Histological changes and hyperlipidemia criteria for triton and therapeutic efficacy of *Punica granatum* husk extract in liver tissue of male white rabbits

Ansam Hussein Ali(1), Ahmed Hamid Ahmed(2), Zinah Ibrahim Khaleel(2)

(1)Department of Pathological Analysis, AL-Dour Technical Institute, Northern Technical University.
(2)Department of Pathological Analysis, College of Applied Sciences, University of Samarra, Iraq.

*Corresponding author email: Mohammed.alsad3@gmail.com*

**Abstract**

**Background:** Triton is a non-ionic surfactant that acts to raise or increase intestinal absorption of fat through its emulsification process. It changes the permeability of cell membranes and thus causes cell lysis and increased susceptibility to infection. Antioxidant effects of pomegranate studies have shown that pomegranate seed extract contains compounds with an antioxidant capacity of 2 to 3 times more than green tea. The present study aimed to study the effect of triton on the histological structure of white rabbit liver and to try to get rid of the effects of triton used alcoholic extract pomegranate husks.

**Methods:** Observational and behavioral symptoms were observed in triton-treated animals, such as lack of water and food intake, inactivity in the movement and tremor with diarrhea.

**Results:** The results of the study in the second group (G2), only injected with triton under the peritoneum at a concentration of 0.1mg/kg, showed the histological changes caused by triton in hepatic tissue with negative changes in tissue represented by degeneration of the majority of hepatocytes. In other regions, hepatic cell size swelling, cytoplasm and Kupffer cell numbers were observed, while the inhibitory effect and cellular repair of the histological structure of the studied organ were observed in the (G3) group, injected with triton under the peritoneum and dosed with pomegranate extract at a concentration of 1mg/kg, while no changes in tissue structure were shown. The liver in the fourth group (G4), dosed with pomegranate extract only at a concentration of 1mg/kg, according to the studied concentration compared to the control group (G1).

**Conclusion:** From the results of the present study we can say that the extract of pomegranate husks has an effect in eliminating the effect of Triton as well as in cellular repair of liver tissue.

**Keywords:** Triton, alcoholic extract, pomegranate husks, liver tissue

How to cite this article: Ali AH, Ahmed AH, Khaleel ZI (2020): Histological changes and hyperlipidemia criteria for triton and therapeutic efficacy of Punica granatum husk extract in liver tissue of male white rabbits, Ann Trop Med & Pub Health; 23:S393. DOI:
http://doi.org/10.36295/ASRO.2020.2317

**Introduction**

Triton is a non-ionic surfactant that acts to raise or increase intestinal absorption of fat through its emulsification process. It changes the permeability of cell membranes and thus causes cell lysis and increased susceptibility to infection. Prolonged exposure to Triton leads to cell death due to the role of the surface tensioner in tearing the polar head groups of hydrogen bonds in the double layer of the cell membrane causing destruction of fatty membranes. Thus, this mechanism can extend to the endothelial cells of the blood vessels, thus increased osmotic pressure within the red blood cells and their dissolution, causing animal death (1). Triton is used by several studies to induce hypercholesterolemia in animals (2,3). Triton acts as a surfactant and inhibits the activity of lipase enzyme to absorb lipoproteins from blood circulation by extracellular tissues which increases the concentration of lipids in the blood (4). The non-archaeological compound, Triton, has been utilized as an extender, which contributes extensively to the absorption of triacylglycerol-rich lipoproteins from plasma by the peripheral tissues to produce hyperlipidemia in animal models, which are often used for a number of targets, particularly for the examination of natural lipid-lowering and chemical drugs (5,6). While there is a marked increase
in the blood levels of total cholesterol, triglycerides, phospholipids, LDL, VLDL and a decrease in the level of HDL cholesterol carrier in rabbits which dealt with Triton (7). Triton is a non-ionic compound that causes the accumulation of serum lipids by blocking the absorption of lipoprotein through extracellular tissues, resulting in an increase in levels of circulating lipoproteins (8). It was used by many studies to induce hypercholesterolemia in animals (2,3).

Pomegranate

The pomegranate plant is a shrub and has many branches growing outward and then hanging down, the height of the pomegranate plant ranges between 1.8-4.6m. It is a perennial plant, there have been some pomegranate trees perennial for more than 200 years in France. The leaves are characterized by being shiny and are constantly falling. The length of the leaf is 7.6cm, the leaves are opposite in order on the stem. The flowers are orange-red and have a conical shape. The flower is 5cm long and is often double and is produced continuously throughout the summer. The fruits are spherical in shape, and the diameter varies between 5-7.6cm. The unripe fruit has a yellowish green color, and the ripe fruit is dark red. The fruits are of the pulp type and are filled with seeds rich in juice. The pulp has an acidic taste and is coated with leather cover (9).

The pomegranates are given many common names, the Carthaginians call it Roman, the Spanish call it Granada and the French call it Grenade (10). The Indians refer to it as Anar. The most common English name for pomegranate is Pomegranate. Punica according to the classification shown by (11):

Kingdom: Plantae
Division: Spermatophyta
Sub division: Angiospermae
Class: Dicotyledonae
Sub class: Polygonae
Order: Myrtales
Family: Lythraceae
Genus: Punica
Species: granatum

Utilized part of pomegranate is a medically important plant where many use its parts (roots, crust, flowers and whole fruits) in the treatment of many conditions. Antioxidant effects of pomegranate studies have shown that pomegranate seed extract contains compounds with an antioxidant capacity of 2 to 3 times more than green tea, where many studies have shown the superior ability of these compounds in preventing oxidation and resistance to free radicals, which exceeded the ability of Natural antioxidants such as beta carotene and ascorbic acid, and even some industrial antioxidants such as BHA (12). Antioxidant compounds in the pomegranate are polyphenolics which are the source of two types of compounds; namely Anthocyanins, which give the juice of pomegranate distinctive red color, and tannins hydrolysable biodegradable, and the most important Tannin is acid Ellagicas this compound has a 92% antioxidant capacity (13). The present study aimed to study the effect of triton on the histological structure of white rabbit liver and to try to get rid of the effects of triton used alcoholic extract pomegranate husks.

Materials and method

Preparation of alcoholic extract

The alcoholic extract of pomegranate husk powder was prepared according to the method of (2). Therefore, 10g of dry powder was added and 200ml of ethyl alcohol at a concentration of 70% in a 500ml glass beaker. The mixture was left for 24 hours to give more room to extract the active substance in the plant sample. After that, we filtered the solution by filter papers and transferred the filter to centrifuge at a speed of 300r/min for 10 minutes to settle the suspended plant parts and obtain a clear solution.

Experimental Design

A total of 20 adult male rabbits were used, ranging in weight from 1000-0015g and aged 3-5 months. The animals were placed in a room with suitable hygienic conditions for raising animals like temperature, good ventilation conditions and lightening in the laboratories of the General Industries
Company, Pharmacokinetics-Samarra. Animals were divided randomly into four groups in each group 5 animals in iron cages lined with sawdust changed two to three times weekly, and all the groups were given a free ready-made diet. All the groups were given free normal drinking water, Substituting drinking water and bush twice a day. Hyperlipidemia was introduced in groups by sub-peritoneal injection of Triton at 0.1mg/kg of animal weight and the animals were divided into four groups:

- **Good control group**: The good control group dealt with water and plain food from vegetables and blackberries.
- **Infected control group**: Animals in this group were only injected with triton under the peritoneum at a concentration of 0.1mg/kg.
- **Group I (G1)**: Control group dealt with water and regular food of vegetables and blackberry ready.
- **Group II (G2)**: Animals in this group were only injected with triton under the peritoneum at a concentration of 0.1mg/kg.
- **Group III (G3)**: Animals in this group were injected with triton under the peritoneum and dosed with pomegranate extract at a concentration of 1mg/kg.
- **Group IV (G4)**: Animals in this group were dosed with pomegranate extract only at a concentration of 1mg/kg.

### Preparation of tissue sections

After dissecting the animals and placing organs in 10% Formalin solution, the samples to be studied were converted to ethyl alcohol at 70% concentration. The following steps were taken:

1. Dehydration
2. Clearing
3. Infiltration
4. Embedding
5. Sectioning
6. Staining
7. Mounting

### Results

#### Macroscopic Changes in Experimental Animals

It was noticed that there were ocular and behavioral changes in triton-treated animals such as lack of water and food consumption, inactivity in movement, trembling and minor bleeding in the eyes in addition to the change of stool color from dark brown to yellow, with diarrhea. The pomegranate husk extract showed an almost normal amount of food and water consumption compared to the control group.

#### Histological examination

**Control group**

The livers of the control animals were characterized by the central vein around which the hepatocytes were arranged in the form of tapes, including the sinuses, and the liver cells appeared to contain one or two nuclei.

**Triton-Treated Group (Figures 1 and 2)**

The results of liver histological examination in this group showed negative changes in the tissue represented by degeneration of the majority of hepatocellular nuclei. In other areas, hepatic cell swelling, cytoplasm and increased numbers of KFPR cells were observed that these changes led to the migration of inflammatory cells, which were observed near the central vein, which appeared containing coagulant erythrocytes.

**Group treated with triton and pomegranate peel extract (Figures 3 and 4):**

The results showed that despite the inhibitory action of pomegranate peels extract on the effect of Triton, hepatic degenerative hepatocytes were observed in some areas, but hepatic tissue appeared predominantly normal.
Group treated with pomegranate husk extract (Figures 5 and 6):

The results of the present study showed that the extract of lemon pomegranate peels was without negative side effects on liver tissue according to the concentration used compared to control group.

Figure (1) Cross-section of the liver of a triton-treated animal showing: (1) Degeneration of hepatocytes, (2) infiltration of inflammatory cells. H&E (100x).

Figure (2) Cross-section of the liver of a triton-treated animal showing: (1) vacuolated cytoplasm of hepatocytes and (2) KPFR cell. H&E(100x).
Figure (3) Cross-section of the liver of a triton-treated animals and pomegranate peel extract showing: (1) hepatic degenerative hepatocytes and (2) central vein. H&E (100x).

Figure (4) Cross-section of the liver of a triton-treated animal and pomegranate peel extract showing: (1) hepatic degenerative hepatocytes and (2) central vein. H & E (400x).

Figure (5) cross-section in the liver of an animal from the treatment group with pomegranate peels extract showing: (1) hepatocytes arranged in straps around the (2) central vein. H & E (400x).
Discussion

Macroscopic changes in experimental animals

It was noticed that there were ocular and behavioral changes in triton-treated animals such as lack of water and food consumption, inactivity in movement, trembling and minor bleeding in the eyes in addition to the change of stool color from dark brown to yellow, with diarrhea. The pomegranate husk extract showed an almost normal amount of food and water consumption compared to the control group.

Some of these symptoms can be explained by loss of appetite, lethargy and dehydration as well as the change in the color of stool to yellow due to the occurrence of a defect in the digestive system caused by contraction and convergence of smooth muscles in the walls of small intestine, which is accompanied by intestinal colic. Also, noticed that Triton has an effect on blood pressure as a result of its direct and indirect effects on blood vessels. It causes atherosclerosis and affects heart muscles, which is responsible for the contraction of blood vessels that affects the elasticity of blood vessel walls. Triton-induced tremor may be due to neurological convulsions caused by inflammation of central nervous system. Triton breaks down the blood-brain barrier and reaches the brain tissue leading to damage to some brain cells.

While the group treated with triton and pomegranate husk extract showed a normal level of water and food consumption and normal motor activity, it may be due to the fact that the components of pomegranate husks acted as inhibitors of triton as well as the possibility of treatment with this extract to reduce the effects of triton in the body tissues where it has been shown to have anti-inflammatory and antimicrobial properties. The antioxidant and its effect on detoxification showed that the group treated with pomegranate husk extract showed no significant changes and this indicated that the extract has no negative side effects related to these changes at this concentration.

Triton-treated group

The results of liver histological examination in this group showed negative changes in the tissue represented by degeneration of the majority of hepatocellular nuclei and in other areas it was noted that swelling of hepatocytes and the outbreak of cytoplasm and increased numbers of Kupffer cells, that these changes led to migration of inflammatory cells, which were observed near the vein. These results were consistent with who indicated that exposure to chemical Triton led to degeneration of hepatocytes due to failure to represent and accumulate protein and inhibition of Triton. For the present work, protein synthesis through its effects on the enzyme protein phosphatase and with the continuing occurrence of infections has increased the severity of changes to infected hepatic cells necrosis which causes programmed death, which included large areas of the liver in the later periods of the experiment. Moreover, also noticed that swelling of hepatocytes, cytoplasmic outbreaks and increased numbers of KPF cells and cellular phagocytes that have been observed in liver tissue were a clear indication of the inflammatory response to triton and its effects on cell death, particularly in the final period of the experiment. Cell-free areas have emerged. The role of inflammatory and Kupffer cells is known to remove damaged cells and clean tissue from the remains of those cells. As a natural reaction to this significant effect, hepatic tissue fills the spaces created by cell death by fibrous tissue.
Group treated with triton and pomegranate husk extract

The results indicated that in spite of the inhibitory action of pomegranate peels extract on the effect of Triton, hepatocellular degeneration was observed in some areas, but hepatic tissue appeared predominantly normal. Studies have shown that pomegranate husk extract contains compounds with antioxidant and free radical resistance that exceed the ability of natural antioxidants such as ascorbic acid and even some industrial antioxidants. Flashing Ellagic acid is characterized by its ability to activate enzymes that break down toxins, and free radicals caused by Triton. This compound has a 92% antioxidant capacity.

In several in vivo studies, indicated the ability of some compounds found in pomegranate husk extract to inhibit cyclooxygenase, which directly affects inflammation through its ability to convert arachidonic acid into prostaglandins which in turn mediate inflammation; pomegranate peel extract can inhibit 37% of these enzymes.

Group treated with pomegranate husk extract

Numerous studies indicated that there were few histological changes due to the treatment of plant extracts, which were minor and non-significant. The histological examination confirmed that pomegranate husk extract had no adverse side effects on liver tissue in the present study. The histological structure of the liver was similar to that of control group. In addition, pointed out that there were no negative effects on the histological structure when using pomegranate husk extract in many studies in which the total activity of antioxidants from selected food stuffs was estimated as pomegranate husks gave high antioxidant content because it contains a high percentage of polyphenols. Research has also indicated that pomegranate husk extract is rich in sugars, unsaturated as well as monounsaturated fatty acids, vitamins and minerals that have antioxidant activity without negative effect on the used tissue.

Conclusion

From the results of the present study we can conclude that the extract of pomegranate husks has an effect in eliminating the effect of Triton as well as in cellular repair of liver tissue.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of interest: The authors declare that they have no conflict of interest.

Funding: Self-funding.

References