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**Antioxidants and Biochemical Effects of Oral Administration
of Red Pepper Aqueous Extract on Rabbit's Liver Treated
with Cholesterol**

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Abstract: This study was made to know the effect of the cholesterol with (250 mg/kg provender) concentration in a number of Histo-biochemical variables for liver tissue of local rabbit's. Aqueous extract of Red pepper was also used in this study with (1000mg/kg B.W) to reduce the negative effects of the rising levels of cholesterol and Malondialdehyde (MDA) in liver tissue and also to enhance its antioxidant efficiency in eliminating the free radicals that are formed inside the body, by containing high level of vitamins A, E, C and Biotin.

This study has included 40 aged male rabbit, randomly divided into four groups, 10 rabbits per each. Treatment with cholesterol alone has led to negative effects in the results of Histo-biochemical tests for the treated local male rabbits liver tissue through the significant increase in the levels of cholesterol and (MDA),



and the significant increase in the levels of Glycogen and Glutathione in possibility level of ($P \leq 0.05$). on the other hand, treatment with the red Aqueous extract of Red pepper with (1000 mg/kg B.W) has led to a reduction in the negative effects for the cholesterol added to the provender, when the result showed a significant decrease in the levels of cholesterol and MDA and increase of Glycogen and Glutathione in possibility level of ($P \leq 0.05$).

Keywords: Red Pepper, Aqueous Extract, Rabbit's Liver, Cholesterol.

INTRODUCTION

Since the dawn of time, Arabs have studied various sciences, Medicine and Pharmacy are among which. They discovered many plants were not recognized by the others and made syrups and extracts. Plant in general was their source for prescriptions, or some parts of it as fruits, seeds, leaves, or even legs. Astonishingly, plenty of these prescriptions are of great value and still up to now used in the modern herbal medicine (Suman, 2001)).

Due to the damages of increased use of the chemicals, we see the world has returned to nature for the enormous amount of plants which considered as a eternal treasure since the old times. However, using of these plants for healing has greatly changed due to the development occurred in the fields of Agriculture, chemistry and pharmacy.

All over the world, herbals and hot spices are considered of the natural additions to the food for giving a distinguished flavour, also used as remedy for many illnesses. Among these plants is Red pepper of *Solanaceae*, known as “ Cayenne” referring the name of the town being seeded in, eastern coast of southern America (Ahuja *et al.*, 2006).

High nutritional value of the Red Pepper is attributed to having huge amounts of vitamin C, Each 100 g of red pepper powder contains 175 mg of this vitamin as well as 1.2 g Protein, 11 mg of Calcium, 0.06 mg of Thymine, 0.30 mg of Riboflavin and 870 global units of vitamin A as well as containing folic acid, B vitamins groups and a high level of active ingredient Capsaicin (MacGillivray, 1961).

1- Cholesterol

Cholesterol is widely spread steroid, found in 18th century as a major components for the gallstones, been partially described by the French Chemical (Cheverul) in 1816 naming it Cholesterin in spite of the steroids are present in most of the body tissues, the Cholesterol ratio is clearly different between the free and ester cholesterol ratio. 60-80 % of Ester cholesterol is present in the blood plasma, but, in Brain and nervous tissues, Cholesterol is a main component that forming the Cerebellar casing of neurons (Al-Chalabi *et al.*, 1985). Cholesterol appears in the tissues and blood



Lipoproteins whether in free or ester shape. It is a component for the Biological membranes and primary material for the Steroid Hormones and Bile acids (David and Basil, 1989). Cholesterol percentage goes more than normal by being diagnosed with number of diseases such as Diabetes Mellitus, Atherosclerosis, Heart Disease, and Hypertension (Young and McEneny, 2001; Al-Hamish, 1999).

Cholesterol is produced in different cells in the body, however, the main spot of producing it is the Liver, it is also being produced in Intestine, Skin, Nervous tissue, Adrenal gland and Gonads, while the other source of it, is the food rich with Cholesterol (Bronk, 1999). Cholesterol Structural formula is rings of phenanthrin with a double bound between carbon atoms 5 and 6 (Al-Zuhairi, 2000).

2- Plant material used in the study

Red Pepper will be dealt with in this study considering it as one of the commonly used Medical Plants in the Arab world in general and Iraq in particular.

English name: Red Pepper

Kingdom: Plantae.

Subkingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Asterales

Order: Solanales

Family: Solanaceae

Genus: Capsicum

Species: Frutescens

3- Medical use of the Red Pepper

The common use of the various medical herbals and spices as Red Pepper, Ginger, turmeric and others has caught the attention to know their health impacts and to know the possibility of using it in the different medical applications for different diseases as an alternative or with chemotherapy putting into consideration potions and their amounts (Yoon *et al.*, 2005).



3-1: Effect of Red Pepper on the biochemical variables

Carbohydrate, Proteins and Fats are one of the important elements for the life of the organism. Thus, it is highly important to monitor changes happen to these important elements when exposed to number of chemical compounds for the sake of determining the physiological status for organism exposed to it. Many studies show spices ability to cure many illnesses by its impact over the chemical variables in blood like lower sugar level, among these spices are: *Zingiber officinae*, *Cinnamum zeylanicum*, *Curcuma longa*, *Capsicum frutescens* and others. Red pepper is one of the most effective spices on sugar level for containing high percentage of vitamin C which turned out to have a big role reducing stress and inhibiting secretion of corticosteroids hormone from the crust of the Adrenal gland, thereby inhibiting Gluconeogenesis process which thereby reduce the sugar level in blood plasma (Khalil, 2003). It is believed that the Capcasin in Red Pepper causing an increase in Calcium receivers, opening Calcium channels to pancreas cells, increase the secretion of Insulin hormone and lowering the sugar level in blood (Tolan *et al.*, 2001).

In a study done on volunteers, turned out that the sugar blood level reduced after (30-45) minutes of taking the Red Pepper compared with normal people, this is attributed to Capcasin, to activate substance Capcasin that function as a transporter (VRS1) which increase the secretion of Insulin resulting in lowering sugar level in blood (Chaiyasit *et al.*, 2009).

The researchers Ibrahim, (2009), showed that oral administration Chicken meat with Aqueous extract the boiled extract for red pepper fruit concentrated as 200g/L distilled water led to significant decrease in blood sugar level in chickens' serum compared with the control group because of an increase in carbohydrates oxidation and in energy burning through Capcasin influence, thus, lower blood sugar level. Turned out that feeding shrimps with provender containing red pepper fruit powder in 6.6% percentage led to increase in the level of the total protein in the muscles of the concerned animals, (Mustafà *et al.*, 2005). In another study for (Abdel Azim *et al.*, 2009) found that giving red pepper fruit powder in 10% percentage with the provender led to an increase in level of total protein, Albumin, Glubioline in Muscovy ducks serum.

The red pepper influence on cholesterol and triglycerides, the researcher Greville (1969), found that red pepper reduces cholesterol and triglycerides by expanding the bloodstream and blood vessels and inhibiting enzymes responsible for producing and construction fats and carbohydrates and they are: Acetylc-CoA-Carboxylase, Glucose -6-Phosphate dehydrogenase, Malic enzyme, Citrate synthetase. The reason behind the decrease of fats in mince fed on provender containing red pepper



is inhibiting fats absorption in the internal intestinal layer and increase throwing them away out of the body (Nopanitaya, 1973).

Reducing the weight of the fats surrounding liver and kidney is achieved by the influence of the red pepper on the metabolic enzymes and undermine fats, increase its secretion and increase fats move form fatty tissues to bloodstream to get metabolic it, and then thrown out of the body (Yoon *et al.*, 2005).

The damaged of liver cells by oxidation and lipid peroxidation is indicated by measuring the level of ALT and AST enzymes. It is found by the study, done by the two researchers, (Pulla and Lokesh, 1996) that injecting concentration of 2% of Aqueous extract red pepper inside rat body led to lower levels of fat in the liver and hence lower lipid peroxidation and the level of ALT and AST enzymes for concerned rats compared with the control group.

Red pepper is one of the most natural antioxidants-containing vegetables, it contains high level of vitamin C, various studies and researches which have conducted over this concern showed that the existence percentage of vitamin C in red pepper is 4-5 times more than it in lemon, that's why, this fruit (red pepper) is undoubtedly considered is top Vegetable Crops containing this vitamin 'C' (Al-Ware'a, 1980), as well as, having lots of red pepper may leads to increase the concentration on vitamin A in serum, because it contains high amounts of B-Carotene, the main source for this vitamin, which is described as one of the most powerful antioxidants against cancer and cardiovascular disease (Antonious *et al.*, 2006).

MATERIALS AND METHODS

1- Chemical materials

1-1: Cholesterol

Pure Cholesterol was used in this study which is extracted from the liver tissue, manufactured by France 'BDH' obtained from the local markets.

1-2: Plants used in the study

Red Pepper dried fruit was used in this study and was obtained from the local markets after ensuring the classification depending on Cheij, (1984), cleaned, grinded in grinder for 1 minute duration, saved in paper sacks till use.



1-3: Kits

The kits use to estimate the biochemical variables, provided from different international companies such as Biolabo (French company), Randox (English company) and Biocon (German company), which have all obtained from the local market.

2- Preparation of Aqueous extract of red pepper

In order to follow a practical not costly acceptable method, simply, the boild extract of the fruit of the red pepper was used without resorting to the complicated extracting methods. Samples of the dried fruit of red pepper were grinded in the grinder for 1 minute duration, it's being prepared based on (Pandit *et al.*, 1979).

3- Experimental Animals

40 local male rabbit were used in the study aged 8-10 months and weights 1200-1500 g, all were obtained from local markets and all placed in aluminium made cages specifically prepared for this purpose. Under suitable circumstances of suitable heat between 25-28° and 14 hours a day of lighting with good ventilation. They were put in a preparatory period of one week in order to get them used to the environment and provender before starting treating them and surely after making sure of their safe of any diseases, provender's were given to the rabbits on plastic made plates in equal quantity and in a stable sequence for all of the treated rabbits. Water was given in containers fixed on the cages to avoid water pouring.

4- Experiment design

Rabbis have randomly divided into four groups, 10 rabbits in each. Rabbit treating phase started after the end of the preparatory period on sequence basis for 8 weeks, treating started in October of 2009, initial weights were taken for the rabbits and then started to get treated through oral administration using gavage needle according to follows:

Control Group

This group was treated with distilled water and fed from a standard provender. The average of the initial weight of it was 1225 g

Cholesterol Group

This group was treated with distilled water and given the cholesterol mixed with the provender in 260mg/kg provender percentage (Ameli *et al.*, 1996). The average of the initial weight for this group was 1225g.



Red Pepper Group

This group was treated with the aqueous extract of red pepper and the dosage was in 1000mg/kg B.W percentage (Kendabie and Adjene, 2007). Fed from a standard provender and the average of the initial weight for it was 1240g.

Cholesterol – Red Pepper Group

This group was treated with the red pepper boiled extract with the same percentage of (1000mg/kg B.W) giving it also a cholesterol containing provender with percentage of 260mg/kg provender. The average of the initial weight was 1220g.

NOTE : The rabbits of both Control and cholesterol groups were oral administration of the distilled water to balance the effort of catching the rabbit. (Batchelor and Giddins, 1995).

6- Histo-biochemical tests

6-1: Estimation of Cholesterol in liver tissue.

The total cholesterol concentration in liver tissue was estimated (mg/g) a wet tissue in the way used to estimate cholesterol in serum itself, fats were extracted as follows: (Folch *et al.*, 1957).

6-2: Estimation of Glycogen Concentration In Liver Tissue.

Glycogen concentration in liver tissue was estimated according to Anthron dectecor (Plummer, 1978).

6-3: Estimation Glutathione's concentration in liver tissue

Glutathione's concentration in liver tissue was estimated using the searchers method (Moron *et al.*, 1979).

Calculations:

Glutathione concentration was calculated as follows:

Glutathione concentration (GSH) in liver tissue = $\frac{0.03 + 0.D \text{ sample's absorption strenght}}{0.113}$ (micromole/g wet tissue)

6-4: Estimation of lipid Peroxidation in Liver Tissue.

Searchers method was followed here Gilbert *et al.*, (1984) to estimate the lipid Peroxidation concentration in liver tissue through measuring Malondialdehyde (MDA) which is one of the outputs of the lipid peroxidation process. This interaction among lipid peroxides and mainly Malondialdehyde and thiobarbatureic acid depends on the (pH).



Calculations :

MDA concentration was calculated according to this rule :

MDA (Nanomole/g of the wet solution)= (sample's absorption strength in 532–standard absorption strength in 532) – 20% (sample's absorption strength in 453–standard absorption strength in 453).

Afterwards, MDA quantity was estimated using molar diffraction factor $1.56 \times 10^5 \text{M}^{-1} \text{cm}^{-1}$

RESULTS AND DISCUSSION

1– Effect of Cholesterol and red pepper's aqueous extract on glycogen concentration in the liver tissue of the local male rabbits.

Figure (2) shows a significant reduction of glycogen concentration in the possibility level ($P \leq 0.05$) in the liver tissue for male rabbits have been treated with cholesterol of 260mg/kg provender compared to control group. Arithmetic average for this group was 2.29 ± 0.01 mg/g liver tissue, while control group arithmetic average was 2.59 ± 0.005 mg/g liver tissue.

While the figure show occurrence of a significant increase in the possibility level ($P \leq 0.05$) in glycogen concentration in the liver of the male rabbits treated with red pepper concentrated as 1000mg/kg B.W compared to control group, the arithmetic average for this group was 3.07 ± 0.02 mg/g liver tissue. As results show significant increase in the possibility level of ($P \leq 0.05$) in glycogen concentration in the liver of the male rabbits treated with cholesterol in concentration of 260 mg/kg provender and red pepper in concentration of 1000mg/kg B.W compared to the cholesterol group alone and the values almost reach control group ones, then the arithmetic average for this group is 2.50 ± 0.02 mg/g liver tissue.

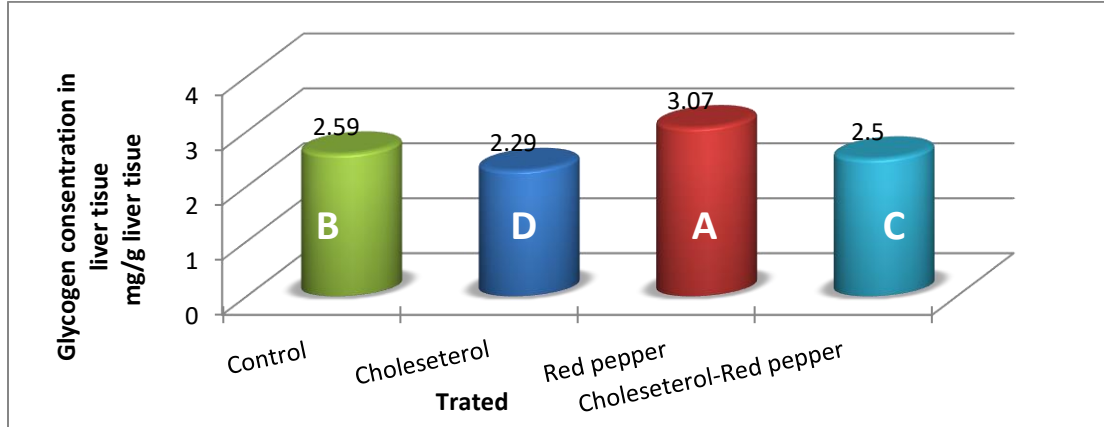


Figure (2) : The effect of treatment with cholesterol (260 mg/kg provender) and red pepper (1000mg/kg B.W) in glycogen concentration in liver tissue.

Treating rabbit with cholesterol led to a significant decrease in glycogen concentration in liver and this result is compatible with (Al-Jubouri, 2009) when cholesterol added to the provender with 260mg/kg provender concentration given to the growing rats. The reason behind this significant decrease in glycogen concentration in liver is that cholesterol has led to an increase in the secretion of the main stress hormone Corticosterone from the adrenal cortex, which increases the catalysed of tissues, including liver tissue, or even the effect of cholesterol in increasing the secretion of Glucagon hormone out of alpha-pancreatic cells, which inhibits the Synthetase phosphatase D enzyme, the one responsible of activating the enzyme Glycogen synthase, and by so, glycogen producing in liver will be decreased, or that cholesterol has increased Glycogenolysis which is one of the reactions and responses to stress resisting (Bander *et al.*, 1984).

While treating with the aqueous extract of red pepper has led to a significant increased in glycogen concentration in treated rabbits livers and this might be attributed to the fact of red peppers containing fibers that reducing the glucose absorption in intestines (Nelson *et al.*, 1998), or fibers have perhaps inhibited the secretion of the hormones responsible of increasing glucose concentration such as Glucagon and Somatostatin, thus increasing the glycogen building by increasing the effectiveness and activity of Glycogen synthase enzyme (Kumar *et al.*, 1993), or because of Capsaicin existence in red pepper that stimulates Insulin secretion through Beta pancreas under the effect of VRS1 leading to reduction in Glucose concentration and increase in glycogen synthesis (Akiba *et al.*, 2004).

2- Effect of Cholesterol and red pepper aqueous extract on the concentration of cholesterol in liver tissue of the local male rabbits

Figure (3) shows a significant increase in the possibility level of ($P \leq 0.05$) in cholesterol concentration in the livers tissues of male rabbits treated with cholesterol with 260 mg/kg provender concentration compared to control group, arithmetic average for this group was 180.59 ± 0.64 mg/g liver tissue and for control group was 178.33 ± 1.03 mg/g liver tissue.

While figure (3) shows a significant decrease in the possibility level of ($P \leq 0.05$) in cholesterol concentration in the livers tissues of the rabbits treated with 1000mg/kg B.W concentration compared to the control group where the arithmetic average for this group was 78.47 ± 0.16 mg/g liver tissue. Results have also proven that treating rabbits with cholesterol 260 mg/kg provender and red pepper of 1000mg/kg B.W has also led to significant reduction at ($P \leq 0.05$) possibility level in cholesterol concentration in the liver tissues of treated rabbits and values reach less level than control group one arithmetic average for this group was 122.30 ± 0.24 mg/g liver tissue.

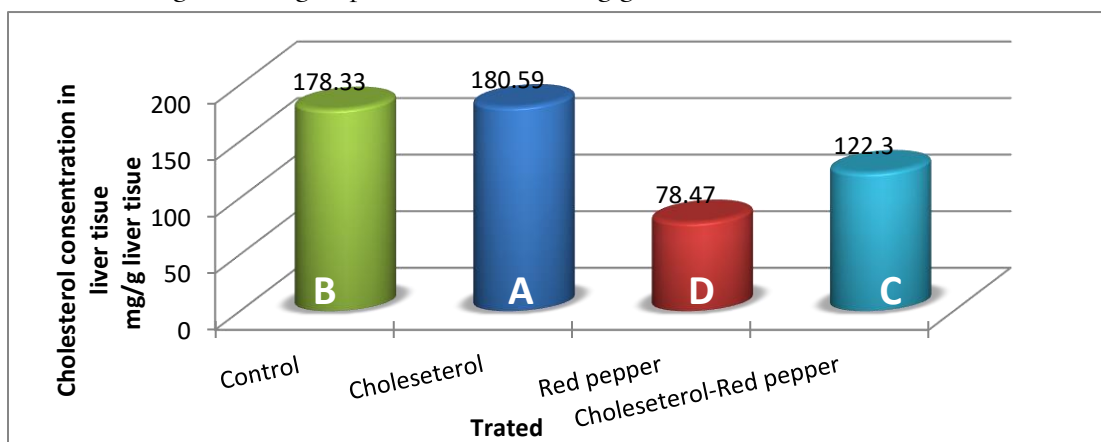


Figure (3): Effect of treatment with cholesterol 260mg/kg provender and red pepper 1000mg/kg B.W in cholesterol concentration in livers tissues.

Treating rabbits with cholesterol has led to a significant increase in cholesterol concentration in liver tissue. This result is compatible with (Al-Jubouri, 2009) has achieved in his study over growing rats which fed on cholesterol containing provender with 260 mg/kg provender concentration. The reason of this increase might be contributed to imbalance or disruption of lipid



metabolism or occurrence of some changes in absorption and steroids throwing or a decrease in the bile salts concentration. This is confirmed by (Pfeuffer, 1989).

The ability of the aqueous extract of red pepper on reducing the cholesterol concentration in the liver is attributed to the fact that it contains fibers and these fibers acts to reduce the absorption of fat and cholesterol, and on the other hand, stimulating oxidation of the internal made cholesterol to produce bile acids (Aprikian *et al.*, 2002). Or the reason might be due to that red pepper fruit contains a high percentage of vitamin C which increases the activity of the thyroid gland and thus increase the secretion of thyroxine and thyroid hormone, which regulate fat's metabolism and oxidation, and in turn reduces the concentration of cholesterol in both blood plasma and liver tissue (Sturkie, 1986).

3- Effect of cholesterol and aqueous extract of red pepper on Glutathione (GSH) concentration in liver tissue of local male rabbits.

Figure (4) shows that treating rabbits with cholesterol with 260 mg/kg provender concentration has led to a significant decrease in the possibility level of ($P \leq 0.05$) in GSH concentration in liver tissue compared to control group. Arithmetic average for this group was 1.15 ± 0.008 mm/g wet tissue while the one for control group was 1.33 ± 0.009 mm/g wet tissue.

While figure (4) shows that treating with red pepper with 1000mg/kg B.W concentration, has led to a significant increase in the possibility level of ($P \leq 0.05$) in GSH in liver tissue compared to control group, arithmetic average for this group was 2.15 ± 0.01 mm/g wet tissue, while treating rabbits with cholesterol with 260 mg/kg provender and red pepper with 1000mg/kg B.W has led to a significant increase in possibility level of ($P \leq 0.05$) compared with cholesterol group alone, and arithmetic increase compared to control group, arithmetic average for this group was 1.35 ± 0.02 mm/g wet tissue.

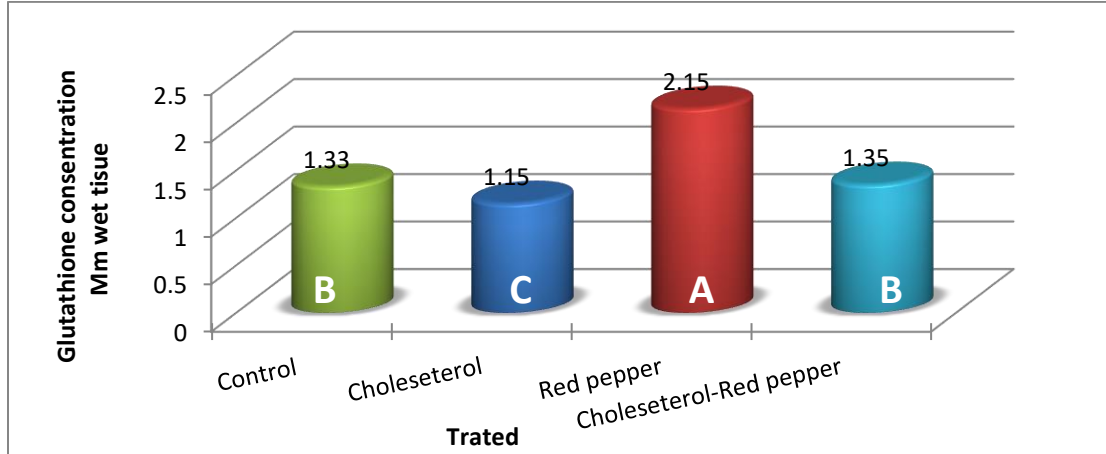


Figure (4): Effect of cholesterol (260 mg/kg provender) and red pepper (1000mg/kg B.W) on Glutathione concentration in local male rabbits serums.

4- Effect of cholesterol and aqueous extract of red pepper on MDA concentration in local male rabbits livers tissues.

Figure (5) shows that treating rabbits with cholesterol (260 mg/kg provender) has led to a significant increase in the possibility level of ($P \leq 0.05$) in MDA concentration for liver tissue compared to control group, arithmetic average for this group was 393.33 ± 6.96 nanomole/g wet tissue, while arithmetic average for control group was 250.36 ± 0.38 nanomole/g wet tissue. Treating rabbits with red pepper with 1000mg/kg B.W concentration has led to a significant decrease in possibility level of ($P \leq 0.05$) in MDA concentration in liver tissue compared to control group, arithmetic average for this group was 193.02 ± 2.63 nanomole/g wet tissue, additionally, the figure shows that treating rabbits with cholesterol with 260 mg/kg provender and red pepper with 1000mg/kg B.W concentration has led to a significant decrease in the possibility level of ($P \leq 0.05$) in MDA in liver tissue compared to cholesterol group alone, values did not reach control group's ones for the arithmetic average for this group was 253.41 ± 3.14 nanomole/g wet tissue

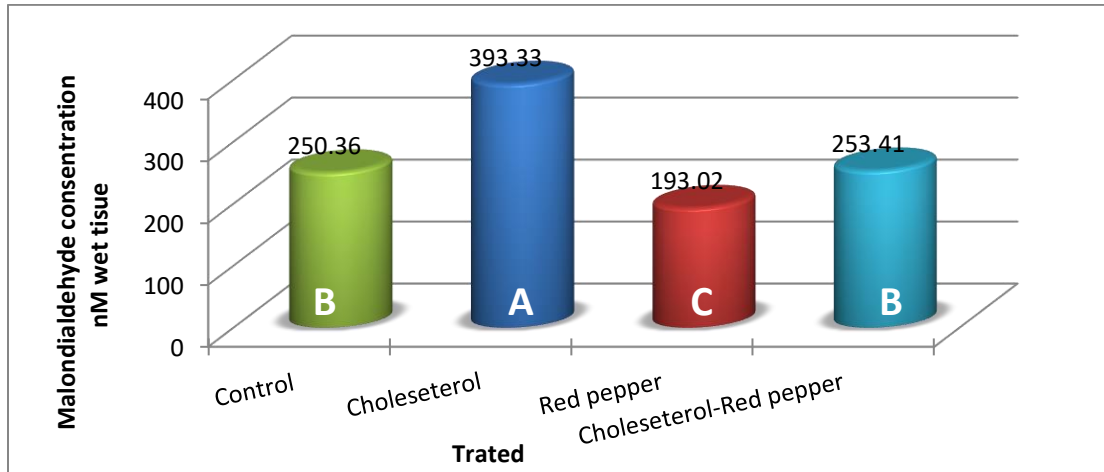


Figure (5): Effect of cholesterol (260 mg/kg provender) and aqueous extract of red pepper (1000mg/kg B.W), in Malondialdehyde concentration in local male rabbits serum.

Treating rabbits with cholesterol of 260 mg/kg provender concentration has led to a significant decrease in GSH and a significant increase in MDA in liver tissue. These results are compatible with what (Al-Kanani, 1998) has concluded when have chick chicken treated with cholesterol added to the provender in 1% for four weeks. GSH concentration decrease in liver tissue might be attributed to the increased degradation and reduced intracellular production or due to the amino acids forming it in the process of building glucose from non-carbohydrate sources in the Gluconeogenesis process or the low concentration of GSH in the cell is an indication that these cells have been oxidatively stressed (McLennan *et al.*, 1991).

The reason of having MDA concentrating increased in the livers tissues of male rabbits treated with cholesterol might be attributed to the reduction of the highly concentrated lipoproteins in the serum, because MDA concentration gets lower when lipoproteins are oxidized LDL-C and with Lipoproteins existence HDL-C (Nenseter *et al.*, 1994), or the cholesterol stimulated Fatty acyle-CoA oxidase enzyme and started oxidizing the fatty acids that lead to the formation of internal made hydrogen peroxide, which in turn contributes in the production of fat peroxides, where MDA is the final result (Basha and Sovers, 1996).

While treating rabbits with the red pepper boiled extract has led to a significant increase in GSH concentration and a significant decrease in MDA concentration all that in liver tissue, this might



be attributed to the fact that red pepper contains many vitamins, A and E and C and Biotin, are among which, those vitamins play a vital role in eliminating the free radicals that are formed inside the body, before they go into a series of cell-harming and cell-destructing interactions (Combs, 2008). By so, enhancing the antioxidants activity inside the body and reducing the fat peroxidation.

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