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The Histological and Some Physiological Effects of Aqueous Extract of *Punca granatum* L. Peel on Rats

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Abstract

. The development in the drug industry stimulates the phytotherapy applications with the advance of research about the active constituents in plants exhibit that use of them in traditional medicine[4], such *Punica granatum*L.(pomegranate) plant. This study is required to establish new scientific proofs for therapeutic use of such traditional medicinal plants and this by experimental work on (16 female rats). The study showed a decrease in body weight in *P. granatum* peel extract administered rats, may be decreasing fat absorption and storing and revealed that peel extract affect lipid profile parameters. The histopathological study no harm effects of peel extracts on liver, kidneys, gastrointestinal tract, but an ingestion in the spleen observed in the high dose treatment.

Key words: *Punica granatum*, pomegranate ,herbal treatment, histopathology.

Introduction:

In traditional medicine, the phytotherapy used to preventing and dealing with simple disease[1]. Phytotherapy means that the chemical material which extracted from plants have therapeutics action on diseases and body disorders[2]. The phytotherapy used by the public due of their biological effect have inspired to their therapeutic use which enhance the pharmacognostic industry[3]. The development in the drug industry stimulates the phytotherapy applications with the advance of research about the active constituents in plants exhibit that use of them in traditional medicine[4], such *Punica granatum*L.(pomegranate) plant.

P. granatum is a deciduous shrub, with often dense thorny branches widely cultured in Asia, the Mediterranean area, and the American region. Its fruits has many chemical compounds include acids, sugars, vitamins, polysaccharides, polyphenols and minerals[5]. Since these components, the pomegranate fruit has medicinal effects such as anti-inflammation, antibacterial inhibitory effect on skin and breast cancers and strong antioxidant activity[6]. This study is required to establish new scientific proofs for therapeutic use of such traditional medicinal plants, as it may possibly be useful source of new active treatments in the drug development process.

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2. Animals and methods

2.1. Animals

16 female rats (150 - 250) were obtained from Faculty of Pharmacy / University of Kufa. These animals were kept under appropriate environmental conditions of 20-25°C in an air-conditioned room and light period of 12 hours daily. The animals were accommodated in plastic cages of dimensions 20 × 50 × 75 cm and had allowed access to water ad-libitum. For at least 2 weeks, earlier for begining the testing, for adaptation the animals were set aside.

2.2. Experimental Design

The animals (16 rats) were separated equally into 4 groups and housed in 4 cages. The control group considered as first group and administered distilled water, while other 3 groups daily administered the extracts of P. granatum peel which earlier prepared, administered orally with 40, 50, and 60 mg/kg body weight respectively. These work was persisted for two weeks. The animals weighted after 24h of the last administration, the animals were scarified , the blood put in serum tubes, the liver spleen and kidney were separated and placed in formalin for 24h. then in ethanol 70% for preservation. After 24h of last administration the animals were weighed, anesthetized using a mixture of ketamine and xylazinei.m., then they were sacrificed[7]. Some organs were isolated for histopathological study, they include spleen , liver, kidneys, and parts of GIT, they were cleaned with normal saline then fixed with 10% formalin for 24h. then put in 70% ethanol for preservation until histological preparations.

2.3. Preparation of crude aqueous extract

The *P. granatum* peels aqueous extract was obtained by using (boiled distilled water) which is considered as very effective in extracting the active ingredients of the plant[8]. A quantity of 40 g plant peels powder was put inside the flask with 200 ml of distilled water and boiled for 10 min with stirring. Then filtered with filter paper and dried by oven at 45-50 °C. The dry extract was used to prepare the stock solution which was kept frozen at –20oC until used to make three concentrations (40, 50 and 60 mg/ml),

2.4. Histological study

The histological slides were prepared in histological unit in Department of Biology / Faculty of science/ University of Kufa. The histologic work were prepared according to Bancroft and Stevens (1982)[9] and stained with hematoxylin and eosin. The prepared slides were studied with compound light microscope for the histological changes occurred by the treatment and compared with control samples.

2.5. Lipid profile

2.5.1. Total Cholesterol: Quantitative-enzymatic1-colorimetric determination of total cholesterol in serum (from Stanbio cholesterol Liquid color®) was performed[10].

2.5.2. HDL- Cholesterol: Low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL)cholesterol fractiond are precipitated from serum or plasma by magnesium chloride / dextran sulfate reagent according to Finely *et al*., 1978[11], high density lipoprotein (HDL) cholesterol is then determined in the supernant fluid, using a cholesterol reagent and the derived dilution factor in the calculation. This precipitating reagent is designed for use with the enzymatic cholesterol assay such as Stanbio cholesterol procedures.

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2.5.3. Serum Triglyceride (TG): Stanbio triglyceride liquid color[12] a quantitative enzymatic-colorimetric determination of triglyceride in serum was performed.

2.5.4.Calculation of LDL-Cholesterol: The calculation occurred by using the following equation[13]:

LDL in mg/ dl = Total cholesterol – (VLDL + HDL- cholesterol)

2.5.5.Calculation of VLDL-Cholesterol[13] The calculation occurred by using the following equation:

VLDL in mg/ dl = Triglyceride / 5

3. Results

3.1. Body weight

The results showed a significant decrease in the body weight after the treatment in comparison with control group.(Figure 3.1).

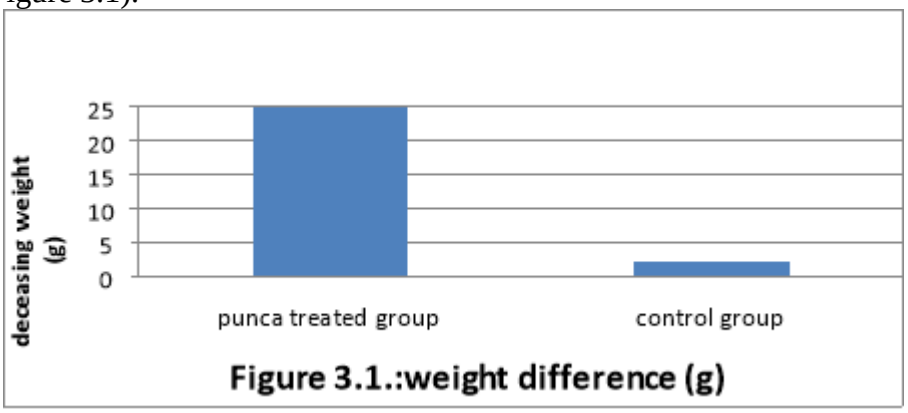

Figure 3.1.:weight difference (g)

3.2. Lipid profile

A decrease in LDL observed in treatment group but it didn't reach to the significance level. (Figure 3.2).

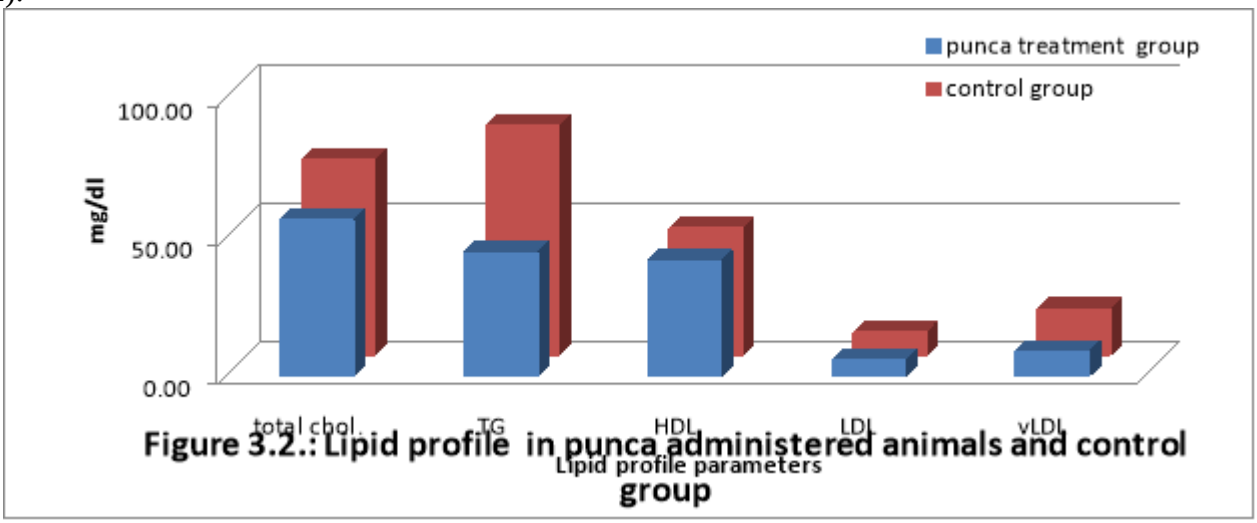

Figure 3.2.: Lipid profile in punca administered animals and control group

3.3. Histopathological study

The histological preparations of treated animals show little changes in high doses of treatment in some organs but most organs were normal when compared with control group. The following figures show

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these sections.

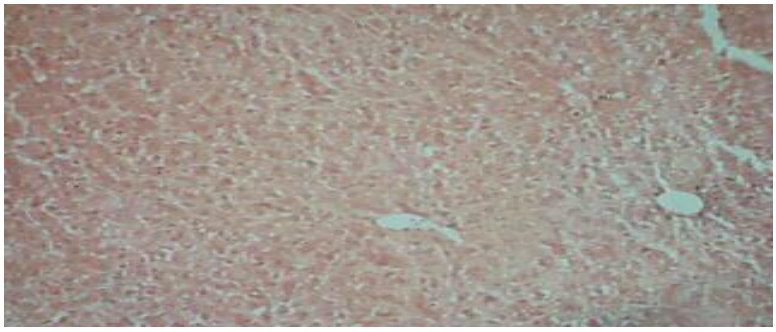

Figure (3-3) Liver section of 40mg/Kg *Punica granatum* L. aqeuas peel extract administrated rats. Hematoxylin eosin stain (100X)

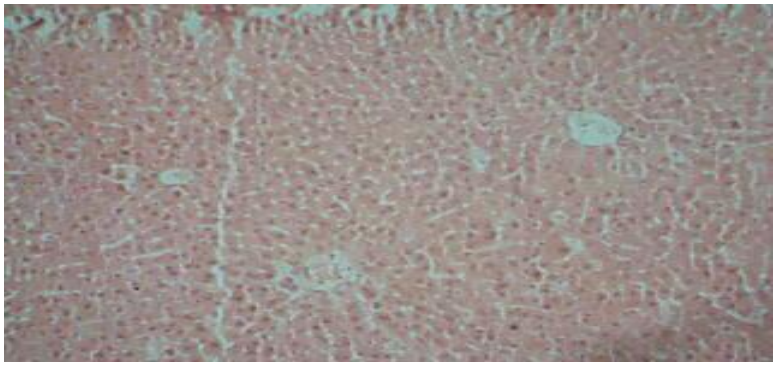

Figure (3-4) Liver section of 60mg/Kg *Punica granatum* L. aqeuas peel extract administrated rats. Hematoxylin eosin stain (100X)

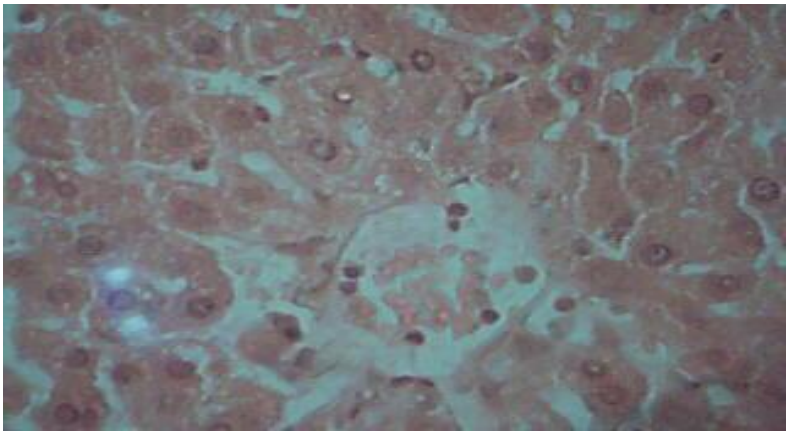

Figure (3-5) Liver section of 60mg/Kg *Punica granatum* L. aqeuas peel extract administrated rats. Hematoxylin eosin stain (400X)

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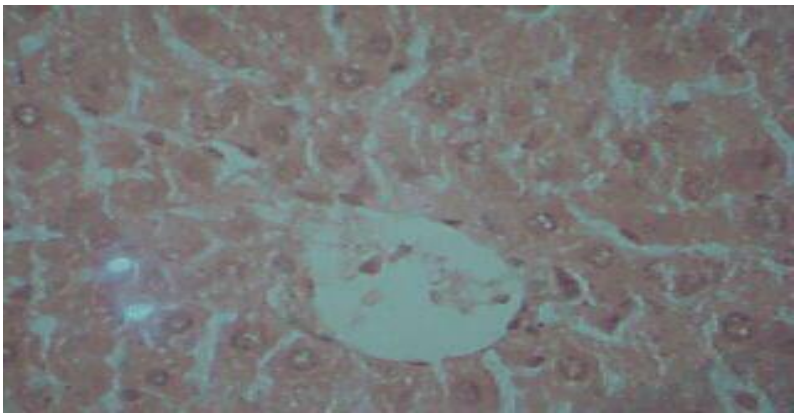

Figure (3-6) Liver section of 60mg/Kg *Punica granatum* L. aqeuas peel extract administrated rats. Hematoxylin eosin stain (400X)

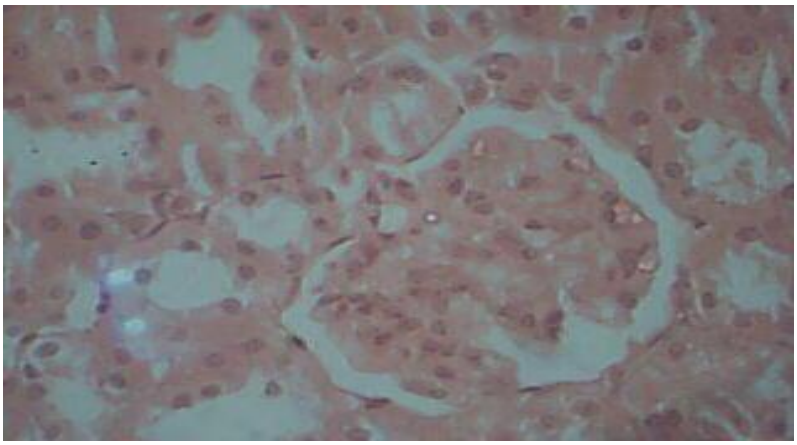

Figure (3-7) Kidney section of 40mg/Kg *Punica granatum* L. aqeuas peel extract administrated rats. Hematoxylin eosin stain (400X)

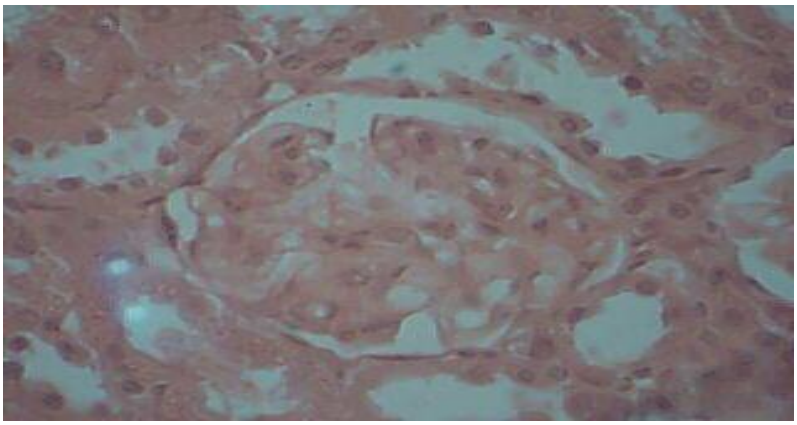

Figure (3-8) Kidney section of 40mg/Kg *Punica granatum* L. aqeuas peel extract administrated rats. Hematoxylin eosin stain (400X)

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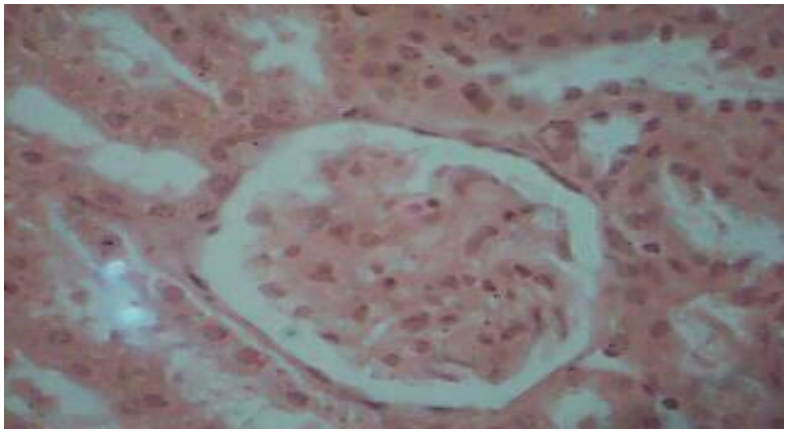

Figure (3-9) Kidney section of 50mg/Kg *Punica granatum* L. aqeuas peel extract administrated rats. Hematoxylin eosin stain (400X)

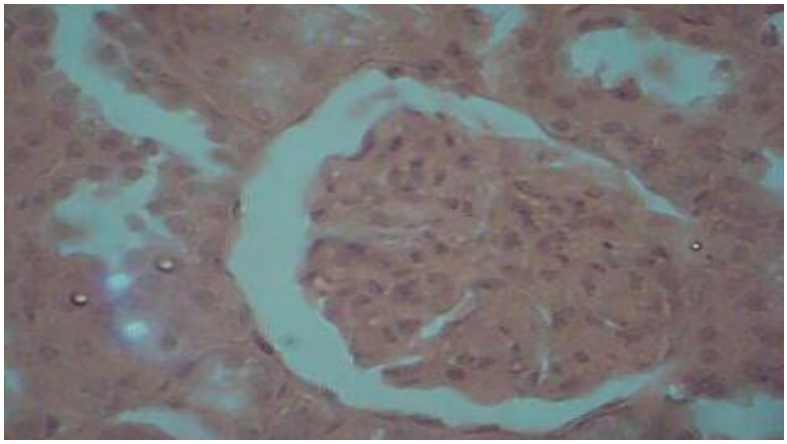

Figure (3-10) Kidney section of 60mg/Kg *Punica granatum* L. aqeuas peel extract administrated rats. Hematoxylin eosin stain (400X)

Figure (3-11) Spleen section of 60mg/Kg *Punica granatum* L. aqeuas peel extract administrated rats. Hematoxylin eosin stain (100X)

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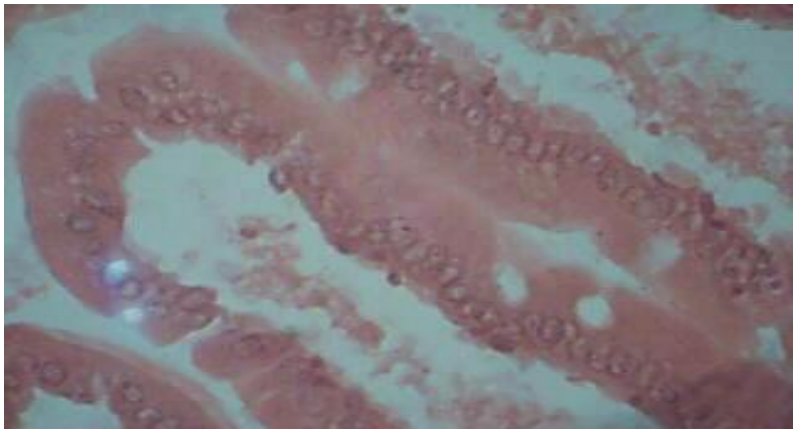

Figure (3-12) Doudenum section of 40mg/Kg *Punica granatum* L. aqeuas peel extract administrated rats. Hematoxylin eosin stain (400X)

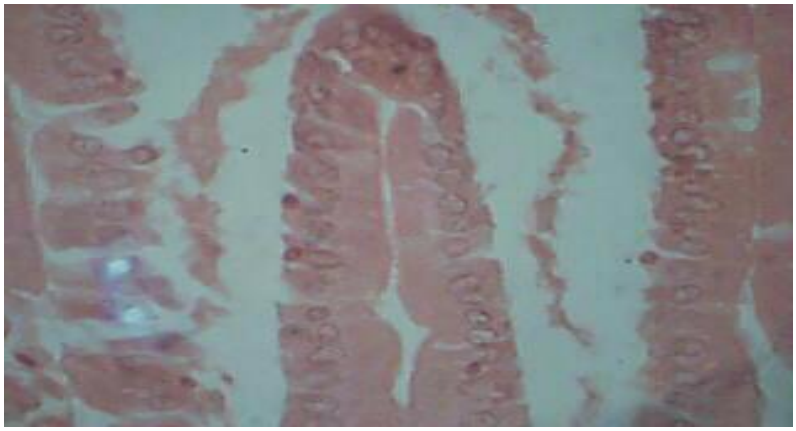

Figure (3-13) Jojenum section of 40mg/Kg *Punica granatum* L. aqeuas peel extract administrated rats. Hematoxylin eosin stain (400X)

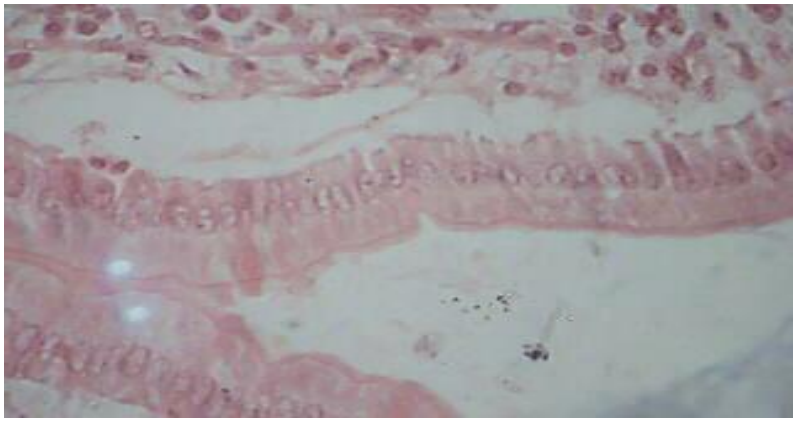

Figure (3-14) Large section of 60mg/Kg *Punica granatum* L. aqeuas peel extract administrated rats. Hematoxylin eosin stain (100X)

4. Discussion

The pomegranate fruits are rich in many compounds especially in the peels which make up about 60% of the fruit such as complex polysaccharides, many minerals, phenolics, flavonoids, ellagitannins

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(including punicalagins), proanthocyanidin compounds[14,15]. The highest antioxidant activity of the fruit due to the peel active constituents which is the part its high content of polyphenols[16].

As the peel extract used traditionally for diarrhea curing, our study designed to investigate any possible harm effects on some organs.

The study showed a decrease in body weight in *P. granatum* peel extract administered rats, may be decreasing fat absorption and storing, other studies like (Chalfoun-Mounayar *et al*., 2012)[17] used extracts from different parts of *P. granatum* to induce weight loss in mice.

Also this study revealed that peel extract affect lipid profile parameters, this result agree with (Hossin, 2009)[18] who suggested that, uptake of pomegranate peel powder or it's extract may alter and decrease the risk of hypercholesterolemia and it have more possible as a health supplement rich in natural sources antioxidants.

Also in another study Mathew *et al*., (2012)[19], the *P. granatum* extract has not acute effect on postprandial lipaemia, but suppressed the postprandial increase in SBP following the high-fat meal.

The histopathological study no harm effects of peel extracts on liver, kidneys, gastrointestinal tract, but an ingestion in the spleen observed in the high dose treatment. These results agreed with Sharifiyan *et al*., (2016)[16] who found that the use of peel extracts cure the harmful changes induced by some atherosclerotic agents, and not affected normal animals. Also in their study, Akter *et al*., (2013)[20], found that peel extract cure induced diarrhea by protecting the mucosa by d the presence of flavonoids and alkaloids that may play key role in its antidiarrhoeal activity.

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