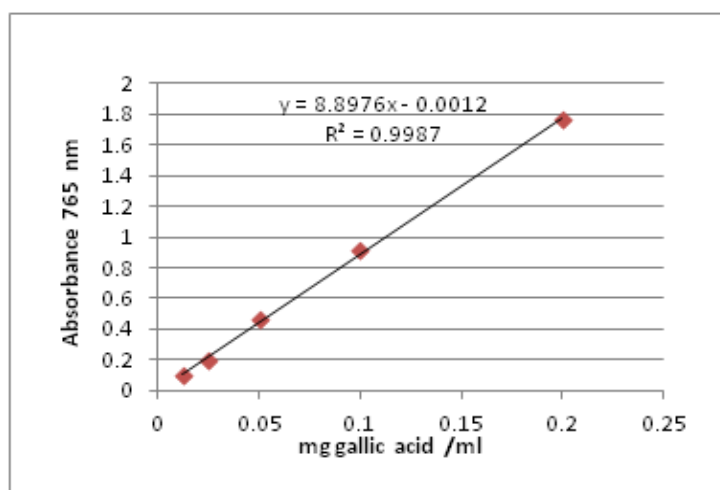


Figure 1. represents the standard curve for Gallic acid

- Determination of total flavonoids of *C. myxa* fruit extracts:**

The total amount of flavonoids was estimated in mg / mL Rutin Equivalent after obtaining the standard curve for the Rutin as shown in Fig 2. The results of Table 2 showed the superiority of the CME, as the flavonoids were estimated by  $2.76 \pm 0.10$  mg / g Rutin from the extract weight, while the remaining extracts came in proportions close to each other starting with the aqueous extract AE,

then the ethyl acetate EAE. Then, the ChE by  $2.32 \pm 0.03$ ,  $2.12 \pm 0.2$ ,  $1.66 \pm 0.04$  mg / g Rutin, respectively, and ended with HxE  $1.94 \pm 0.01$  mg / g Rutin from extract weight. These results were not agreed with what was reported by (Murthy *et al.*, 2019). Flavonoids are part of the total phenols (Garg *et al.*, 2019). This explains the presence of other phenolic compounds such as tannins that were detected in the qualitative examination and other compounds such as phenolic acids.

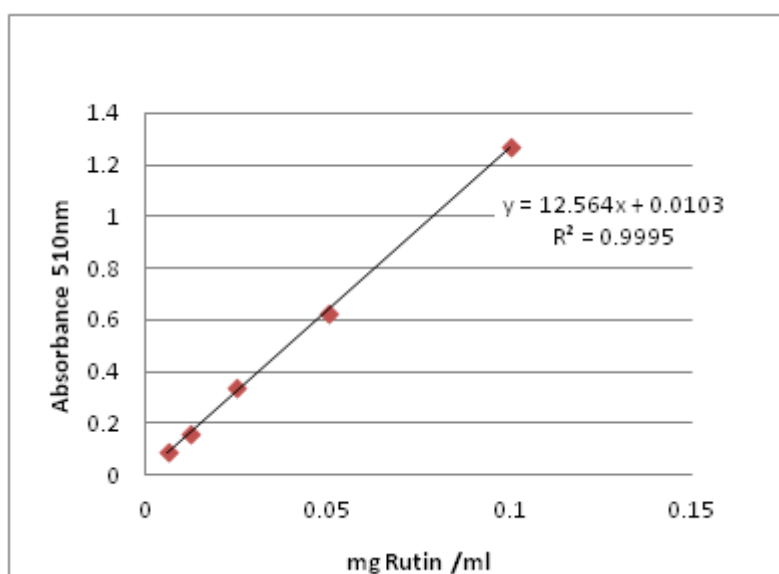


Figure (1) represents the standard curve for Rutin

However, this variation in the values of flavonoids calculated in the five extracts is due to the nature of the solvent used to extract the different phenolic compounds such as the mono-, di- and tetra - glycosides flavonoids.

- Inhibition ability of *C. myxa* fruit extracts to DPPH radical :

The CME showed a high ability to scavenge free radical DPPH as the value of IC<sub>50</sub> was 0.22 ± 0.01 mg/ml. The ability of the alcoholic extract was approximately equal to control AA (Ascorbic acid), which its value was 0.2 ± 0.01 mg/ml. Moreover, the ability of AE to scavenging free radical DPPH was also observed with twice the amount of control AA by 0.41 ± 0.04 mg/ml, whereas, the ChE , EAE needed twice as much CME and control AA to scavenge 50% of the radical, represented by 1.09 ± 0.05, 1.37 ± 0.1 mg/ml, respectively, while HxE extract had the highest value of 1.46 ± 0.12 mg/ml, which reflects the weakness of the extract on scavenging, as shown in Table 2. The results were not agreed with (Murthy *et al.*, 2019). This difference between the results of the aforementioned study may be due to the presence of high amounts of compounds specialized in scavenging free radicals, the most important of which is the phenolic compounds in the extract. Furthermore, the results clearly showed that all the extracts have a scavenging activity of the radical DPPH that increases with increasing concentration. Since phenolic compounds are among the important components that are responsible for inactivating free radicals based on their ability to donate hydrogen atoms to free radicals (Amarowicz *et al.*, 2004), as well as giving CME extract higher potency. It can be concluded that the quantitative content of phenolic compounds such as phenolic acids as well as flavonoids may be the main reason for the ability to scavenge the DPPH radical. This is consistent with a number of studies that have confirmed the existence of a linear relationship between the phenolic content of plant extracts and their activity in scavenging free radicals, including the study of (Aryal *et al.*, 2019). In addition to the quantitative effect of phenolic compounds, they also have their ideal

structural properties for scavenging free radicals, This was confirmed by (Shrestha and Dhillion 2006) by the presence of additional groups of OH on carbon C<sub>5</sub> and C<sub>3</sub> from the ring of flavonoids such as Quercetin increases the activity of scavenging free radicals (Williams *et al.*, 2004). The results showed that the AE was ranked second in terms of antioxidant activity, despite its quantitative approach in the proportion of phenols and flavonoids present in the chloroform extract, as it needed to double the amount of the aqueous extract to scavenge 50% of the DPPH radical. The activity of the aqueous extract can be attributed to the structural properties of some of its components, as it contains tri and tetra - glycosides flavonoids, while the chloroform extract contains non-glycosides flavonoids. This is confirmed by (Mishra *et al.*, 2003) that the presence of additional sugar units for flavonoids helps to increase the activity of inhibition of the free radicals than of non- glycosides flavonoids. Also, when the ability of the alcohol extract to be close to the ability of the control (AA ascorbic acid) to a large extent is evidence of the activity of this extract in scavenging free radicals.

- The Total Antioxidant Capacity Test (TAC):

Table (2) showed that CME extract obtained the highest antioxidant activity, which was expressed in mg / g of ascorbic acid from the extract, which was 23.65± 0.9 mg / g. However, the other extracts recorded in succession AE, EAE, ChE, and HxE, with a total capacity of 15.34±1.2 ,10.91 ± 1.061 , 8.02±1.08 , 1.68 ±0.1 mg / g, respectively. Once comparing these results with the antioxidant activity of the *C. myxa* leaves extracts, the total antioxidant capacity using the ethyl acetate solvent was reached 103.40 ± 0.05 (Abdel-Aleem *et al.*, 2019), which is four times what was obtained in the CME in this study. This reflects the high amount of phenols in the leaves . Table 2 indicates the superiority of the alcoholic and aqueous extract over the rest of other extracts in their content of antioxidant compounds. Therefore, CME extract and AE extract were selected to test their protective antioxidant efficacy in vivo.

Table 2. Shows the quantitative determination of phenols and flavonoids and the antioxidant activity of *C. myxa* fruit extracts through the TAC and DPPH test

| Extract | Total phenols mg / g Gallic acid | Flavonoids mg / g rutin | TAC test mg / g ascorbic acid | DPPH test IC <sub>50</sub> mg / mL |
|---------|----------------------------------|-------------------------|-------------------------------|------------------------------------|
| CME     | 24.86±0.93                       | 2.76 ± 0.10             | 23.65±0.9                     | 0.22± 0.01                         |
| HxE     | 13.81±0.68                       | 1.66 ±0.04              | 1.68±0.1                      | 1.46±0.12                          |
| ChE     | 18.21±1.06                       | 1.94 ±0.01              | 8.02±1.08                     | 1.09±0.05                          |
| EAE     | 15.46± 1.19                      | 2.12 ± 0.2              | 10.91±1.06                    | 1.37±0.1                           |
| AE      | 21.21 ± 0.55                     | 2.32 ± 0.03             | 15.34±1.2                     | 0.41 ±0.04                         |
| AA      | -                                | -                       | -                             | 0.20±0.01                          |
| LSD     | 4.71*                            | 0.705*                  | 4.59*                         | 0.463*                             |
| *P≤0.05 |                                  |                         |                               |                                    |

\* is represent the presence of significant differences at the level of (P≤0.05), NS represents no significant differences. Note that the values represent the mean ± standard deviation

• **The effect of oral administration of alcoholic and aqueous extract on the activity of liver enzymes (ALP, AST, ALT) in male rats induced by paracetamol:**

Table 3 showed the effect of forced oral administration for the experimental animals of CME extract, AE, and paracetamol on the activity of liver enzymes ALP, AST, ALT. A significant increase in the activity of ALP, AST and ALT enzymes was observed in the animals serum of the C + group treated with paracetamol. The high levels of the three liver enzymes upon exposure of animals to a high dose of paracetamol are due to the toxic metabolism of paracetamol, leading to a series of disturbances ending with hepatic cell necrosis. Thus, it leads to permeability of the liver cell membrane and leakage of these enzymes into the bloodstream (El-Senosiy *et al.* 2015; Salman *et al.*, 2020).

• **The effect of oral administration of alcoholic and aqueous extract on the activity of liver enzymes (ALP, AST, ALT) in male rats induced by paracetamol:**

Table (3) shows the effect of forced dosing of experimental animals of CME alcoholic extract, aqueous AE, and paracetamol by mouth on the activity of liver enzymes ALP, AST, ALT. A significant increase in the activity of ALP, AST, and ALT enzymes was observed in the blood serum of animals of the C + positive control group which treated with paracetamol. Table 3 also showed the results of treating experimental animals of groups A and B with different concentrations of the aqueous and alcoholic extract of *C. myxa* fruits, and concurrently with paracetamol, the levels of liver enzymes, ALP, AST, ALT closed to their normal level that observed in the C- group.

Table 3. The effect of oral administration of CME and AE on the activity of ALP, AST, ALT enzymes and oxidative stress indicators in male rats induced by paracetamol

| Treatment type                    |                 | Concentration<br>mg / kg | ALP<br>(IU/L) | AST<br>(IU/L) | ALT<br>(IU/L) | CAT<br>IU\ml | MDA<br>nmol/ml |
|-----------------------------------|-----------------|--------------------------|---------------|---------------|---------------|--------------|----------------|
| Group C-<br>(Distilled water)     |                 | 0                        | 76.28±5.17    | 24.17±4.04    | 20.73 ±1.29   | 4,87±0.87    | 2.32 ± 0.45    |
| Group C +<br>(Paracetamol)        |                 | 500                      | 91.45±3.91    | 48.25± 6.68   | 40.91 ±3.6    | 2.16 ± 0.26  | 5.06 ± 0.87    |
| Group A<br>(AE +<br>paracetamol)  | First<br>group  | 100                      | 79.82±3.61    | 42.02± 3.16   | 35.43±2.48    | 2.23 ± 0.38  | 3.94 ±0.86     |
|                                   | Second<br>group | 200                      | 78.99±5.64    | 37.85±3.14    | 34.43±1.07    | 2.74 ± 0.33  | 2.95 ±0.35     |
|                                   | Third<br>group  | 300                      | 77.35±4.87    | 36.59 ±1.61   | 33.81 ±3.15   | 3.27 ± 0.44  | 2.9 ±0.14      |
| Group B<br>(MCE +<br>paracetamol) | First<br>group  | 100                      | 82.72±5.43    | 34.29 ±3.89   | 29.69 ±2.14   | 3.32 ± 0.35  | 4.1±0.65       |
|                                   | Second<br>group | 200                      | 74.96±4.85    | 29.94 ±2.55   | 27.97 ±2.43   | 4.03 ± 0.13  | 2.75 ±0.44     |
|                                   | Third<br>group  | 300                      | 74.8±4.07     | 24.17 ±1.38   | 23.77 ±2.06   | 4.03 ± 0.78  | 2.07±0.12      |
| LSD                               |                 | -                        | * 8.922       | * 6.527       | * 6.839       | * 0.872      | * 1.066        |
| *P≤0.05                           |                 |                          |               |               |               |              |                |

\* It represents the presence of significant differences at (P≤0.05).

NS represents no significant differences.

Values represent the mean ± standard deviation.

The alcoholic extract played a positive preventive role, where it was noticed that the level of these enzymes activity in the serum decreased, whenever the concentration of the alcohol extract dosed to the animals increased, and the best results were observed at the concentration of 300 mg of the alcoholic extract, while the aqueous extract played a moderate protective role. This protective efficacy or ability of the alcoholic and aqueous extracts is attributed to the presence of the active antioxidant compounds directly related to the prevention of oxidative stress by scavenging free radicals.

Also, it acts as part of the non-self defense system of these radicals represented by the phenolic compounds, the most important of which are the flavonoids (Yakubu *et al.*, 2013; Beh *et al.*, 2016; Ahmed *et al.*, 2019). These extracts containing phenolic compounds such as flavonoids work to maintain the normal state of the liver by preserving cell membranes from necrosis resulting from free radicals that follow depletion of antioxidants (Abdel Azim *et al.*, 2017). Thus, preventing leakage of liver enzymes ALP, AST, ALT to the bloodstream.

• **The effect of oral administration of CME and aqueous-alcoholic extract AE on indicators of oxidative stress.**

The results in Table 3 showed an increase in the level of MAD in the serum of male rats for the C + group by more than twice its level compared with the C- group, as its level reached  $5.06 \pm 0.87$  nmol/ml at treatment with paracetamol. However, its level in the C- group was estimated at  $2.32 \pm 0.45$  nmol/ml. The results also showed a decrease in the MAD level after treating group A and B of the experimental animals with aqueous and alcoholic extract respectively at the highest concentration of subgroups 100, 200, 300 mg/kg compared with the C + group. The results values were close to the MAD level in group A and B toward normal control group values whenever the concentration increased. The results also showed a decrease in the level of CAT activity in the C + group, where the enzyme level reached  $2.16 \pm 0.26$  IU / ml, compared with the C- group, in which the enzyme level reached  $4.87 \pm 0.84$  IU / ml, where the activity decrease was more than 50%. But after treating groups A and B with AE and CME, the enzyme level began to return to the normal level at groups B, especially at a concentration of 300 and 200 mg/kg b.w. of the CME, and close to the C- group with a level of  $4.03 \pm 0.78$  and  $4.03 \pm 0.13$  IU / ml respectively.

Further, the concentration of 100 mg/kg b.w. of CME achieved an improvement in the enzyme level than from the enzyme level in the C + group at  $3.32 \pm 0.35$  IU / ml. However, the aqueous extract showed a significant improvement at the highest concentration in the enzyme level, but lower than the alcoholic extract, where the enzyme level increased with the increase in the three concentrations 100, 200, 300 mg/kg and the level of 2.23

$\pm 0.38$ ,  $2.74 \pm 0.33$ ,  $3.27 \pm 0.44$  IU / ml, respectively. The results were consistent with the (Teng *et al.*, 2012 and Abdel Azim *et al.*, 2017) findings, where the level of MAD increased and the level of CAT in the serum of laboratory animals after treatment with paracetamol in a long-term dose. These results are also agreed with the study of (Alwan *et al.*, 2015), which indicates that the concentration of MAD decreased to its normal levels in the animal's serum when dosed with alcoholic extract of *C. myxa* fruit concurrently with dosing with carbon tetrachloride ( $\text{CCl}_4$ ) that forms free radicals. Table 3 also showed that the low level of CAT in the C + group compared with the C- group indicates a failure in the defense mechanisms against oxidizing agents (Ramachandran and Raja, 2010; Hanafy *et al.*, 2016), it is also evidence of the formation of a chain of free radical, (Rasyid *et al.*, 2012). The above results indicated that the treatment of male rats with paracetamol resulted in a continuous chain of free radicals that contributed to the depletion of antioxidant enzymes, including the catalase enzyme, which caused the occurrence of oxidative stress that caused damage to various parts of liver cells such as their membranes.

• **Histological study of male rat liver:**

The results of microscopic examination of the male rat's livers treated with paracetamol, the group C +, at a concentration of 500 mg/kg for 14 days showed clear tissue changes represented by the occurrence of shattering of the liver lobules, irregularity of the hepatic cord with loss of the radial arrangement of hepatocytes around the central vein, swelling and necrosis of hepatocytes, hemorrhagic areas, sinus congestion, swelling, and hemolysis in different areas of tissue as shown in Figure 3.

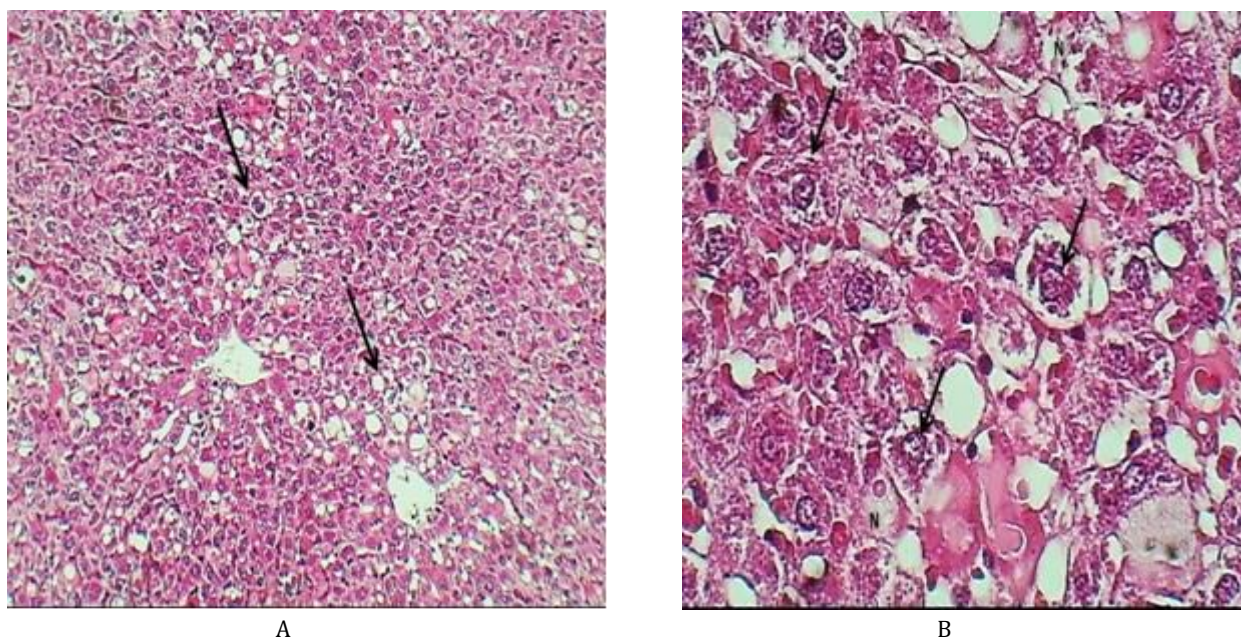


Figure 3: Section of the liver group of paracetamol (A) shows: lobular degeneration of hepatocytes (Arrows) with disarranged hepatic cords. H&E stain.100x (B) shows mild swelling and necrosis of hepatocytes (Arrows) with sinusoidal congestion and hemolysis. H&E stain.400x

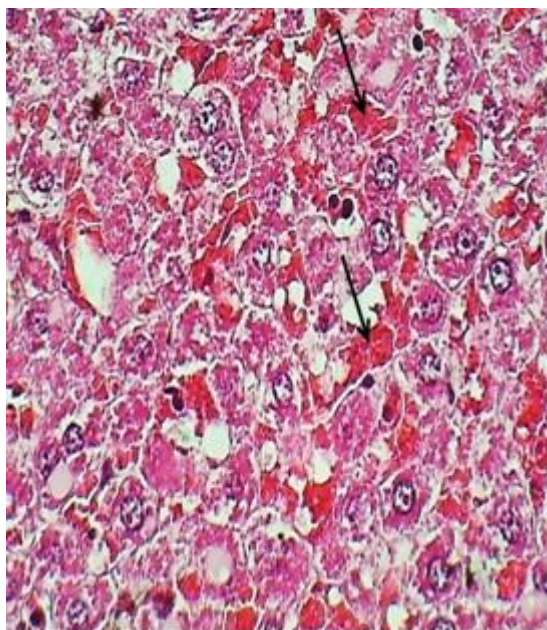


Figure 4: Section of the liver (the group of 100 mg/kg b.w. AE ) shows: extensive parenchymal hemorrhagic hepatitis (arrows). H&E stain.400x

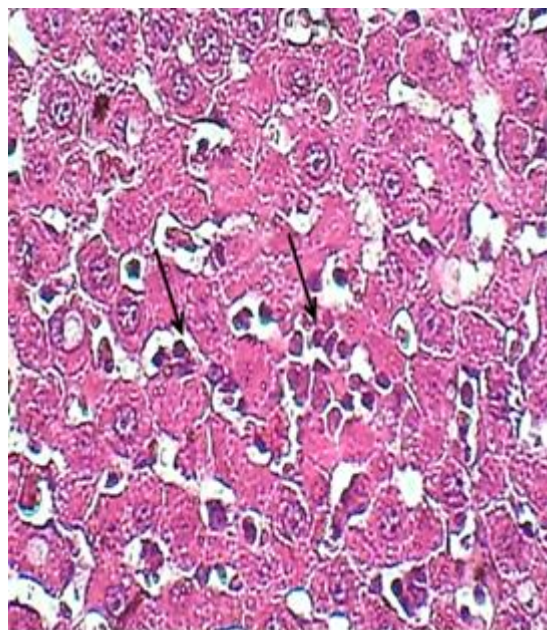
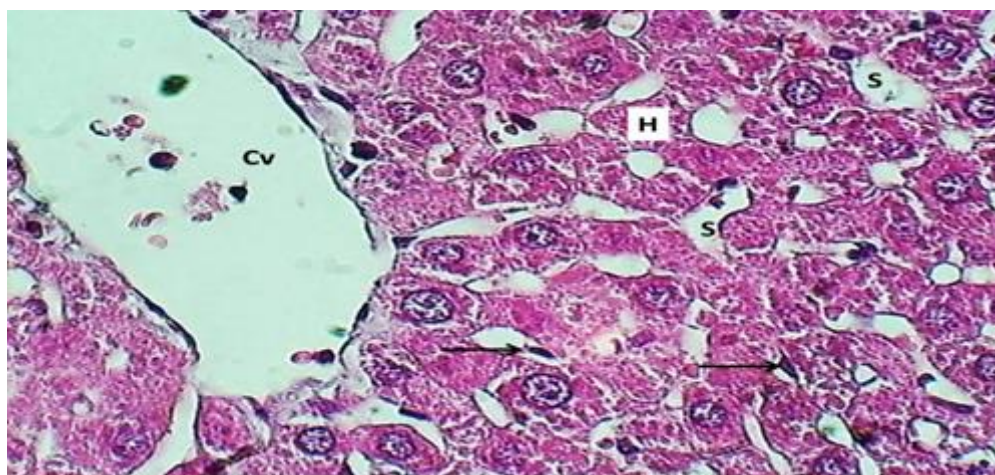


Figure 5: Magnified section of the liver (the group of 200 mg/kg b.w. AE ) shows: normal hepatocytes with mild sinusoidal infiltration of mononuclear leukocytes (arrow). H&E stain.400x

The results of treatment with aqueous extract shown in Figures 4 and 5 and treatment with alcoholic extract shown in Figures 7 and 8. It was observed that the oral administration of alcoholic or aqueous extracts at a concentration of 100 and 200 mg/kg of body weight of each extract concurrently with a dose of paracetamol at a

concentration of 500 mg/kg did not lead to complete protection of the rat liver from the toxic effects of paracetamol on the liver compared with the encouraging results of the dosing process at a concentration of 300 mg/kg of body weight of the aqueous extract shown in Fig. 6.



**Figure 6:** Section of the liver (the group of 300 mg/kg b.w. AE ) shows: normal appearance of central vein (Cv), hepatocytes (H), hepatic sinusoid (S) & kupffer cells (Arrows). H&E stain.400x.

Besides, the alcoholic extract was shown in Figures 9 and 10 concurrently with the paracetamol dose at a concentration of 500 mg/kg, which confirms the protective action of the aqueous and alcoholic extract of

the *C. myxa* fruit in protecting liver tissue concerning the central vein, hepatocytes, hepatic sinusoids, and Kupffer cells

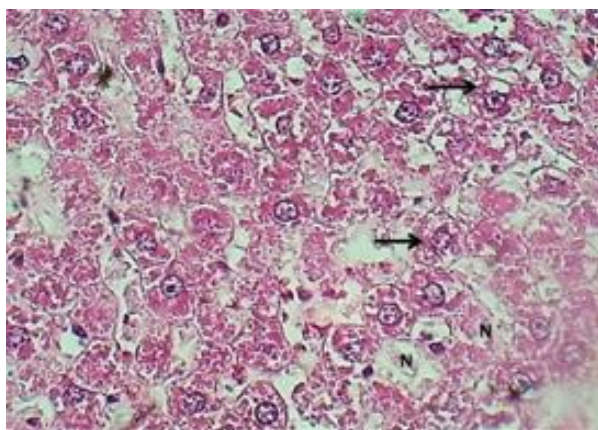


Figure 8: Section of the liver (a group of 100 mg/kg (bw) of CME ) shows: moderate cellular swelling (arrow) and necrosis (N). H&E stain.400x.

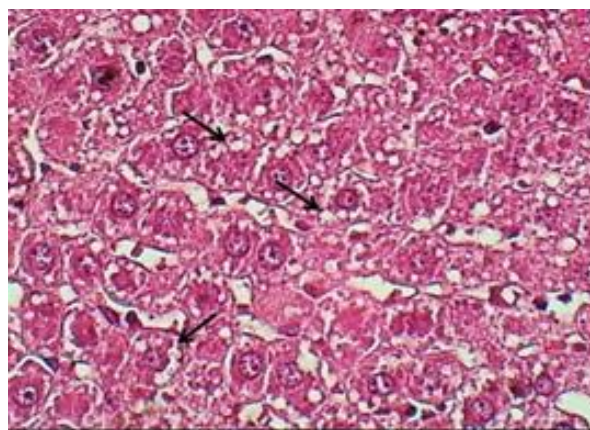


Figure 8: Section of the liver (a group of 200 mg/kg (bw) of CME ) shows: microvacuolation of hepatocytes (arrow). H&E stain.400x.

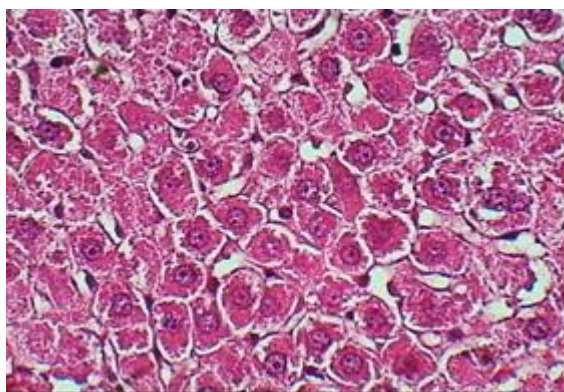


Figure 9: Section of the liver (a group of 300 mg/kg b.w. CME ) shows: the normal appearance of hepatocytes. H&E stain.400x.

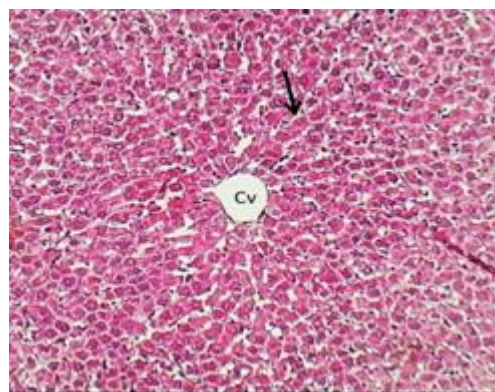


Figure 10: Section of the liver (a group of 300 mg/kg b.w. CME ) shows: the normal appearance of hepatic lobules, central vein (Cv)& hepatic cord (arrow). H&E stain.100x.

These results are consistent with (Ikechukwu *et al.*, 2019) indicated that liver sections of male rats treated with paracetamol suffer from degeneration of the liver lobules. Also, loss of radial arrangement around the hepatic vein, irregularity of the hepatic cord with swelling of the affected liver cells, and the appearance of small gaps within the cytoplasm as well as congestion of hepatic sinuses. On the other hand, it also agreed with the findings of (Shahriari and Moghadamnia 2019) for the ability of *C. myxa* fruit extracts to protect male rat liver tissues upon inducing oxidative damage by the toxic cadmium chloride CdCl<sub>2</sub>. These changes in liver cellular tissue in a group treated with paracetamol can be attributed to the formation of reactive oxygen species ROS from free radicals (Dash *et al.*, 2007). It causes damage to various components of membranes and organelles such as the mitochondria of the tissue cells

receiving the drug paracetamol. As for congestion and swelling of the sinuses, it may be attributed to an increase in the pressure of the hepatic portal vein as a result of injury (Karkar *et al.*, 2007). In terms of small gaps within the tissue, it may be relapses or resulting from oxidation in the liver tissue (James, 2020). The pathological changes observed through the microscopic images were associated with liver function tests, as increased values of the activity of liver enzymes ALP, AST, and ALT in blood serum were associated with more pathological changes, that is, the increase in normal tissue damage is accompanied by an increase in the leakage of liver enzymes into the bloodstream. These changes are associated with an increase in the level of lipid peroxidation, which in turn leads to cell damage (Turgut *et al.*, 2006). The apparent improvement in the liver health status of male rats treated with aqueous and

alcoholic extract at a concentration of 300 mg/kg of body weight is due to the protective role of the aqueous and alcoholic extract with a preference for the above concentration based on liver function tests and measurements of catalase activity. Where the active substances in the extracts work to protect liver cells from oxidative stress by balancing free radicals and antioxidants in addition to their role in treatment (Amin *et al.*, 2007). Especially flavonoids present in the two extracts, in addition to their important role as a preventive substance that prevents the process of fat oxidation (Jagadeesan and Kavitha, 2006). As well as, the presence of some other types of phenolic compounds such as Caffeic acid and Rutin which (Shahriari and Moghadamnia 2019) indicated in the *C. myxa* fruit extract had a protective effect on rat liver tissues.

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