International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.9, pp 60-72, 2017

Comparative study for the Preventive effect of Quercetin and Trigonella fenum graecum extract in carbon tetrachloride induced hepatotoxicity at male rats

Noor Hassoon Al-Qaraghuli[1]*,SaherMahmood Al-Jammali[2]
Mohammed Dakhil Al-Rekabi[3]

[1]University College of Humanities / Pharmacy Department,
[2]College of Education for Girls / Kufa University,[3]University College of Humanities / Pharmacy Department,Iraq

Abstract : The Liver is considered the main organ that is responsible for the detoxicating of the human body, which makes it the first defense in facing the different chemicals entering the body from the Digestive System. According to that, this study was designed to study Quercetin And *Trigonella fenum gracum* Seeds' extracts preventive effect in the carbon tetrachloride poisoned hepatic tissue of the male rats liver and knowing their effects if used together. The study is conducted at the Faculty of Pharmacy, Kufa University, that include using 35 (Sprague-Dawley)-rats which were divided into five groups: Control Group, Positive Control Group, Extract-Treated Group, Quercetin-Treated Group, and Dually treated Group. The dosage lasted for 7 days, and on the 8th day, they were poisoned with carbon tetrachloride, and killed after 24 hours. The study continued from April 2013, to July 2013.
The livers of the treated animals were weighed and the effectiveness of the transferase enzymes (ALT and AST), Alkaline Phosphatase enzyme (ALP) and the overall bilirubin level in the antidote. The results showed an increased weight of the liver and effectiveness of the transferase and phosphatase enzymes, and the bilirubin level in the antidote of the treated animals when compared to the Positive Control Group.
The results of the study showed that the Trigonella Fenum Gracum Seeds' extracts and the Quercetin showed a preventive effective in protecting the hepatic tissue against the carbon tetrachloride toxication through maintaining the liver enzymes and the bilirubin level. Moreover, using both of the materials, the Trigonella Fenum Gracum Seeds' extracts and the Quercetin, helped to double protect the high efficiency hepatic tissues because of the positive effect they have when used together.

Introduction

The Liver plays a critical part in performing the main functions of the organism. It is prone to be affected by chemicals considering its broad metabolic capacity as well as the cellular changes in its physiological synthesis(1). It is the main organ responsible for detoxing the bodies of the Vertebrates, and plays a critical role in the carbs, protein, and fat metabolism and helps removing the toxics of various materials. It is also capable of controlling synthesizing and production the Bile(2).

Many Chemicals such as Acetaminophen, yellow phosphorous, and Carbon tetrachloride can reflect serious damage to the hepatic tissue in a predictable way that depends on the dosage. The most common ways

through which the liver can be affected include the formation of the harmful metabolic products via Cytochrome system p450 (3). Liver is considered one of the most organs prone to be affected by toxics because all the absorbed substances after being swallowed are moved to the liver first, because it is responsible for the body metabolism and the removal of many harmful substances (2).

The carbon tetrachloride is known to be poisonous to the liver. It triggers the toxicity of the liver through producing free roots and hyper fat oxidization in the hepatic tissues, which leads to a the convulsive liver damage (4). Also, the liver is the main target of the carbon tetrachloride toxicity because it contains large amounts of Cytochrome P-450 (5).

The Trigonella fenum gracum Seeds' extract is considered one of the important herbs that is used in traditional medicine. This herb possesses a wide therapeutic properties like being a fat blood sugar reducer, galactagogic, and diuretic (6). Chinese herbs doctors found that it has a very effective role in treating kidney and liver diseases (7).

Quercetin is of the Flavonoids that are called (Flavonals). They are the spine of other flavonoids including citrus fruits and flavonoids compositions that are like Retin and Narangin (8). One of the Quercetin most important characteristics is its capacity to work as an anti-oxidant by searching for the free roots and removing them (9). It also proven that Quercetin also prevents the molecules from sticking on the endothelial cells (10).

Aim of the Study

1- Evaluating the Liver's protective effects by Quercetin and organic Trigonella Fenum Gracum Seeds' extract in the liver cells through the estimate the effectiveness of the ALP, AST, and ALT enzymes and the bilirubin level.
2- Studying the preventive capacity and efficiency of the liver after mixing the Trigonella Fenum Gracum Seeds' extracts with the Quercetin together.

Materials and Methods

Materials

1- The Lab Animals Preparation

Thirty white lab rats of the Sprague-Dawley were used in this study. Their weights ranged from 200-250 gm, and are all younger than three months. These rats were brought from the animal house at the Faculty of Pharmacy, Karbala University and were admitted into that of the Faculty of Pharmacy, Kufa University. They were taken care of until they became mature enough (three months old).

2- Seeds of Trigonella Fenum Gracum

The powder of the Trigonella Fenum Gracum were brought from the department of drugs and medical of the Faculty of Pharmacy, Kufa University. They were previously defined and sorted by the National Iraqi Herbal Center.

3- Quercetin

The Quercetin was bought from Jarrow Formulary, USA, and it was a clear powder.

4 - Methods

4-1 The Trigonella Fenum Gracum Seeds Extracts Preparation

One Kilogram of the Trigonella Fenum Gracum Seeds were smashed and grounded in the electric grindery in order to get the powder of these seeds. Then, after adding 1500ml of Hexane and the extraction was made by the Sequential Extraction device. The process took 6 hours at 40^{O} centigrade (till the yellow color disappeared). After that, what is left of the seeds was taken, and the extract now is oil-free and was left to cool and dry for 24 hours. The remaining is the powder is the extract of the Trigonella Fenum Gracum Seedsfrom

which the active ingredients were extracted using the same device after adding 2 liters of Ethel Alcohol (80%) for 6 hours at 40 centigrade.

Then the mixture is left to cool down and filtrated using filtration paper. Then, the leached in vaporized using the evaporator device at 40 centigrade until the extract is completely free of Ethel alcohol but containing all the effective ingredients of the Trigonella Fenum Gracum seeds. The total weight of the resulting product is 173.641 gm (At a rate of 173.641mg active extract ingredient for each 1gm of the seeds powder. The extract was dissolved in 1000ml of distilled water (D.W), so each 1ml of the resulting liquid contains 1gm of the extract. The subjects were injected with the resulting according to their weights (11).

4-2 Quercetin Preparation

The used Quercetin dose was 10mg/kg and given to the rats according to their weights by intraperitoneally injecting them in the blood stream (12).

4-3 Experimental Groups

First Group: This group was injected in the peritoneal membrane with 2ml/kg of the normal saline for seven days. This group was considered a control group.

Second Group: This group was injected in the peritoneal membrane with 2ml/kg of the normal saline for seven days. And it was given a mixture of equal volumes 1:1 of Carbon Tetrachloride and olive oil, 2ml/kg injected in the peritoneal membrane in order to inflect damage to the rats' livers(13). And this was considered a Positive Control Group.

Third Group: This group was injected with 147.282mg/kg (of the rats' weight) of Trigonella Fenum Gracum Seeds' extracts in the peritoneal membrane (14) every day for seven days. On the eighth day, they were given a mixture of equal volumes 1:1 of Carbon Tetrachloride and olive oil, 2ml/kg injected in the peritoneal membrane in order to inflect damage to the rats' livers(13). And this was considered an Evaluation Group of the Preventive Effectiveness of the Trigonella Fenum Gracum Seeds' extract in the poisoned rats.

Fourth Group: This group was injected with 10mg/kg (of the rats' weight) of the Quercetin drug in the peritoneal membrane consequently for seven days. On the eighth day, they were given a mixture of equal volumes 1:1 of Carbon Tetrachloride and olive oil, 2ml/kg injected in the peritoneal membrane in order to inflect damage to the rats' livers(13). And this was considered an Evaluation Group of the Preventive Effectiveness of the Quercetin drug in the poisoned rats.

Fifth Group: This group was injected with 147.282mg/kg (of the rats' weight) of Trigonella Fenum Gracum Seeds' extracts and 10mg/kg (of the rats' weight) of the Quercetin drug in the peritoneal membrane respectively for seven days. On the eighth day, they were given a mixture of equal volumes 1:1 of Carbon Tetrachloride and olive oil, 2ml/kg injected in the peritoneal membrane in order to inflect damage to the rats' livers. And this was considered an Evaluation Group of the Preventive Effectiveness of the Trigonella Fenum Gracum Seeds' extracts and the Quercetin drug in the poisoned rats.

4-4 Sacrificing subjects and collecting samples to estimate the hepatic tissue damage:

After 24 hours of injecting the animals with Carbon tetrachloride, they were anaesthetized with diethyl ether and they were dissected from the abdominal cavity. The blood was drown from their hearts using the Heart Puncture method in order to get a decent amount of blood to put it in the test tube that does not include an anticoagulant. The liver was removed from each rat and their weights were taken using a sensitive scale.

4-5 Preparing Serum Samples

The serum samples were prepared after splitting it from the collected heart blood (by means of blood puncture method), they were put in the Centrifuge device at 3000 rpm for 15 minutes. The floating layer was taken to measure the effectiveness of the ALP, ALT, AST enzymes as well as the total Bilirubin level (15).

4-5-1 Evaluating the Transferase Enzymes Effectiveness in the Serum

The ALT and AST enzymes were estimated through the use of personal kit from France Biomerieux.

4-5-2 Evaluating the Alkaline Phosphatase (ALP) in the Serum

The Alkaline Phosphatase (ALP)was estimated through the use of personal kit from France Biomerieux.

4-5-3 Evaluating the total Bilirubin Level in the Serum

The total Bilirubin Level was estimated through the use of personal kit from France Biomerieux.

4-7 The Statistical Analysis

The 20 edition of the statistical program Statistical Package Social Sciences, known as SPSS, was used. It uses ANOVA (Data Analysis) to compare the different data and the least significant difference (L.S.D) at the highest possibility ($P<0.05$).

Results

1- The effect of different treatment on live weight

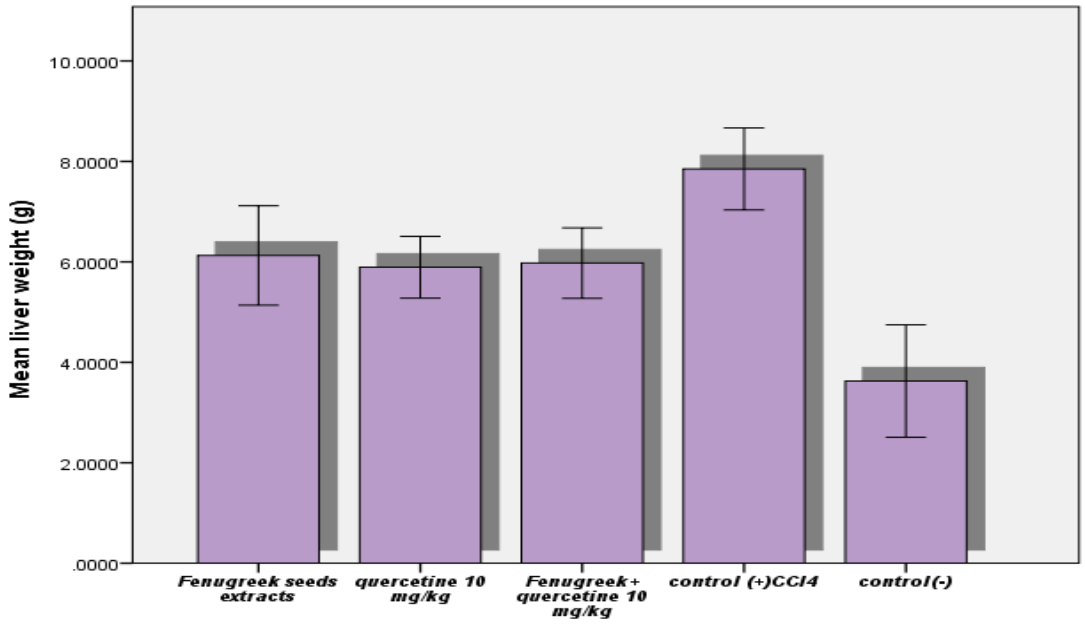
The results in table (1) shows a significant increase ($P<0.05$) of the liver weight for the groups, the positive control group, the Trigonella Fenum Gracum Seeds' extract-injected, the Quercetin-injected group, and that injected with both of the extract and the drug when compared to the control group. The results in the same table showed a significant decrease ($P<0.05$) of the liver weight for the Trigonella Fenum Gracum Seeds' extract-injected, the Quercetin-injected group, and that injected with both of the extract and the drug when compared to the positive control group.

The results also showed no significant differences ($P>0.05$) of the liver weight for the Trigonella Fenum Gracum Seeds' extract-injected compared to that Quercetin-injected group and that injected with both of the extract and the drug. It also showed no significant difference ($P>0.05$) when comparing the Quercetin-injected group, and that injected with both of the extract and the drug, as indicated in figure 1.

Table (1): The effect of different treatment on liver weight at male rats

Groups	Average± Standard erorr
	liver weight(g)
Control	0.43±3.626
Positivecontrol	0.31±7.851*****
Fenugreek seeds extracts	0.38±6.1275*****
quercetine 10 mg/kg	0.23±5.8928*****
Fenugreek extract+quercetine 10 mg/kg	0.27±5.976*****

*** Significant difference (P>0.05).**



Figure(1) comparison between different groups regarding liver weight

2- The effect of different treatments ALT in activity in the serum

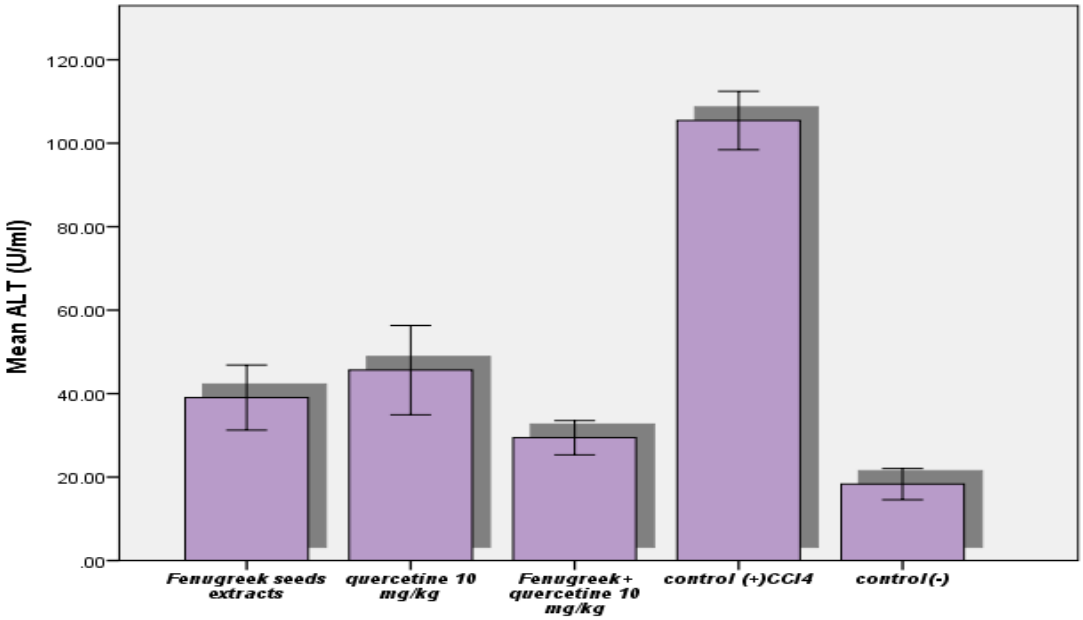
The results mentioned in table 2 showed a significant increase (P<0.05) in the ALT effectiveness for the groups: Positive Control Group, the Trigonella Fenum Gracum Seeds' extract-injected, the Quercetin-injected group, and that injected with both of the extract and the drug when compared to the control group, Whereas it showed a significant decrease (P<0.05) in the ALT effectiveness for the Trigonella Fenum Gracum Seeds' extract-injected, the Quercetin injected-group, and that injected with both of the extract and the drug compared to the positive control group.

The results showed no significant difference in the ALT effectiveness for the Trigonella Fenum Gracum Seeds' extract-injected compared to the Quercetin-injected group, but have showed a significant difference (P<0.05) for the same group compared with that injected with both of the extract and the drug.

The results mentioned in the same table showed significant differences (P<0.05) when comparing the Quercetin-injected group and that injected with both of the extract and Quercetin, as shown in figure 2.

Table (2): The effect of different treatment on ALT activity at male rats

Groups	**Average±Standard erorr**
	ActivityALT (U/ml)
Control	1.45±18.31
Positive control	2.71±105.43*****
Fenugreek seeds extracts	3.03±39.045*****
quercetine 10 mg/kg	4.16±45.63*****
Fenugreek extract+quercetine 10 mg/kg	1.59±29.433*



Figure(2): comparison between different groups regarding ALTactivity.

3- Effect of different treatment on AST activity in the Serum

Table no.3 showed a significant increase (P<0.05) in the AST effectiveness in the groups: The positive Control Group, the extract-injected group, and the drug-injected group when compared to the control group. However, the results showed no significant difference (P>0.05) for the extract and drug treated group when compared to the control group.

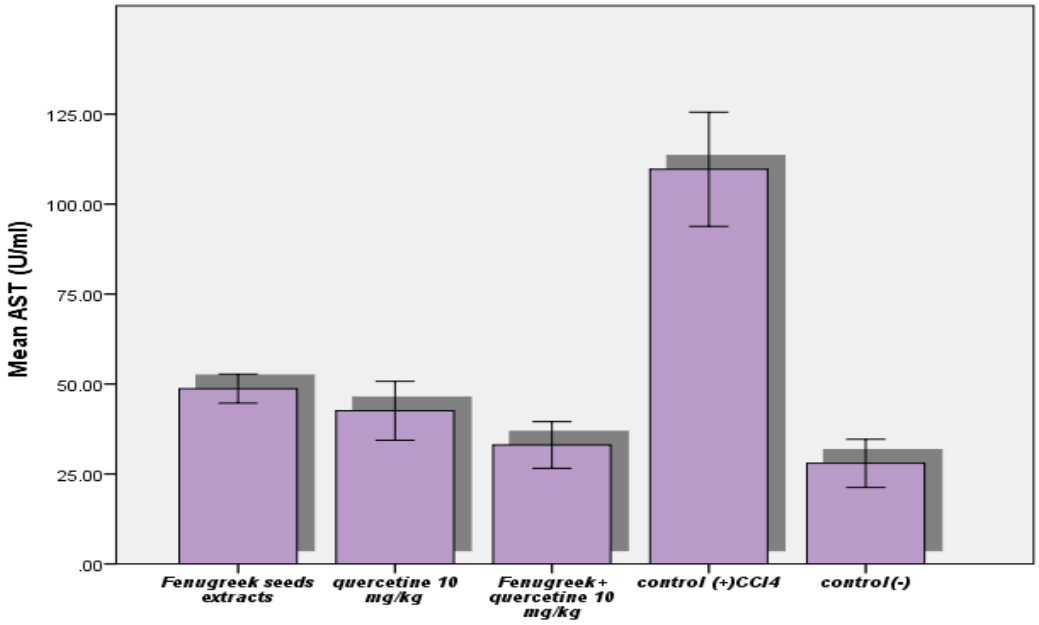
It showed a significant decrease (P<0.05) for the groups, the extract-injected group, the drug-injected , and both extract and drug-injected group compared to the control group.

The results also showed no significant differences (P>0.05) in the AST effectiveness of the extract-treated group compared with the drug-treated group, whereas it showed a significant difference (P<0.05) for the same group compared with that treated with both the drug and extract together.

However, the comparison between the Quercetin-injected group and that treated with both materials showed no significant difference between them (P>0.05), and as it is shown in figure (3).

Table (3): The effect of different treatment on AST activity at male rats

Groups	**Average± Standard error**
	Activity AST (U/ml)
Control	2.60±27.95
Positive control	6.16±109.68*****
Fenugreek seeds extracts	1.57±48.7*****
quercetine 10 mg/kg	3.19±42.55*****
Fenugreek extract+quercetine 10 mg/kg	2.51±33.05



Figure(3) comparison between different groups regarding AST activity.

4- The Effect of Different Treatments in the Serum ALP activity

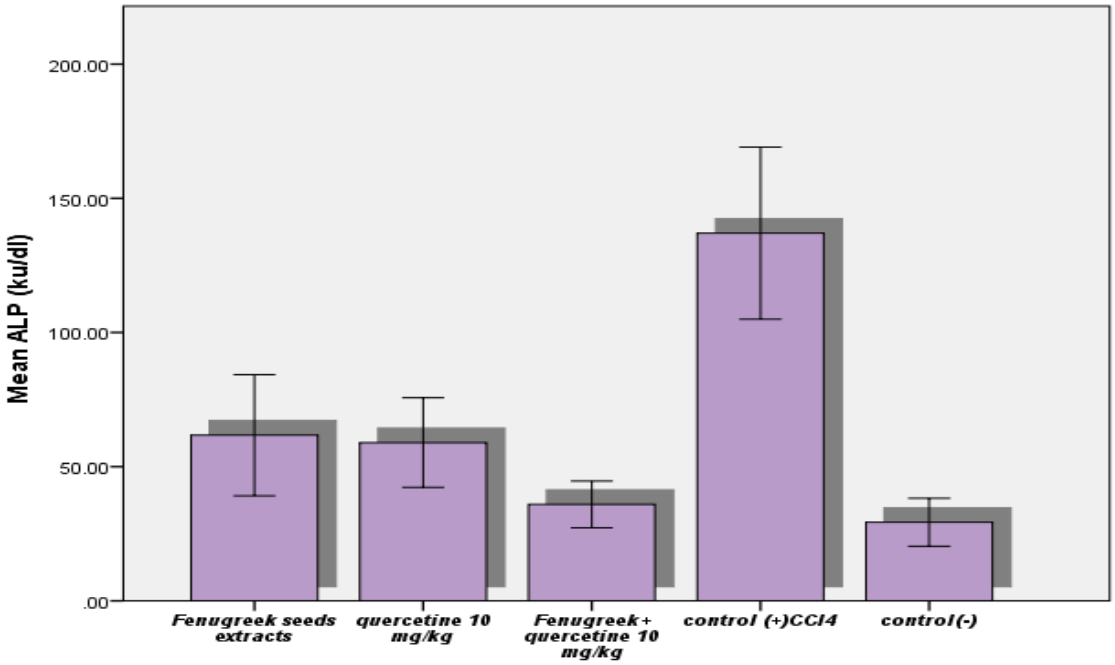
As the results show in table (4), a significant increase (P<0.05) was noticed in the ALP effectiveness for the groups: positive control group, extract-treated group, and drug-treated group when compared to the control group. However, the extract and drug-treated group showed no significant difference (P>0.05) when compared to the control group.

A significant decrease (P<0.05) in that effectiveness was found in: extract-treated group, Quercetin-treated group, and both the extract and Quercetin treated group.

The results showed no significant differences (P.0.05) in the ALP effectiveness for the extract-treated group compared with the Quercetin treated group. However, it showed a significant difference (P<0.05) for the same group compared to that treated with both the Quercetin and Extract together. A significant difference (P<0.05) was noticed when comparing the Quercetin-treated group with the extract and Quercetin-treated group, and as displayed in figure (4).

Table (4): The effect of different treatment on ALP activityat male rats

Groups	**Average±Standard error**
	activity ALP(U/l)
Control	3.47±29.31
Positive control	12.48±137*
Fenugreek seeds extracts	8.77±61.73*
quercetine 10 mg/kg	6.49±58.98*
Fenugreek extract+quercetine 10 mg/kg	3.38±35.93*



Figure(4) comparison between different groups regarding ALP activity.

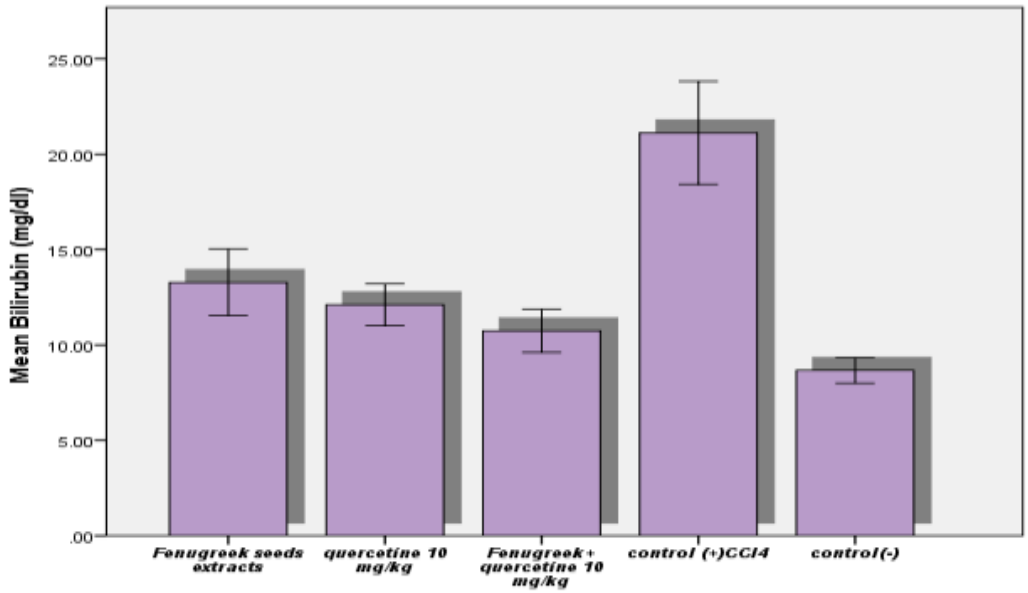
5- The Effect of Different Treatments on total Bilirubin level in the serum.

From table (5), a significant increase (P<0.05) is noticed in the bilirubin level for the groups: positive control group, Extract-treated group, Quercetin-treated group, and extract and Quercetin-treated group when compared to the control group. A significant decrease (P<0.05) of the bilirubin level for the groups: extract-treated, Quercetin-treated, and extract and Quercetin-treated group when compared to the positive control group.

The results showed no significant differences (P>0.05) in the bilirubin levels for the extract-treated groups compared to the Quercetin-treated groups. It also indicated significant differences (P<0.05) for the same group when compared to the extract and Quercetin-treated group. However, the Quercetin-treated group indicated no significant differences (P>0.05) when compared to the extract and Quercetin-treated group, see figure (5).

Table (5): The effect of different treatment on Bilirubin levelat male rats

Groups	**Average±Standard error**
	Bilirubin level(mg/dl)
Control	0.25±8.666
Positive control	1.04±21.121*****
Fenugreek seeds extracts	0.67±13.2767*****
quercetine 10 mg/kg	0.42±12.1017*****
Fenugreek extract+quercetine 10 mg/kg	0.44±10.74*****



Figure(5) :comparison between different groups regarding bilirubin level

Discussion

1- Effect of Different Treatments on Liver Weight

The results showed a significant increase in the liver weights of the carbon tetrachloride-treated animals compared to those treated with the extract, the Quercetin, or both of them. This was due to hepatic cells hypertrophy and swelling tissues surrounding the blood vessels of these cells, the congestion of their nuclei, increasing in their weight, and ultimately increasing the liver weight.

The current study showed an increase of the liver weight for the groups treated with the extract and the Quercetin and both of them together, compared to the control group. This is due to the effect of the Trigonella Fenum Gracum Seeds' extract as it contains carbs, proteins, and amino-acids, as well as steroids, alkaloids, flavonoids, and the different vitamins, especially Beta-carotene, and Thiamine (16), which may have triggered a significant increase in the extract-treated animals' liver weight.

This increase may also be explained due to the fact that this extract contains lots of mineral salts that include: Calcium, Sodium, Potassium, Iron, and Phosphates ions (16) which may have indirectly led to an increase in the liver weight due to their contribution in the different hepatic protein building process, especially the enzymes.

It is possible that injecting the rats with the extract increased the food consumption rates of these rats. Some sources showed that the extract is widely used in medicine in general, and as an aperitif in particular (17).

The liver weight increase may partially be referred to the fact that the extract contains more than 8% of water (18) which may have positively affected the liver and increased the aquatic content of the liver, which significantly increased their weights. And this increase may also be due to all the facts and possibilities mentioned above.

However, as for the increase in the liver weight for the Quercetin-treated animals, it may have happened due to the biological effects of the Quercetin contents. It is considered one of the Polyphenol Flavonoids (19, 20) which may have caused a significant increase in the weight of the studied rats' livers.

This increase may be due to the preventive effect of the Quercetin in the hepatic tissue (21) which may have triggered the tissue construction in these cells, their growth rate, node cell divisions, as well as their reproductive rate.

As for the significant increase noticed in the weight of the livers in the extract and Quercetin-treated animals, it may be due to the collective positive effects of the extract and the protective impact of the Quercetin in the hepatic tissues, which results in the activation of these cells' bioactivity and stimulate their reproduction rates as well as their renewal speed, and ultimately, a significant increase in the animals' liver weights included in this study.

2- The Effects of Different Treatments on ALT and AST in Serum

The results showed an increase in the effectiveness of both the ALT and AST for the groups treated with carbon tetrachloride. This may be due to the fact that this substance is of great toxicity for the hepatic tissues. These two enzymes are highly sensitive regarding the hepatic injuries and they are released in big amounts for the serum after any oxidative effect done to the hepatic tissues (22).

The noticed increase in the effectiveness of the (previously mentioned) two enzymes was reduced for the extract-treated group when compared with the control group, and the decrease when compared with the positive control group. What Said found out (23) which illustrated that the extract have showed a high efficiency in protecting the hepatic cells from the damage inflected by the oxidative stress due to the effectiveness of the antioxidants this extract includes in facing the free roots resulting from the stress. The effectiveness of the liver enzymes have dropped significantly for the extract-treated group, but they were still above the control group level.

The increase and decrease in the enzymes effectiveness for the extract-treated group compared with the control group and the positive control group respectively may be due to the fat accumulation which indicates tissue changes because these enzymes leak out from the hepatic tissues to the blood circulation when these cells are prone to be infected (24). Their effectiveness increase in the serum in all types of the hepatic injuries (25), including hepatotoxicity with carbon tetrachloride (26).

Or may be the cause of reduction in the enzyme effectiveness is in the group of the fenugreek seeds extract , along with the active elements that these seeds contain which are carbs, protein, amino acids, vitamins, flavoind compounds, mineral salts and other elements(16), which may do a vital important role to protect the structural construction for liver's cells from being poisoned with carbon tetrachloride ; and do work on reducing the range of its poisoning effectiveness upon these cells , and thereby reduce the effectiveness of these enzymes within the animal's serum that had been treated with such extract.

Or may the efficiency of fenugreek extract to maintain the effectiveness of enzymes in the animals' livers that were exposed to poison, it relates to all reasons mentioned above.

The results showed an increasing as well in the effectiveness of ALT and AST for those groups which had been treated with quercetin, comparing them with controlling group. While it showed decrease in its efficiency for the same group comparing it with controlling positive group, and that goes along with a study is made by(12)which had proved that the biological effectiveness of quercetin prevents the increase of serum's enzymes in the rats poisoned by Carbon tetrachloride. Or maybe this effect relates to the fact that quercetin has a protective effect for liver cells as was proved by (21) which protected it from poisoning despite being exposed to Carbon tetrachloride, which might be what actually happened in this study.

As for the group that was treated with the extract and quercetin combined, the results showed an increase in the effectiveness of the enzymes in compare with the control group and a decrease in the same group compared with positive control group. This result might be because of the multiplied effect in the treated animals which was a result of using the extract and quercetin combine, thus, reaching a higher liver protectiveness as a result of the effective ingredients of fenugreek and quercetin combined.

3- Effect of Different Treatments on ALP Activity in Serum:

The current study's results pointed to a significant increase in the effectiveness of ALP on the groups that were treated by Carbon tetrachloride, and this might be because of the high poisoners levels in this matter, where this enzyme is considered to be one of the most sensitive enzymes to hepatic injuries, that is why it is released by large amounts to the blood stream in case if liver is exposed to any oxidative stress (22).

The increase in the effectiveness of the enzyme in the group treated by the extract went the same way with the control group, and the decrease in the same group in compare with the positive control group with (23) which showed that the fenugreek's extract has shown an indeed positive action in protecting the liver cells from the damage caused by oxidative stress, thanks to the viral effectiveness of antioxidants facing the resulting free roots from such stress, as well as the decrease of the enzyme effectiveness of all liver enzymes in the extract group.

The effectiveness of the enzyme also increases massively in the serum because of the bile stagnation which maybe resulted from liver cells being exposed to a damage caused by carbon tetrachloride, where big amounts of this enzyme are released into the blood in cases of hepatic injuries(27).

The increase and decrease in the enzyme levels, in the group treated by the extract compared to the control group and the positive control group respectively, caused by the increase of fat concentration in the liver cells, which might lead to making negative changes in the histological structure of these cells .

Or this decrease of effectiveness in the extract group might be because of the effective ingredients of the fenugreek seeds which maybe helped on a big scale in protecting and nourishing liver cells and preserving its cytoplasmic and nuclear contents from the damage resulted by the poisonous carbon tetrachloride, which lead to these cells to maintain its form and its enzyme content, which resulted in not being affected by the biological effectiveness of those enzymes.

The results of the current study showed an increase of ALP levels in the quercetin treated groups in compare with the control group, also a decrease in the same group in compare with the positive control one, and that might be because of the protective effect of the quercetin on liver cells and tissues (21) as previously mentioned.

The results showed a drop in ALP's effectiveness the in the group treated by the extract and quercetin combined in compare with the control group, which might be trailed to the synergistic action of the active chemical constituents of the ring seed extract and quercetin together in the protection of hepatic cells and the preservation of their enzymes from the toxic effect of exposure to carbon tetrachloride.

4-Effect of Different Treatments on Total Bilirubin Level in Serum:

The results showed an increase in total serum bilirubin in the group treated with carbon tetrachloride, which may be due to the toxic effect of this substance, which may have caused the destruction of hepatocellular membranes by its production of free radicals, resulting in the release of superoxide fats from the membranes of these cells(28), which would cause a significant increase in the total level of bilirubin in the bloodstream (29), possibly in the current study.

The results of the current study showed an increase in the level of bilirubin in the treated group compared with the control group. It also showed a decrease in the level of bilirubin in the same group compared to the positive control group. This result was agreed with (23), which indicated a significant decrease in the total bilirubin level in the treated group. This decrease may be due to the positive effect of the extract of the fenugreek seeds from antioxidants which have shown high biological efficacy in reducing the level of superoxide fat which is likely to be a major cause of severe damage to hepatic cells and tissues, resulting in disruption of hepatic secretion of bilirubin and therefore a biliary stagnation in these cells as confirmed (29).

This decline may be delayed by the biological efficacy of the pre-indicated fenugreek seed components, which may have had a positive effect on the protection of the histological structure of the hepatic cells and the maintenance of their various activities and thus significantly reduced serum bilirubin levels. The decline in the level of bilirubin observed in the present study may explain the two reasons cited.

As for the observed decrease in bilirubin level in the quercetin-treated group compared to the positive control group, it is likely that the quercetin is likely to have a protective effect on hepatic cells (21), as it is a flavonoid (19,20) ,which may have shown high bioactivity in protecting the histological structure of liver cells by their effective chemical composition.

The decrease in the total bilirubin level in the treated group with the extract and quercetin compared to the positive control group may be due to the combined effect of the ring constituents and the protective effect of quercetin together in the treated animal livers, thus protecting and maintaining serum bilirubin levels despite exposure to carbon tetrachloride.

Conclusions:

1. Quercetin in the rat model showed an invariant protective effect of liver cells from carbon tetrachloride poisoning by maintaining the effectiveness of liver enzymes.
2. The organic extract of the seeds of the ring plant in the model of rats has a protective effect of hepatic cells of carbon tetrachloride poisoning by maintaining the effectiveness of liver enzymes.
3. Using the extract of the seeds of the fenugreek and quercetin together have resulted in the protection of liver tissue from poisoning and biological efficiency higher than their efficiency alone, indicating that one of them supported the other in showing the protective effect of the liver.

Recommendations:

1. Recommend more verification of results to the same health problem by adopting more precise standards at the molecular level and supported by modern techniques such as PCR and specific biomarker.
2. Using a wider sample of animals and a more accurate poisoning model.
3. Adoption of clinical trials of compounds with therapeutic correct in humans.
4. Adopting the therapeutic method as well as the preventive method.
5. Evaluation of therapeutic attempts to poison the liver with natural compounds and the selection of vehicles with high efficiency and safety to solve the problem of hepatic poisoning.
6. purification and evaluation of the protective effect of the components of the ring seeds dissolved organically in the liver.

References

1. Khan, T. and Sultana, S. (2009). Antioxidant and hepatoprotective potential of *Aegle marmelos* Correa against CCl4-induced oxidative stress and early tumor events. J. Enz. Inhib. Med. Chem., 24 (2): 320-7.
2. Casarett andDoull's. (2008). toxicology; the basic science of poisons, 7th ed. Mc-Graw Hill, pp: 557-576.
3. Sunita, T.; Vupta, V. and Sandeep, B. (2008). Comparative study of antioxidant potential of tea with and without additives, Indian. J. physiol. Pharmacol., 44: 215-219.
4. Ram, V.J. and Goel, A. (1999). Curr. Med. Chem., 6, 217-254.
5. Södergren, E.; Cederberg, J.; Vessby, B. and Basu, S . (2001). Vitamin E reduces lipid peroxidation in experimental hepatotoxicity in rats. Eur.. J. Nutr., 40 (1):10-6.
6. AL-atwi, L.F. (2010). Clinical evaluation for the diuretic effect of the alcoholic extract of *Trigonella faenum- gracum* seeds (fenugreek) on rabbits. Kufa. J. for Veterinary. Med. Sci., 1(1): 116-121.
7. Prasanna M. (2000). Hypolipidemic effect of fenugreek. A clinical study Indian. J. phramcol., 32:34-6.
8. Madhavan P.N.; Nair, L.; Zainulabedin, M.; Saiyed; NimishaH. ;Gandhi and Ramchand, C.N. (2009). The flavonoid, quercetin, inhibits HIV-1 infection in normal peripheral blood mononuclear cells. Am. J. Infec. Dis., 5 (2): 142-148.
9. Hubbard, G.P.; Wolffram, S. and de Vos, R. (2006). Ingestion of onion soup high in quercetin inhibits platelets aggregation and essential components of collagen-stimulated platelet activation pathway in man: A pilot study. Br. J. Nutr.,96(3):428-8.
10. Lakhanpal, P. and Kumar, D. (2007). Quercetin: a versatile flavonoid.Inter. J. Med. Update., 2.
11. AL-Hakeemi, A.A. (2002). Isolation of Trigonelline from Iraqi fenugreek seeds and studying its effect on blood glucose level and lipid profile in normal and alloxan-diabetic rabbits. M.Sc. thesis. Dept of clinical pharmacy, College of Pharmacy, University of Baghdad.
12. Janbaz, K.H.; Saeed, S.A. and Gilani, A.H. (2004). Studies the protective effects of caffeic acid and quercetin on chemical-induced hepatotoxicity in rodents. Phyto. Med., 11: 424-430.

13. Fadhel, Z.A. and Amran, S. (2002). Effects of black tea extract on carbon tetrachloride-induced lipid peroxidation in liver, kidneys and testes of rats. Phytother. Res., 16(1): 28-32.
14. Devasena, T. and Menon, V.P. (2002). Enhancement of circulatory antioxidants by fenugreek during 1,2-dimethyl hydrazine-induced rat colon carcinogenesis. J. Biochem. Mol. Biol. Biophys., 6(4): 289-292.
15. Shenoy, A.K.; Somayaji, S.N. and Bairy, K.L. (2001). Hepatoprotective effects of *Ginkgo biloba* against carbon tetrachloride induced hepatic injury in rats. Indian. J. Pharmacol., 33: 260-266.
16. Bralles, J.; Anderson, L.; and Phillipson, J.D. (2002). Herbal medicine 2nd ed. Pharmaceutical Press, Publication division of the Royal Pharmaceutical Society of Great British. pp: 209-211.
17. Bouaziz, A. (1976).Veterinary drags. French Demande. 20:4-8.
18. Duke, J.A. (1992). Handbook of phytochemical constituents of GRAS herbs and other economic plants. BocaRaton, FL CRC Press. Florida.
19. Hollman, P. C.; van Trijp, J. M.; Mengelers, M. J.; de Vries, J. H. and Katan, M. B. (1997). Bioavailability of the dietary antioxidant flavonol quercetin in man. Cancer. Letters., 114:139-140.
20. Sampson, L.; Rimm, E.; Hollman, P. C.; de Vries, J. H. and Katan, M. B. (2002). Flavonol and flavone intakes in US health professionals. J.Am. Diet. Ass., 102: 1414-1420.
21. Pavanato,A.; Tunon, M.J.; Sanchez-Campos, S.; Marroni, C.A.; Llesuy, S.; Gonzalez-Gallego, J. and Marroni, N. (2003). The effects of quercetine on liver damage in rats with carbon tetrachloride-induced cirrhosis. Dig. Dis. Sci., 48(4): 824-829.
22. Uskokovic- Marcovic, S.; Milencovic, M.; Topic, A.; Kotur-Stivuljevic, J.; Stefanovic, A. & Antic-Stancovic, J. (2007). Protective effect of tungsto phosphoric acid and sodium tungstate on chemically induced liver necrosis in wistar rats. J. Pharm. Phamaceut. Sci.,10(3): 340-349.
23. Said, A.M. (2005). The hepatoprotective activity of fenugreek seeds extract against carbon tetrchloiride-induced liver toxicity in rats. A thesis. College of pharmacy. University of Baghdad.pp:4-51.
24. Plaa, G.L. and Charbonneau, M. (2001). Detection and evaluation of chemically induced liver injury. In: principles and methods of toxicology 4th ed. A. Wallace Hayes (Ed.). Taytor and Francis. pp: 1145-1187.
25. Cadman, B.E. (1999). Adverse effect of drugs on liver. In: clinical pharmacy and therapeutics 2nd ed. Walker, R. and Edwards, C. (Ed.). Churchill Livingstone, London. pp: 183-194.
26. Obi, F.O.; Omogbai, L.A.; Oriafo, O.S.J. and Ovat, O.D. (2001). Effect of a short time post carbon tetrachloride treatment interval on rat plasma enzyme levels and percentage mortality. J. Appl. Sci. Environ. Mgt., 5(1): 5-8.
27. Hewawasam, P.R.; Jayatilaka, K.A.P.W.; Pathirana, C. and Mudduwa, L.K.B. (2004). Hepatoprotective effect of *Epaltes diraricata* extract on carbon tetrachloride induced hepatotoxicity in mice. Indian. J. Med. Res., 120: 30-34.
28. Lee, K. J.; Terada, K.; Oyadomari, S.; Inomate, Y.; Mori, M. &Gotoh, (2004). Induction of molecular chaperones in carbon tetrachloride-treated rat liver: implications in protection against liver damage. Cell –stress chaperon., 9 (1): 58-68.
29. Sethuraman, M.G.; Latitha, K.G. and Rajkapoor, B. (2003). Hepatoprotective activity of Sarcotstemma brevistigma against carbon tetrachloride-induced hepatic damage in rats. Curr. Sci., 84(9): 1186-1187.

*****