Phyto-pharmaceuticals and biological study on graviola (*Annona muricata L.*) fruit and dietary supplement of graviola sold on the Libyan market as a cancer cure against TCA induce hepatotoxicity in mice

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Abstract: *Annona muricata* (Graviola) has many medicinal properties and used widely in traditional medicine for treatment various disorders. The present study was conducted to evaluate phytochemical and quality control (QC) of random sample of graviola dietary supplement capsules (DS) which sold in the Libyan market as anticancer product. As well as the present work designed to evaluate heatoprotective effects of aqueous extract of graviola fruit pulp or aqueous DS of graviola capsules against trichloroacetic acid (TCA) induced hepatotoxicity in albino mice. Quality control parameters were determined on random samples of graviola DS using standard methods. A total of 120 female mice were divided into 6 groups and were used for biological screening to determine biochemical and histopathological alteration in liver of mice treated with TCA with or without aqueous extract of *A.muricata* fruit pulp or DS of graviola. The results of quality control and phytochemical screening revealed that all quality control tests conducted on the random sample of DS capsules of graviola were within normal values according to the standards of the Quality Control Center for Pharmaceuticals in Tripoli, only few samples showed slight increase in the moisture content. However, all samples appeared free from microbial contamination. While, growth of fungal contamination (Pencillium Spp) in the same samples were detected but all samples appeared free from aflatoxins contamination. Also, all samples were free from industrial radioactive contamination. Phytochemical study revealed presence of alkaloids, tannins, steroids, glycosides, falvonoids, anthraquinones, saponin and coumarins in extracts of graviola fruit pulp and graviola DS capsules. However, absence some phytochemical components in DS capsules was detected. The result of biological screening revealed that no clinical signs and abnormalities in behavior and external feature in mice treated with aqueous extract of graviola fruit pulp or aqueous extract of graviola DS capsules. However, the treatment with aqueous extract of graviola fruit pulp and DS of graviola reduced the abnormal changes in behavior and external features in female mice intoxicated with TCA, markedly reduced the mortality in TCA administrated mice and induced slight improvement in the final body weight comparing to TCA only intoxicated group. Biochemical study revealed that administration of aqueous extract of graviola fruit pulp or aqueous extract of DS of graviola significantly decreased the elevated serum activities of AST and ALT compared to TCA only intoxicated mice. Histological examination revealed that administration of aqueous extract of graviola fruit plup or aqueous extract of DS of graviola with TCA induced ameliorative changes and disappearance of the most pathological changes in the liver tissue compared to of TCA only intoxicated mice and the ameliorating changes were more obvious in the mice treated with aqueous extracts of DS of graviola and TCA. The present results demonstrate that *A. muricata* play an important role in the protection against TCA induced hepatotoxicity. It can be concluded that the present study provide some pharmacological and therapeutical informations about extract of the graviola fruit pulp and DS of graviola capsules which can use in future investigations and applications and demonstrated presence of important phyochemical constituents in the graviola fruit pulp extract and DS of graviola capsules. The extract of the graviola fruit pulp and DS of graviola capsules have protective effects against TCA induced liver toxicity in mice.

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Key words: Annona muricata fruit (graviola), Dietary supplement of graviola, Quality control, Phytochemical, histological, trichloroacetic acid, Liver and mice.

1. Introduction

The use of herbs and herbal products, in both developing and developed countries, for the treatment of various diseases has increased dramatically in recent years. However the major drawback in promoting the use of medicinal plants is the lack of standardization as well as the confusion in the identification of the plant and their substitutes or

adulterants. To ensure reproducible quality of herbal plants, authentication is invaluable. The pharmacognostical studies not only give the authentication but also quality and purity standards of the plant **(Annan *et al*.,2013)**. Many dietary compounds have been identified as a potential chemopreventive agent. These include vitamins, minerals, carotenoids, and the large class of phytochemicals (polyphenol, isothiocynates, organosulfer compounds**) (Montes de oca *et al.,* 2017)**. Chemoprevention includes the use of natural or pharmacological agents was used to suppress arrest or reverse carcinogenesis at its early stages **(Montes de oca *et al.,* 2017)**. Other investigators have focused on the progression, treatment and prevention of cancer with such compounds **(Ioannis *et al*., 2015).**

Annona muricata Linn. is an evergreen plant which distributed in tropical and subtropical regions. *A.muricata* fruit belongs to the family of Annonaceae is also commonly known as sirsak, graviola, soursop and gunbanana **(Desmiaty *et al*.,2017 & and Patel and Patel,2016)**. Traditionally, all parts of the graviola tree are used in natural medicine in many countries for the treatment of number of diseases, including the bark, leaves, roots, fruit, and fruit seeds **(Onyechi *et al.,* 2012 and Kedari and Khan, 2014)**. It is used as a strong diuretic for swollen feet (edema) and as a tonic used for dysentery, mouth sores, fever, liver problems, for an anthelmintic and antirheumatic, for neuralgia, rheumatism, arthritis pain and as an antiparasitic, intestinal colic, antidiabetic, high blood pressure and diarrhea hypertension and parasites **(Kedari and Khan,2014)**. Furthermore, pharmacological studies showed that *A. muricata* has been showed to have biological and pharmacological activities such as antifungal, antibacterial, antioxidant and anticancer properties on multidrug resistant cancer cell line, **(Vieira *et al*., 2010; Heinrich *et al*., 1992; Antoun *et al*., 1993; Baskar *et al*.,2007 and Luna *et al*.,2006)**. *A. muricata* also was found to have many pharmacological activities as antimicrobial, antiinflammatory, antiprotozoan, antioxidant, insecticide**,** anti ulcer, free radical scavenging activity, anticancer, antirthritic, hepatoprotective, antidibetic, immune enhancing affects, cytotoxicity, chemopreventive and antiproliferative **(Kedari and Khan,2014 and Coria-Tellez et *al*., 2016)**. Graviola also expresses analgesic and antiinflammatory effects, promotes apoptosis (programmed cell death) and cytotoxicity on cancer cells that may result from the presence of alkaloids, essential oils and acetogenins **(De Sousa *et al*., 2010; Kossouoh *et al*.,2007; Chang *et al*.,2003 and Leboeuf *et al*.,1982).** Extensive phytochemical screening on different parts of the *A. muricata* plant have shown the presence of various phytoconstituents and compounds, including alkaloids (acetogenins), carbohydrates, coumarins, flavonoids, glycosides, phenolic compounds, phytosterols, proteins, quinones, saponins, steroids and terpenoids tannins, and essential oils **(Nawwar *et al.,* 2012; Vijayameena *et al*.,2013; Jiménez *et al.,* 2014 and Yang *et al*., 2015)**. However, *Annona* species, including *A. muricata*, have been shown to be a generally rich source of annonaceous acetogenin compounds which it is known to have a potent anticancer activity **(Moghadamtousi *et al*.,2015; Ioannis *et al*.,2015 and Najmuddin and Romli, 2016)**. More than 100 annonaceous acetogenins have been isolated from leaves, barks, seeds, roots and fruits of *A. muricata* **(Moghadamtousi *et al*.,2015)**. These acetogenins demonstrated to be selective and toxic against various types of cancer cells without harming normal and healthy host cells **(Ekaprasasti *et al*.,2012; Gajalakshmi *et al*.,2012; Ragasa *et al*.,2012; De Sousa *et al*., 2010 and Zeng *et al*., 1996)**. Also, **Leslie Taylor (2005)** reported that more than 200 chemical compounds have been identified and isolated from this plant; the most important being alkaloids, phenols and acetogenins. The presence of different major minerals such as K, Ca, Na, Cu, Fe and Mg suggest that regular consumption of the *A. muricata* fruit can help provide essential nutrients and elements to the human body (**Moghadamtousi *et al*.,2015**). However, study by **Minari and Okeke (2014)** supported that the graviola leaf extract could act as a cancer prevention agent. In recent years graviola is sold in Libya and other countries as a popular adjunctive natural therapy for cancer. There assurance of the safety and efficacy of a dietary supplement requires monitoring of the quality of the product. The various phytochemicals such as phenols, phenolic acids, alkaloids, flavonoids, carotenoids, and vitamins play a major role in boosting the immunity **(Wang *et al*., 2012)**. *A. muricata* known as the cancer killer has been widely used in the traditional medicine for the treatment of cancer and tumors **(Moghadamtousi *et al*.,2014)**. The alcoholic extract of *A.muricata* renders an overall protection against CCl4 induced toxicity by scavenging the free radicals produced by CCl4 metabolism. Thus it provides protection against increase in serum glutamic oxaloacetic transminase (SGOT), serum Glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SALP), liver and brain lipid peroxidation (LOP) levels and decrease in liver and brain protein levels **(Padma *et al*.,1999).**

Trichloroacetic acid (TCA) is a colorless to white crystalline solid with a sharp, pungent odor **(NIOSH, 2003)** formed from organic material during water chlorination **(IPCS, 2000; Coleman *et al*., 1980)** and has been detected in groundwater, surface water distribution systems, swimming pool water and

drinking water **(U.S. EPA, 2000 and IARC, 2004)**. TCA was detected in vegetables, fruits, and grains **(Reimann *et al*., 1996)** and can be taken up into foodstuffs from the cooking water **(U.S. EPA, 2005)**. Therefore, human exposure to TCA can also occur via food tap water consumption. TCA administered at dose level 2,000 ppm (300 mg/kg-day) in drinking water for 50 days significantly increased serum AST, ALT **(Celik, 2007). Celik (2007)** concluded that elevated serum marker enzymes probably resulted from damage to liver cells by TCA and subsequent leakage of the enzymes into plasma Further, **Celik (2007)** found that TCA treatments caused different effects on antioxidant defense system and lipid peroxidation in various tissues of rats at the end of the TCA treatment. In the literature, it is reported that TCA exposure caused different effects on gross and microscopic examinations, serum chemistry, haematology, biochemical analysis **(Celik and Temur, 2009; Poon *et al*., 2000**). **Poon *et al*. (2002)** performed gross and microscopic examinations, serum chemistry, hematology, biochemical analysis, neurogenic amine analysis and serum TCA analysis at the end of the treatment period. **Mather *et al*. (1990)** reported that TCA and DCA (dichloroacetic acid) produced substantial systemic organ toxicity to the liver and kidney during a 90-day sub-chronic exposure. It was observed that trichloroacetic acid (TCA) caused histological alterations in the liver such as centrilobular necrosis, vacuolation in hepatocytes and loss of hepatic architecture **(Acharya *et al*.,1997)**.

Liver is a target organ and primary site of detoxification and is generally the major site of intense metabolism and is therefore prone to various disorders as a consequence of exposure to the toxins of extrinsic as well as intrinsic forms. The liver plays important role in metabolism to maintain energy level and structural stability of body **(Guyton and Hall, 1996).** It is also site of biotransformation by which a toxic compound has been transformed in less harmful form to reduce toxicity **(Hodgson, 2004).** Liver is a major organ attacked by reactive oxygen species (ROS) **(Sanchez-Valle *et al*.,2012).** Many natural agents possessing antioxidative properties have been reported to prevent and treat liver damages caused by free radicals induced by toxic substance **(Adefolaju *et al*., 2009).** Therefore, the present study was conducted to evaluate quality control (QC) of graviola dietary supplement capsules (DS) and phytochemical of methanolic extract of graviola fruit pulp and aqueous extract of DS of graviola. As well as the present work designed to demonstrate the effect of aqueous extract of graviola fruit pulp or aqueous extract of DS of graviola against trichloroacetic acid (TCA) induced hepatotoxicity in albino mice.

2. Material and Methods

2.1. Materials for quality control (QC) and phytochemical screening

2.1.1. Experimental graviola samples:

Fresh plant sample of graviola fruits (*Annona muricata*) were randomly collected from local markets in Tripoli Libya. The fruit were washed with tap water, then the fruit pulp were collected by clean spoon without seeds and stored in clean jar in the refrigerator. The amount of fruit pulp about 500 grams was dried by freeze-drying technique, this step done in National Oil Corporation Libyan Petroleum Institute. About (50 grams) of the powder of the freeze-dried fruit were kept in plastic bag. The freeze-dried fruit was used to prepared methanolic extract according to methods of **Banoti (1980)**, then kept until used. The fruit extract was applied in phytochemical screening.

2.1.2. Graviola dietary supplement capsules (DS):

DS capsules of gravel which contained graviola pulp fruit extract were bought in 2014 from a pharmacy in Tripoli Libya and all label data (Trade name, Composition,. Manufacture, Manufactured date, Expired date, Register at the Ministry of Health A disclaimer) were recorded and evaluated.

2.1.3. Parameters of quality control of graviola DS capsules:-

2.1.3.1. Packaging and labeling

The contents of the package were observed and recorded. Comparison of the prescribed labeling and packaging of this product was made with prescription labeling and packaging requirements in the guidelines **(USP, 2008)**.

2.1.3.2. Physico-Chemicals constants:

The values of physical constant like loss on drying and weight variation were determined according to **USP (2008)**, moisture content was determined according to **(AOAC,2008)** and the moisture content percentage was calculated according to **WHO (1998)**, ash values (Total ash, acid- insoluble ash and water-soluble ash) were determined according to **WHO (1998)**, crude fibers was determined according to **AOAC (2005).** Microbial contamination included detection of bacterial contamination (detection total bacterial and fungal count, detection specific bacteria detection salmonella and detection of fungi (yeasts and molds) according to **AOAC (2008)** were determined. Also, detection of Aflatoxin has been done for the graviola DS, by spotting the sample on a thin layer chromatography (TLC) plate and compared with standard toxins B1, B2, G1 and G2 using chloroform: methanol (10:90) as a mobile phase. The plate then detected under long UV (366nm) **(Berthiller *et al*., 2017)**. Radioactive contamination was determined qualitatively for graviola DS in Tajora Nuclear Research Center in

Tripoli-Libya. The extract of *A.muricata* fruit and graviola DS capsules were subjected to UV-Visible Spectrophotometric analysis in the wavelength range of 190-350nm using GBC UV/VIS spectrophotometer and the characteristic and values of peaks detected of spectral analysis of solvents used for dissolving the extracts for analysis like distilled water, methanol, ethanol, chloroform etc were recorded according to **Giridhar (2015)** to confirm the presence of a number of chemical constituents to support the chemical test. Analysis was done in Food and Drug Control Center, Tripoli, Libya. The methanolic extracts of *A. muricata* fruit and graviola DS capsules were subjected to thin layer chromatography analysis (TLC) to confirm the presence of a number of chemical constituents to support the chemical test according to **Kamaruz and Kalyani (2013)**.

2.1.3.3. Phytochemical study:

Annona muricata fruit sample and methanol extract of graviola DS capsules were tested for the presence of active principles such as Steroids, Saponins, Tannins, Alkaloids, Flavonoids according to **Adetunji *et al.* (2011)**, Glycosides according to **Bhandary *et al.* (2012)**, Anthraquinone according to **Ayoola *et al.* (2008)** and Coumarins according to **Kumar *et al.* (2013)** were determined.

Test for steroids

0.5g of each extract was dissolved in 10ml of chloroform and equal volume of concentrated H2SO4 was added by the sides of the test tubes. Reddish upper layer and yellowish sulphuric acid layer with green fluorescence indicate the presence of steroids.

Test for Saponins

0.5g of sample was added to 5ml of distilled water in a test tube and the solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed in the formation of an emulsion.

Test for tannins

To 10 ml of freshly prepared 10% KOH in a beaker, 0.5g of sample was added and shaken to dissolve. A dirty precipitate observed indicated the presence of tannin.

Test for alkaloids

Three milliliter of extract was stirred with 5ml of 1%HCl on a steam bath for twenty minutes. The solution obtained was cooled and filtered and the filtrate was added to a few drops of Mayer's reagent/picric acid. A cream precipitate indicated the presence of alkaloid.

Test for flavonoids

To a volume of 3ml of the extract 1ml of 10% sodium hydroxide was added. A yellow coloration indicated the presence of flavonoids**.**

Test for glycoside

Test solution was dissolved in bromine water and observed for the formation of yellow precipitate to show a positive result for the presence of glycosides.

Test for anthraquinones

A 0.5g of the extract was boiled with 10ml of H2SO4 and filtered while hot. The filtrate was shaken with 5ml of chloroform; the chloroform layer was a pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for coumarins

A 0.5g of each extract were dissolved in NaOH, spotted on a Wattman's filter paper and exposed to long UV light (366nrn). The appearance of a florescent spot indicates the presence of coumarins.

2.2-Biological screen:

2.2.1. Preparation of the aqueous extract of *Annona muricata* (graviola) fruit:-

10 g of graviola fruit extract were mixed mechanically with 5 ml of distilled water in an electric grinder. The crude aqueous extracts were freshly prepared before administration and each mouse received orally 0.2ml/mouse at dose level 16000mg/kg/ bw.

2.2.2. Preparation suspension of DS of graviola:-

One capsule (500mg) of dietary supplement of Graviola was suspension in 15.4 ml of distilled water. The suspension were fresh prepared before administration and each mouse received orally 0.1ml/mouse at dose level 130 mg/kg bw.

The aqueous extracts were prepared according to the prescription given by traditional healers and the dose equivalent daily prescribed dose for human was determined according to **Paget and Barnes (1964).**

2.2.3. Trichloroacetic acid (TCA):

Trichloroacetic acid (TCA) from (Sigma Co, Germany) was used in the present study. The mice were given TCA trichloroacetic acid in drinking at dose level 500mg/kg for 7 weeks. TCA was chosen because it has been reported to increase liver growth, cell proliferation, and induce cancer and tumor in liver of mice and using as hepatocarcinogenesis material for mice **(Bull *et al*., 1990 and Pereira *et al*., 2001).**

2.2.4. Animals and treatment

Female Swiss albino mice 6-7 week old and weighing between 23-25 gm were used in this study. Those were obtained from the animal breeding house of faculty of veterinary medicine, Omar Al mukhtar University, AL Bayda Libya. They were housed in plastic cages and kept under a controlled conditions of 20 ± 2, 12 h light/dark cycle, 50±10% humidity and fed commercial standard diet and allowed tap water *ad libitum* for seven days before starting the experiment for acclimatization Throughout the experiments, all animals received human care

according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals. A total 120 adult female albino mice were divided into 6 groups 20 mice each. Group (I) was kept as normal control received orally by oral gavage distilled water at dose level 4 ml/kg for 10 successive weeks, Group (II) were given orally by oral gavage aqueous extract of graviola fruit at dose level 16000 mg/kg bw by for 3 successive weeks, Group (III) were given orally by oral gavage suspension of dietary supplement of graviola at dose level 130 mg/kg bw for 3 successive weeks. Group (IV) were given TCA in drinking water at dose level 500 mg/kg body weight in drinking water for 7 successive weeks (Doses were estimated based on default drinking water intake values for mice), then were left to self recovery for 3 successive weeks, Group (V) were given TCA at dose level 500 mg/kg body weight in drinking water for 7 successive weeks then were treated with aqueous extract of graviola fruit at dose level 16000 mg/kg bw by oral gavage for 3 successive weeks, Group (VI) were given TCA at dose level 500 mg/kg body weight in drinking water for 7 successive weeks then were treated with suspension of dietary supplement of graviola at dose level 130 mg/kg bw by oral gavage for 3 successive weeks. The biological screening comprises morphological and clinical signs study, biochemical study and histopathological study.

At the end of the experimental period, the animals from both control and treatment groups were dissected without anesthesia. A minimum of 6 animals from each group were necropsied after sacrificed by cervical dislocation to evaluate hematological biochemical and histopathological changes. The biological screen in the present investigation comprises the following studies.

I- Morphological and clinical signs study:

Animals were observed daily to note and record any changes in the behavior, depression, food intake and signs of difficulty breathing, salivation, diarrhea, muscular weakness and any signs of toxicity and mortality. Also, body weights of mice in all groups were measured at the beginning and the end of the experiment. Body weights were also recorded at weekly intervals using electronic balance. Weight gains and the body weight changes (%) were calculated according to **Tülüncü *et al*. (2010)**.

II- Biochemical study:

For biochemical parameters, the blood samples were collected into free anticoagulated containers and centrifuged at 3000 rpm for 10 minutes and the superanated serum was collected in eppendorf and utilized for estimation various biochemical parameters. Serum activities of alanine aminotransferas (ALT) and asparatate aminotransferease (AST) were determined colorimetrically using kits obtained from Analyticon Biotechnologies (Germany) according to the methods of **Reitman and Frankel (1957).**

III-Histopathological studies

For the light microscopic examination, the liver tissues were collected from all the animals' groups. A portion of the liver were cut into small pieces of approximately 3-5 mm size and fixed in aqueous Bouin's fluid for -18-20 hour. After embedding in paraffin wax, thin sections of 5 µm thickness of liver tissues were cut and stained with Harri's Hematoxylin and Eosin (H & E) according to **Bancroft & Gamble (2008).** The thin sections of liver were made into permanent slides and examined under high resolution microscope with photographic facility (Nikon Eclipse E400, Japan) and histopathological changes were recognized and photographed

2.2.5. Statistical analysis

The data were expressed as means ± Standard Error of Mean (SEM), analyzed through one way analysis of variance (ANOVA), followed by the post hoc Duncan's test for comparison of various treatments using the SPSS software version 19.0. A p-value of less than 0.05 ($P<0.05$) was considered statistically significant. Excel programs was also used for analysis and draw the figures.

3. Results

3.1. Quality control (QC) and phytochemical screening

Examination of **graviola** D.S label revealed presence primary packaging label (name of product, name of active ingredient, dosage form, direction of use, indication, dosage, duration of use, age group limitation, batch number, production date, expiry date, storage condition, name and address of manufacturer or importers and registration or notification listing number).

Determination of percentage of ash value of graviola dietary supplement

Revealed that the total ash, acid insoluble ash and water soluble ash werefound to be 1.6%, 0.89% and 0.90% respectively. Our result also revealed that the average value of three replicates of moisture content of graviola DS was 6.04%, loss on drying percentage by gravimetric method was 5.7%. the crude fiber percentage of graviola DS was 2.7%, the weight variation of graviola SD sample was 90-110% and this result should approve to the manufactured capsules have an uniform weight. **Results for microbial contamination of graviola DS** revealed that the total viable aerobic microbial count in analytic sample was 90000cfu/gm. While, the Total Yeast & Mould Count was found to be 120000cfu/gm (Specification was 10000000cfu/gm and 100000cfu/gm respectively).

Examination of agar culture

For growth microbial contamination of graviola DS sample revealed absence of *Escherichia coli, Salmonella Spp, Staphylococcus Spp* and *Psuedomonas aeroginosa*. While, fungus growth were detected in the same sample.

Results of total aflatoxins (B1, B2, G1 and G2)

Were revealed free of aflatoxins contamination because the concentration of aflatoxin in the sample was undetectable level and the value was less than the smallest standard.

The qualitative determination of radioactive contamination

Which carried out by using High-Purity Germanium (HPGe) detector with relative efficiency: 30% in Gamma-Ray Spectrometry in TNRC in Tripoli-Libya revealed that all examined sample was found to be free from of industrial radioactive contamination.

Thin Layer Chromatography (TLC) Screening of graviola DS

Using graviola fruit extract sample as a standard were revealed the presence of shared some components in the both samples, their spots showed the same Rf values which indicates some active constituents in the graviola fruit powder extract and graviola DS. Only few components were not present in graviola dietary supplement.

Ultraviolet-Visible (UV-VIS)

Spectrophotometric of graviola DS by using graviola fruit extract sample as a standard were determined between 190 nm to 350 nm. The UV-VIS Spectrophotometric of graviola fruit powder extract showed peak wavelengths of maximum absorbance (λmax) at 202,203,204 and 277nm (Fig.1). The UV-VIS Spectrophotometric of graviola DS sample showed absorption maxima at 202,203,204and 281 nm (Fig.2).

Qualitative phytochemical screening tests

For both samples (extract of *A. muricata* fruit and graviola DS capsules revealed that the graviola DS sample was found to be contained alkaloids, tannins, flavonoids, glycosides and anthraquinone. While, saponins and coumarins were not detected. It was also found that the graviola fruit extract contained alkaloids, tannins, flavonoids, saponin, glycosides, coumarins and anthraquinone were detected.

3.2. Biological screening observation

3.2.1. Result of morphological and clinical study:

The animals intoxicated with TCA for 7 weeks showed hypoactivities and slight decrease in food intake. While, the changes in behavior and external features were less in female mice intoxicated with TCA and than treated with aqueous extract of the graviola fruit pulp or DS of graviola. No behavioral changes and abnormal signs in the external features (including food and water consumption, eye color, body furs, feces, and activities) of female mice treated orally with aqueous extract of the graviola fruit pulp only or DS of graviola and during the experimental period compared to control group.

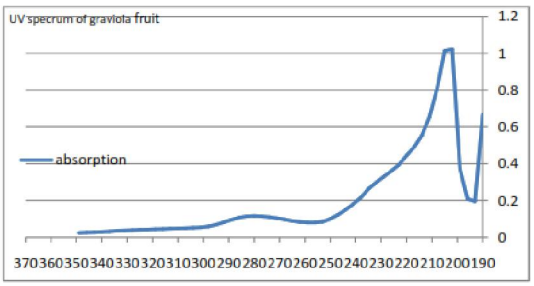

Fig. (1): UV spectrum of *Annona muricata* fruit extract

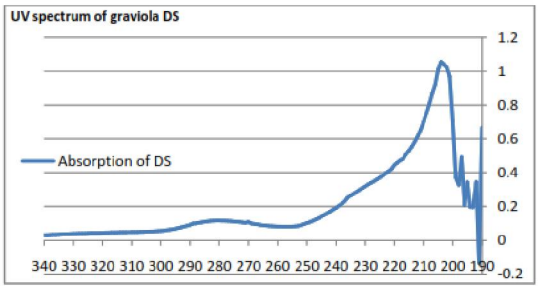

Figure (2): UV spectrum of graviola DS samples

No deaths were recorded during the experiment period in mice of normal control and aqueous extract of the graviola fruit pulp and dietary supplement of graviola treated groups. While, the mortality were 15% in trichloroacetic acid (TCA) treated group during the experimental period. However, administration of aqueous extract of the graviola fruit pulp (16000 mg/kg bw for 3 weeks) and dietary supplement of graviola (130 mg/kg bw for 3 weeks) reduced the mortality to 5% during the experiment period.

Our data on body weight were illustrated in Table (1). A statistical analysis revealed that there were insignificant changes in the initial body weight of control and all experimental groups. The body weight increased gradually in normal control group through the experimental period. The final body weight gain, increased by 9.7% above initial body weight of mice in the control group. The animals treated with aqueous *Annona muricata* extract or those treated with the graviola dietary supplement showed slight, insignificant changes in the final body weight gain compared to initial body weight and final body weight of the control group. The final body weight gain, decreased by 16.8% compared to initial body weight of mice in TCA treated group. It was also

found that the treatment with TCA and graviola dietary supplement or TCA, and aqueous extract of *Annona muricata* fruit pulp induced insignificant decrease in the final body weight comparison to control group.

Table (1): Effect of *A. muricata* fruit pulp and DS of graviola with and without trichloroacetic acid (TCA) on body weight gain of female Swiss albino mice.

Groups / Time	Control	Aqueous extract of graviola fruit pulp	Dietary supplement of graviola	Trichloroacetic acid (TCA)	Aqueous extract of graviola fruit pulp & TCA	Dietary supplement of graviola & TCA
Mean of Initial body weight (gm)	25.1± 0.5^{a}	25.1±0.5^{a}	25.0±0.5^{a}	25.0±0.4^{a}	25.1± 0.4^{a}	25.1± 0.4^{a}
Mean of body weight (gm) after 6 weeks	27.1±0.5^{b}	27.0±0.5^{b}	27.0±0.3^{b}	20.0±0.3^{c}	20.7±0.3^{c}	20.6±0.2^{c}
Mean of final body weight (gm) weeks	27.54± 0.4^{b}	27.4±1.0^{b}	27.5±0.3^{b}	20.8±0.4^{ce}	22.3±0.4^{d}	22.0±0.6^{de}
The change in the body weight (%)	9.7 %	9.1%	10%	- 16.8%	-11.15%	-12.35%

Each value represents the mean ±S.E. of body weight of survival animals in each group. Data are statistically significant at P<0.05 compared to a normal control group and the values with no common superscript (a, b, c, d) in each row and column are statistically significant at P≤ 0.05

3.2.2. Results of the biochemical study:

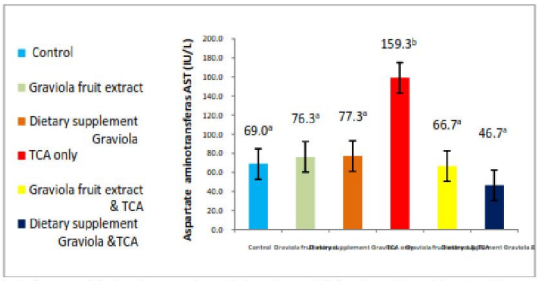

Fig. (3): The influence of fruit pulp *A.muricata* fruit pulp and DS of graviola with and without trichloroacetic acid on Aspartate aminotransferase (AST IU/L) activity in blood serum of mice. (Values with no common superscripts are statistically significant at P≤ 0.05).

Results of the biochemical analysis revealed that the treatment with aqueous extract of graviola fruit pulp or graviola DS alone showed insignificant alterations in the serum level of AST (76.3±13.9 and 77.3±11.9 IU/L) and ALT (63.3±8.8 and 66.7±6.9 IU/L) respectively compared to AST and ALT values of the control group (69.0±2.1 and 69.3±10.9 IU/L) respectively. As expected administration of TCA at 500mg/kg body weight resulted in a significant (P<0.05) elevation of serum hepatic biochemical markers (AST: 159.3±29.7and ALT: 139.7±32.5 IU/L) compared to the control group. Whereas, administration of aqueous extract of graviola fruit pulp or aqueous extract of supplement of graviola DS significantly (P<0.05) decreased the elevated serum activities of AST (66.7±8.7 and 44.7±1.8 IU/L) and ALT (76.7±4.4 and 47.7±4.1 IU/L) respectively compared to in TCA only intoxicated mice. It was noticed the aqueous extract of graviola fruit pulp or graviola DS proved significantly improvement in restoring the altered activity of serum markers of liver functions (AST, ALT) (Figs. 3 and 4).

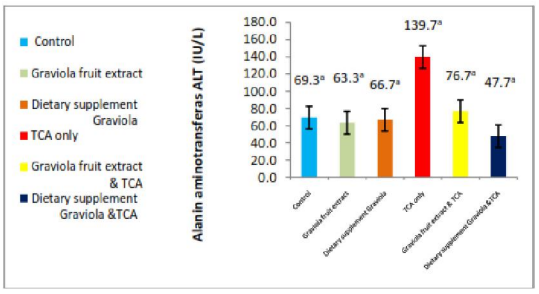

Fig. (4): The influence of fruit pulp *A.muricata* fruit pulp and DS of graviola with and without trichloroacetic acid on Alanin aminotransferas (ALT IU/L) activity in blood serum of mice. (Values with no common superscripts are statistically significant at P≤ 0.05).

3.2.3. Result of histopatological study:

Histological examination revealed that the liver of control mice formed of hepatic cords radiating from a central vein and separated by narrow blood

sinusoids. The hepatocytes are polyhedral in shape with acidophilic granulated cytoplasm and rounded centrally located nuclei with prominent nucleoli. Blood sinusoids are lined with endothelial and Kupffer cells (Figs.5 and 6). The livers of mice treated with aqueous extract of graviola fruit pulp only exhibited normal histological architecture. Compared to control group normal liver structure with central vein and radiating cords of polyhedral hepatocytes separated by blood sinusoids were seen (Fig.7). The livers of mice treated with aqueous extract of DS of graviola capsules only showed no obvious histopathological changes and ormal histological architecture in most liver sections was detected. However, mild vacuolated hepatocytes, congested blood sinusoids and few inflammatory cells infiltration around portal area were noticed (Figs. 8 and 9).

Mice treated with TCA developed significant hepatic damage as compared to controls. The liver sections of mice treated with TCA only showed many pathological alterations include loss of normal histological architecture, mild vacuolated and hypertrophic hepatocytes, congested blood sinusoids, and proliferation of Kupffer cells. Free radical formation during the metabolism of TCA leading to the necrosis of many hepatocytes as evident by pyknotic, karyorrhexis or karyolysis nuclei and vaculated cytoplasm. Also, administration of TCA leads to hepatic cord destruction and stenosis of hepatic sinusoids. In addition many hepatocytes appeared with irregular nuclei or binucleated. Also, hypertrophic nuclei were noticed. As well as mitotic figures could be seen in some hepatocytes. However, many inflammatory cell infiltration and many diffuse necrotic foci with different size were markedly observed (Figs.10 - 13).

An ameliorative effect was obtained in mice treated orally with either TCA or aqueous extract of graviola fruit or TCA with dietary supplement of graviola capsules. The ameliorative changes were more obvious in the mice treated with aqueous extracts of dietary supplement graviola capsules and TCA. The histological examination of liver sections of mice from TCA and aqueous extract of graviola fruit or TCA and dietary supplement of graviola treated group revealed disappearance of most pathological changes, although few inflammatory cells infiltration, disorganized and necrotic hepatocytes as evident by the presence of pyknotic, karyorrhexis or karyolysis nuclei accompanied with vacuolated cytoplasm around portal area and stenosis of hepatic sinusoids were persisted. However, congested blood vessels and nuclei with abnormal chromatin feature were rarely observed (Figs.14-18).

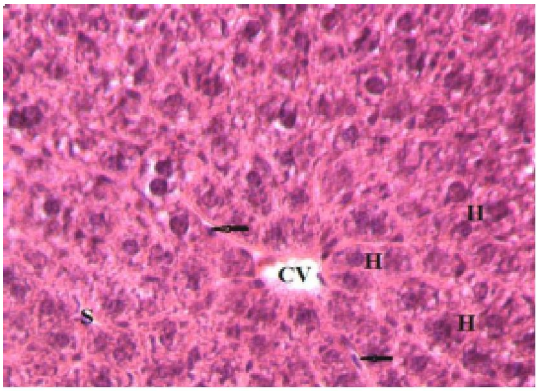

Fig. (5): A section of liver of mouse from control group showing histological architecture, central vein (CV) from which cords of hepatocytes (H) with rounded nuclei are radiating, Kupffer cells (Arrows), Hepatic sinusoids (S) (H & E, X 400).

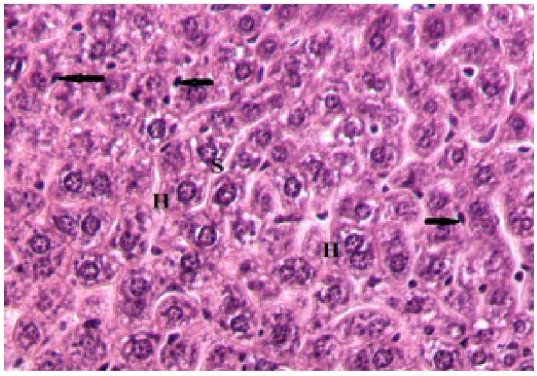

Fig. (6): A section of liver of mouse from control group showing normal histological architecture, hepatocytes (H) with rounded nuclei, Kupffer cells (Arrows), Hepatic sinusoids (S) (H & E., X 400).

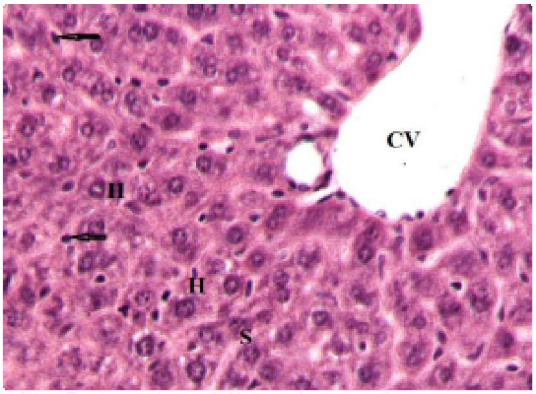

Fig. (7): A section of liver of mouse from aqueous extract of graviola fruit pulp treated group showing normal histological architecture, central vein (CV), hepatocytes (H) with rounded nuclei, Kupffer cells (Arrows), Hepatic sinusoids (S) (H & E., X 400).

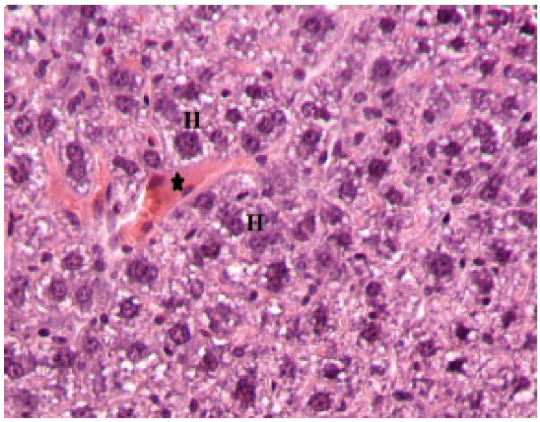

Fig. (8): A section of liver of mouse from aqueous extract of graviola DS only treated group showing mild vacuolated hepatocytes (H), few congested blood sinusoids (Star), (H & E., X 400).

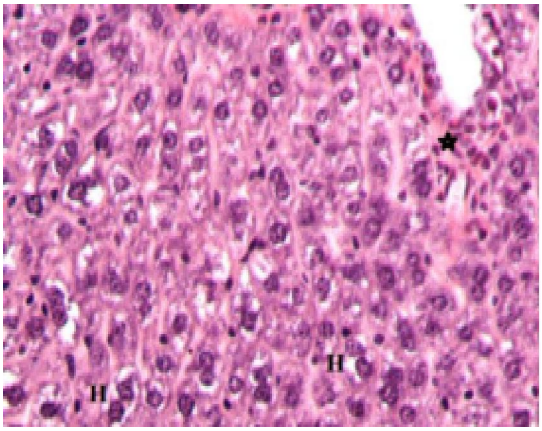

Fig. (9): A section of liver of mouse from aqueous extract of graviola DS only treated group showing mild vacuolated hepatocytes (H), few inflammatory cells infiltration around portal area (Star), (H & E., X 400(.

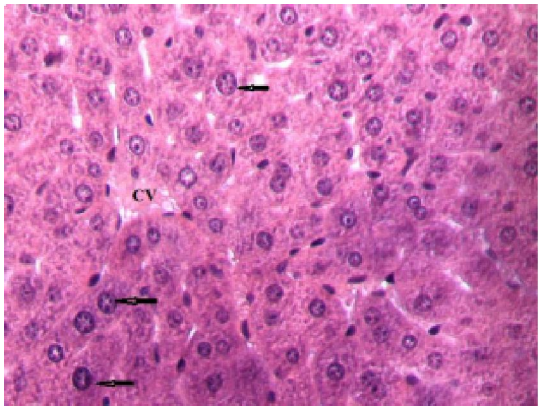

Fig. (10): A section of liver of mouse from TCA only treated group showing hepatocytes hypertrophy nuclei (Arrows), congested central vein (CV), stenosis of hepatic sinusoids and hyperplasia of Kupffer cells (H & E., X 400).

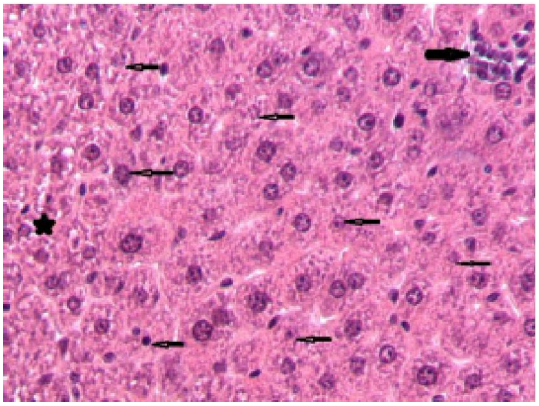

Fig. (11): A section of liver of mouse from TCA only treated group showing stenosis hepatic sinusoids and destruction hepatic cord, many necrotic hepatocytes with pyknotic, karyorrhexis or karyolysis nuclei and vaculated cytoplasm (Star). Note hepatocytes with irregular nuclei or nuclei with abnormal chromatin feature (Arrows), necrotic area with many inflammatory cell infiltration (Thick Arrow) (H & E, X 400).

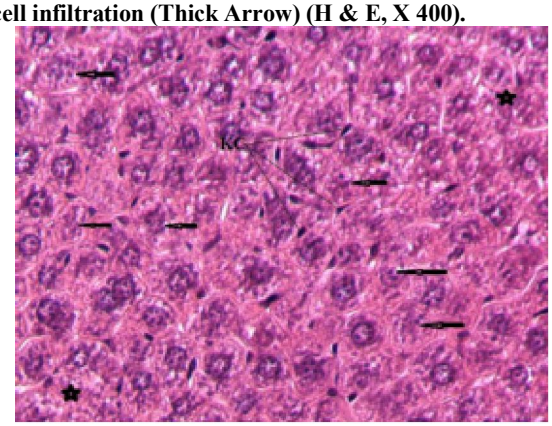

Fig. (12): A section of liver of mouse from TCA only treated group showing stenosis hepatic sinusoids, hyperplasia of Kupffer cells (KC). many necrotic hepatocytes with vaculated cytoplasm (Star), Note hepatocytes with abnormal nuclei and chromatin feature (Arrows) (H & E., X 400).

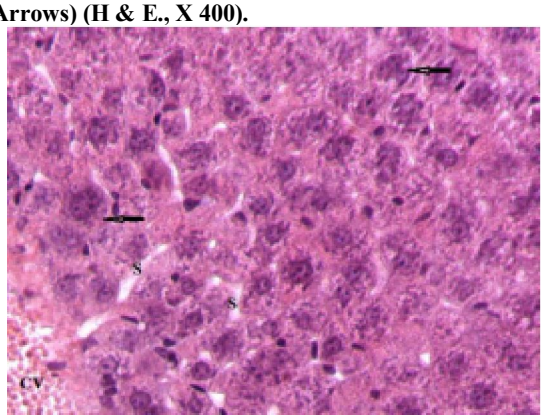

Fig. (13): A section of liver of mouse from TCA only treated group showing congested central vein (CV), hypertrophied hepatocytes with abnormal nuclei displaying abnormal chromatin feature (Arrows), hepatic sinusoids (S) (H & E., X 400).

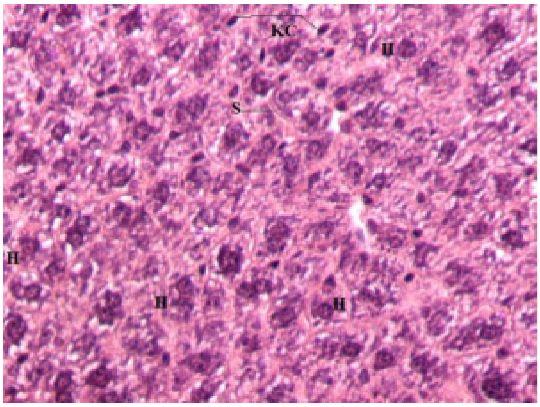

Fig. (14): A section of liver of mouse from TCA and aqueous extract of graviola fruit pulp treated group showing hepatocytes (H) with an improvement in nuclear feature, Kupffer cells (Arrows), mild stenosis hepatic sinusoids (S) (H & E, X 400).

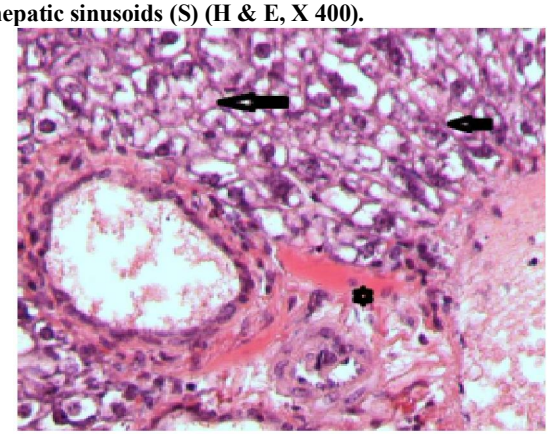

Fig. (15): A section of liver of mouse from TCA and aqueous extract of graviola fruit pulp treated group showing necrotic hepatocytes with vaculated cytoplasm (Arrows), congested blood vessels and few inflammatory cell infiltration around portal area (Star) (H & E, X 400).

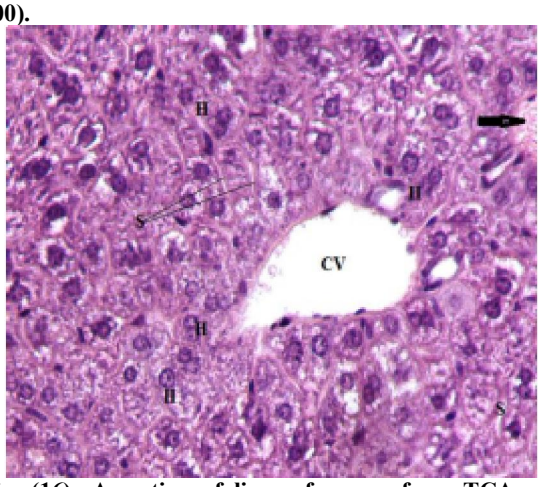

Fig. (16): A section of liver of mouse from TCA and aqueous extract of graviola DS capsules treated group showing many hepatocytes (H) with normal nuclear feature and mild vaculated cytoplasm, central vein (CV), Hepatic sinusoids (S), congested blood vessel (Thick Arrow) (H & E, X 400).

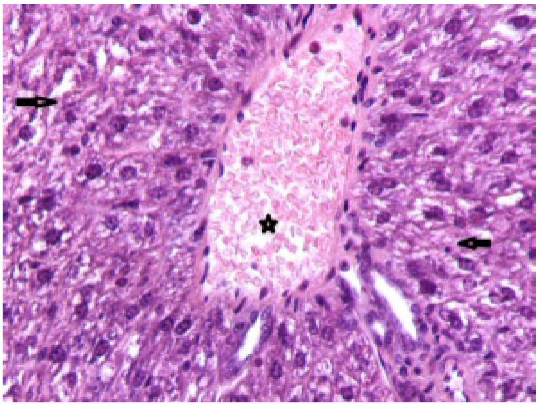

Fig. (17): A section of liver of mouse from TCA and aqueous extract of graviola DS capsules treated group showing congested blood vessel in portal area (Star), disorganized necrotic hepatocytes around portal area (Thick Arrows) H & E, X 400).

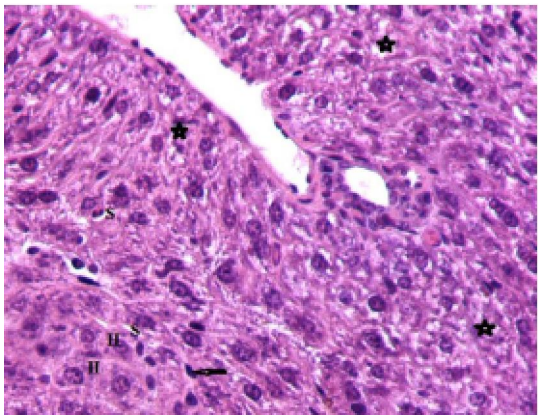

Fig. (18): A section of liver of mouse from TCA and aqueous extract graviola DS capsules treated group showing hepatocytes (H) with normal feature with an improvement in nuclear feature and disappearance of mitotic feature, Hepatic sinusoids (S), Kupffer cell (Arrow), necrotic hepatocytes around portal area (Stars) (H & E, X 400).

4. Discussion

4.1. The Quality control (QC) and phytochemical screening

Herbs are alternative medicines for treatment of various diseases due to their assumed acceptability, effectiveness, affordability, safety and low cost **(Arya *et al*.,2012)**. There is also an emerging increase in the consumption of herbal formulations by the public because of the strong belief that these products are natural; hence, they are safe for the treatment of ailments **(Said *et al*.,2002)**. However, herbal preparations assumed to be safe may contain

contaminants such as heavy metals **(Abou-Arab and Abou Donia.,2000)**, aflatoxins and pathogenic microbes due to the manner in which they are prepared or as a result of acquisition of metals (e.g. cadmium) from the soil **(Thanaboripat *et al*.,2007 and Kneifel *et al*.,2002).** Approximately 80% of the world's population exclusively plants for various htealing proposes. In the industrially developed countries almost 35% of drugs contain active principle of natural origin **(Irvine, 1995)**. Herbal remedies consist of portions of plants or unpurified plant extracts containing several constituents, which often work together synergistically. Quality control for the efficacy and safety of herbal products is essential. The quality control of phytopharmaceuticals may be defined as the status of a drug, which is determined either by identity, purity, content, and other chemical, physical or biological properties, or by the manufacturing process. Compared with synthetic drugs. Many sites on the internet advertise graviola capsules as a cancer cure, but none of them are supported by any legal authorized from Food and Drug Administration (FDA) or reputable scientific cancer organizations (**Cancer Research UK, 2015**). In most countries herbal products are launched into the market without proper scientific evaluation, and without any mandatory safety and toxicological studies. Therefore, the present study was evaluated the quality control of graviola DS capsules and phytochemical of extracts of graviola fruit pulp and DS of graviola.

In the current study evaluation packaging and labeling of random selected sample of graviola DS product container collected from a pharmacy in Tripoli- Libya revealed, accepted dietary supplement product container according to FDA guidelines for industry. The quality of consumer information about the product is as important as the finished herbal product. Warnings on the packet or label will help to reduce the risk of inappropriate uses and adverse reactions **(De Smet *et al*., 1997)**. The primary source of information on herbal products is the product label. Currently, there is no organization or government body that certifies herb or a supplement as being labeled correctly. It has been found that herbal remedy labels often cannot be trusted to reveal what is in the container **(Folashade *et al*., 2012)**. The ash value is a validity parameter describes and to assess the degree of purity of the sample and ash values reflecting the heavy metal contamination level **(Folashade *et al*., 2012)**. In the current study the average values of 3 replicates of physicochemical parameters including total ash, acid - insoluble ash, water soluble ash and the crude fiber percentage of the graviola DS capsules were good enough within the limit which indicate the quality and purity of graviola DS capsules. Total ash and acid insoluble ash contents are important indices to determine quality and purity of herbal medicines. The total ash and acid insoluble ash give an idea of inorganic composition and other impurities present along with drug **(Kalaskar *et al*., 2012; Gandagule *et al*., 2013; Shrestha *et al*., 2014 and Yazdinezhad *et al*., 2016).** Whereas the high water soluble ash indicated the presence of cellulosic substances and the high acid insoluble ash indicated the presence of silicacious substances **(Yazdinezhad *et al*., 2016).** In the current study few sample of graviola DS capsules showed slight increase in the moisture content. The moisture content of herbal drug is directly related to chance of microbial growth, chemical deterioration in sample materials and consequently with the less shelf life of crude drug **(Gandagule *et al*.,2013; Pandavadra and Chanda,2014; Alam and Saqib, 2015 and Yazdinezhad *et al*.,2016).** The moisture content was obtained in the determination of quantitative standards met the pharmacopoeial limits of water content for vegetable drugs, which is between 8–14 % **(African Pharmacopoeia, 1986).** From the foregoing, the plant material can be conveniently stored at room temperature without the deterioration of its active constituents **(Abere and Onwukaeme, 2012).** In the present study all samples of graviola DS capsules appeared free from microbial contamination, while, some samples revealed growth of fungal contamination (*Pencillium Spp*) but all samples appeared free from aflatoxins contamination. Few contaminated with fungi may be from storage. The contamination of herbal drugs by microorganism not only cause bio deterioration, but also reduces the efficacy of herbal drugs. The toxin produces by microbes makes herbal drugs harmful for human consumption because the contaminated drug may develop unwanted disease instead of disease being cured **(Khurana *et al.,* 2012)**. Quality of herbal drugs may be decreased by the microbial contamination. Therefore, World Health Organization, Geneva **(WHO, 2007)** laid down the limits for microbial contamination depending on the use of plant material as a drug **(Agarwal *et al*., 2014)**. The total aerobic viable count of bacteria in herbal drugs is an important quality control parameter to assess the hygienic conditions. Microbial contamination in the crude drug may occur through handling by personnel who are infected with pathogenic bacteria during harvest/collection and post-harvest processing. WHO has developed a series of technical guidelines and documents relating to the safety and quality assurance of medicinal plants and herbal drugs **(Agarwal *et al*., 2014)**. According to the findings of this study, it can be concluded that qualitative radioactive contamination evaluation using small quantity of herbal supplement lead to negative result. The result

indicates acceptable product without radioactive contamination. A certain amount of exposure to ionizing radiation is unavoidable because many sources, including radio-nuclides occur naturally in the ground and the atmosphere **(WHO, 2007).** The present study provides evidence that the methanolic extract of graviola (*A. muricata*) fruit and DS capsules of graviola contains bioactive compounds as alkaloids, tannins, steroids, flavonoid, saponin, coumarins, glycosides, and anthraquinone. Likewise, our results are also in line with the previous report who published that extensive phytochemical screening on different parts of the *A. muricata* plant have shown the presence of various phytoconstituents and compounds, including alkaloids (acetogenins), carbohydrates, coumarins, flavonoids, glycosides, phenolic compounds, phytosterols, proteins, quinones, saponins, steroids and terpenoids tannins, and essential oils **(Nawwar *et al.,* 2012; Vijayameena *et al*., 2013; Jiménez *et al.,* 2014 and Yang *et al*., 2015).** Treatment with dietary phytochemicals and/or relatively non-toxic therapeutic drugs on cancer cells may induce positive results, including cell cycle arrest, apoptosis, and differentiation, and may block tumor development **(Lee *et al.,* 2012).** Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities **(Sofowora,1993).** Analysis of the plant extracts revealed the presence of phytochemicals such as tannins, flavonoids, saponins, glycosides, steroids and alkaloids. Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability **(Roa *et al.*, 1995)**. Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation **(Just *et al.*, 1998; Okwu and Emineke, 2006, Liu and Henkel, 2002).** However, *A. muricata* fruit pulp extract was contained powerful secondary metabolites that can improve the quality of our health and phytochemicals protect us against many diet related diseases. The plants rich in tannins have significant activity in cancer prevention and are used in treating intestinal disorders **(Ruch *et al*.,1989; Motar *et al*., 1985 and Dharmananda,2003)**. Flavonoids and phenolics acids are the most important groups of secondary metabolites and bioactive compounds in plants and good sources of natural antioxidants in human diets **(Kim *et al*.,2003).** They are also a kind of natural product and antioxidant substance capable of scavenging free superoxide radicals, reducing the risk of cancer and protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA **(Halliwell and Gutteridge,1990 & Ghasemzadeh and Ghasemzadeh, 2011)**. It has been reported that flavonoids are free radical scavengers that prevent oxidative cell damage, and have strong anticancer activities **(Hanneken *et al*., 2006 and Pham-Huy *et al*., 2008).** All above recorded pharmacological properties of graviola (*A. muricata*) confirmed and explained the hepatoprotective effect in histopathological study in this work. In the present study the results of TLC plates under UV light screening by using graviola fruit extract sample as a standard revealed the presence of shared some components in graviola fruit extract sample and graviola DS samples. Only few components were not present in graviola dietary supplement. Thin-layer chromatography still remains an important tool in the analysis of plant extracts and herbal preparations. Planar chromatography, along with other chromatographic techniques, are commonly applied for constructing fingerprints for the quality control of plant extracts and plant derived products **(Cieúla *et al*., 2008).** A simple chromatography technique such as TLC may provide valuable additional information to establish the identity of the plant material. Thus, TLC is a convenient method of determining the quality and possible adulteration of herbal products **(Nasiruddin *et al.*, 2014).**

4.2- Biological study:

In the present study aqueous extract of graviola fruit pulp or aqueous DS of graviola capsules have been investigated for its protective action against hepatotoxicity induced by commonly used substance like TCA using as carcinogenic agent according to many authors **(Bull *et al*., 1990; Pereira, 1996; Pereira & Phelps, 1996; Channel *et al*., 1998 and Pereira *et al*., 2001)**. In the current study no visible abnormalities in behavioral and external features were observed in female mice treated orally with aqueous extract of graviola fruit pulp only or DS of graviola capsules. Whereas treated with TCA only showed hypoactivities and slight decrease in food intake. While, these changes in behavior and external features were lees in female mice intoxicated with TCA and than treated with aqueous extract of graviola fruit pulp or aqueous extract of dietary DS capsules. As well as no mortality was recorded in dietary supplement of graviola treated group. While, the mortality were 15% in TCA only treated group during experimental period. Administration of aqueous extract of graviola fruit pulp or DS of graviola markedly reduced the mortality in TCA intoxicated mice. The findings of the present experiment in accordance with **Larbie *et al*. (2011)** who reported that in oral acute and sub-acute toxicity studies, no untoward clinical signs were observed in the mice and rats administrated aqueous extract leaves of *A. muricata* at doses 100, 1000, 2500 and 5000 mg/kg. There were no changes in the nature

of stool, urine and eye colour. No mortality was observed at all dose levels from the critical 24 hours post administration to the end of the seventh day and 14 days. Orally, 5000 mg/kg of aqueous extract leaves of *A. muricata* was well tolerated in mice even after 7 days. Although, **Champy *et al.* (2005) and Lannuzel *et al.* (2006)** reported that in murine models annonacin (It is active compound in *A. muricata*) enters the brain parenchyma, decreases ATP levels and induces neurodegeneration in the basal ganglia. According to these authors, this neurodegeneration induced no change in the behavior or locomotor activity in rodents.

Administration of aqueous extract of graviola fruit pulp or aqueous extract of DS of graviola capsules induced insignificant changes in the final body weight comparing to control group. The mice intoxicated with TCA only showed obvious significant decrease in the final body weight. While, treated with aqueous extract of graviola fruit pulp or aqueous extract of DS of graviola capsules induced slight improvement in the final body weight comparing to TCA only intoxicated mice. The body weight changes are indicators of adverse effects of drugs and chemicals and it will be significant if the body weight loss occurred is more than10% from the initial weight **(Raza *et al.*,2002 and Teo *et al.*, 2002).** According to **Manson and Kang (1994) and Chahoud *et al.*, (1999)** the body weight alterations are a usually observed indicative of toxicity in mice. The protective antioxidant mechanisms maintain the cellular oxidation-reduction potentials required for normal metabolism and to prevent free radical attack of amino acids, proteins, and the lipid components of cell membranes necessary for functional and structural integrity of cells and tissues **(Feldman *et al.*, 1980)**. Also, body weight changes serve as a sensitive indication of the general health status of animal **(Salawu *et al.*,2009)**, and used as an indicator of adverse effect of drugs and chemicals **(Mukinda and Syce, 2007).** The findings of the present experiment, are fully in agreement with previous reports demonstrating that the mice administered TCA for 79 weeks had body weights that were depressed 11% relative to concurrent controls **(Bull *et al.*,2002)**. Similar finding has been previously described by **Acharya *et al.* (1995)** who reported that body weight was decreased by approximately 17% in the absence of changes in food consumption in young male rats exposed to TCA in drinking water at dose level 3.8 mg/kg-day for 10 weeks. Also, **Alzergy *et al.* (2015)** demonstrated that administration of TCA in drinking water at dose level 500 mg/kg for 6 weeks induced a significant decrease in the final body weight of male mice comparing to the control group. Exposure to TCA in drinking water at dose level 0.5,4. Or 5g/L for 60 or 104 week decreased body weight by 15% in the high-dose group relative to the control **(De Angelo *et al.*, 2008).** The reduction in body weight gains may be due to oxidative stress **(Mansour and Mossa,2010 & Saafi *et al.*,2011)** and/or due to the increased degradation of lipids and proteins as direct effects of toxic compound exposure **(Heikal and Soliman, 2010; Goel *et al.*,2005 and Mossa *et al.*, 2011).** The protective action against TCA induced alternations in mice body weight may be attributed to the antioxidant effect of *A. muricata* and its bioactive compounds.

Aspartate aminotransferase (AST), Alanine aminotransferase and (ALT) Alkaline phosphatase (ALP) are important liver enzymes and responsible for detoxification processes **(Abbassy and Mossa, 2012)** These enzymes are secreted into the blood circulation during hepatocellular injury and their levels constantly increase in the blood. Also, **Sharon (2007)** reported that to diagnose hepatotoxicity, serum enzymes AST, ALT and ALP are the most sensitive markers employed**.** In the present study TCA at 500mg/kg body weight resulted in a significant elevation in the hepatic biochemical markers (serum level of AST, ALT) compared to the control group. Whereas, administration of aqueous extract of graviola fruit pulp or aqueous extract of DS of graviola significantly decreased the elevated serum activities of AST and ALT compared to TCA only intoxicated mice. It was noticed the aqueous extract of graviola fruit pulp or DS of graviola proved significantly improvement in restoring the altered activity of serum markers of liver functions (AST, ALT). The results of current study are fully agreement with the earlier reports, since pretreatment with different concentrations of aqueous extract of *A. muricata* (50, 100, 200, and 400 mg/kg) for 7 days prior to liver damage restored liver function toward normal hemostasis, which was shown by biochemical and histological analyses **(Arthur *et al.*,2012)**. Potential hepatoprotective effect of graviola (*A. muricata*) explained previously by **Usunomena (2014)** who evaluated the protective role of pretreated ethanolic leaf extract of A. muricata (400 mg / kg body weight) for 7 days on dimethylnitrosamine (DMN) (single dose orally, 12 mg/kg; on day 8 and sacrificed 48hrs after DMN intoxication) induced hepatotoxicity in rat model recorded that pretreatment with ethanolic leaf extract of A. muricata significantly caused a decrease in serum ALT in comparison to DMN alone treated group. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin. Annona muricata ethanolic leaf extract decreased DMN-induced elevated ALT levels in tested groups, indicating the protection of structural

integrity of hepatocytic cell membrane or regeneration of damaged liver cells **(Palanivel *et al*., 2008). Usunomena (2014)** also reported that flavonoids are known to be antioxidants, free radical scavengers and antilipoperoxidant leading to hepatoprotection and the mechanism by which A.muricata exerts protection against DMN induced alterations in the liver may be due to the antioxidative and acetogeninic effect of the plants extract. The administration of aqueous extract of graviola fruit pulp or aqueous extract of DS of graviola markedly attenuated TCA induced hepatotoxicity in mice, as indicated by the significant decrease in AST activity in mice intoxicated with TCA then treated with A. muricata. The abnormal high level of serum AST and ALT in this study is a consequence of TCA -induced liver dysfunction. However, the result of this study demonstrated that treatment with aqueous extract of A. muricata significantly caused a decrease in serum ALT in comparison to TCA alone treated group. The increase serum level of AST and ALT have been attributed to the damaged structural integrity of the liver. This is because they are cytoplasmic in their location and are released into circulation after cellular damage **(Huang *et al*., 2007)**. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin. Regarding protective effects in the literature, it is reported that *A. muricata* ethanolic leaf extract decreased DMN-induced elevated ALT levels in tested groups, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells **(Palanivel *et al*., 2008).** The alcoholic extract of *A.muricata* renders an overall protection against CCl4 induced toxicity by scavenging the free radicals produced by CCl4 metabolism. Thus it provides protection against increase in serum AST, ALP, ALP, liver and brain lipid peroxidation levels and decrease in liver and brain protein levels. All these data suggest that the plant drugs possess possible antihepatotoxic activity **(Padma *et al*., 1999)**. In the present study liver sections of mice treated with aqueous extract of graviola fruit pulp or aqueous DS of graviola showing normal architecture and no necrosis with negligible cytoplasmic vacuolation and inflammatory cells infiltration around portal area. While, histopathological examination of the liver section of mice intoxicated with TCA only showed many pathological lesions include abnormal architecture, hypertrophic, necrosis and vacuolization hepathocytes, congested blood sinusoids, proliferation of Kupffer cells, many inflammatory cells infiltration, many diffuse necrotic foci with different size, binucleated hepatocytes, many hepatocytes with irregular nuclei and hepatocytes with mitotic chromatin figures. Administration of aqueous extract of graviola fruit pulp or aqueous extract of DS of graviola with TCA induced ameliorative changes and disappearance of most pathological changes in the liver tissue compared to of TCA only intoxicated mice. The findings of the present study clearly indicate the hepatoprotective activity of aqueous extract of graviola fruit pulp or aqueous DS of graviola against TCA induced hepatotoxicity. The liver via the portal vein is the first organ exposed to internally absorbed nutrients and other xenobiotics. The liver is composed of highly active metabolic tissue containing huge complement of detoxification machinery system **(Al-Ubaidy *et al*., 2006 and Metwally *et al*.,2015)**. The resulting hepatic injury was characterized by leakage of cellular enzymes into the blood stream and by centrilobular necrosis **(Recknagel *et al*.,1989 and Recknagel *et al*.,2001)**. Many natural agents possessing anti oxidative properties have been reported to prevent and treat liver damages caused by free radicals induced by toxic substance **(Adefolaju *et al*.,2009)**. In human and animal body, reactive oxygen species (ROS) can be neutralized by antioxidant defense systems including antioxidant enzymes **(Fang *et al*., 2002)** and antioxidant compounds **(Catapano *et al*., 2000)**. Many investigators reported that the decrease of glutathione (GSH) levels leads to elevation of lipid peroxidation **(El-Maraghy *et al*., 2001)**. Furthermore, **Adewole and Ojewole (2009)** reported that *A. muricata* leaf aqueous extract possesses antioxidant activity which is able to inhibit and/or prevent hepatic oxidative damage produced by streptozotocin (STZ) induced oxidative stress in rats treatment. treatment with *A. muricata* leaf aqueous extract at dose 100 mg /kg /day (administered orally by intra-gastric intubation) significantly increased antioxidant enzymes' activities. The same author also suggest that *A. muricata* extract has a protective, beneficial effect on hepatic tissues subjected to streptozotocin induced oxidative stress in rats, possibly by decreasing lipid peroxidation and indirectly enhancing production of endogenous antioxidants **(Adewole and Ojewole, 2009)**. Accumulating evidence demonstrated that ROS could lead to protein modification, lipid peroxidation, DNA damage and therefore acts as the initiator or promoter of carcinogenesis **(Wang and Feng, 2015 and Tan *et al*.,2014)**. As the first line defense in suppressing tumor initiation, antioxidants are treated as one of the promising strategies to prevent liver cancer. Furthermore, it has been reported that the combination of certain chemotherapeutic drugs and antioxidants could reduce drug resistance, sensitizing the liver cancer cells to chemotherapeutics and therefore improving the efficacy of anti-cancer

therapy **(Xu *et al*.,2014). Celik (2007)** found that TCA treatments caused different effects on antioxidant defense system and lipid peroxidation in various tissues of rats administered TCA at dose level 300 mg/kg-day in drinking water for 50 days. The results of present study were in harmony with **Acharya *et al*. (1997)** who found that the liver of rats exposed to TCA in drinking water at dose level 3.8 mg/kg-day for 10 weeks showed vacuolation and necrosis of hepatocytes and loss of hepatic architecture. It was also observed that TCA caused histological alterations in the liver such as centrilobular necrosis, vacuolation in hepatocytes and loss of hepatic architecture as recorded by **De Angelo *et al.* (2008)**. **DeAngelo *et al.* (2008)** noticed significant increase in the severity of inflammation in the liver of male mice exposed to TCA in drinking water at dose level 5g/L for 60 weeks. **Bull *et al*. (1990)** suggested that TCA appears to increase lipid peroxidation, and the production of free radicals may be responsible for its effects. Moreover, **Hassoun and Ray (2003)** reported that TCA induced both lipid peroxidation and oxidative DNA damage following administration of a single oral dose. An excess production of ROS is harmful to cells, which is finally altering physiochemical properties of the membranes leading to its functional and structural disruption **(Recknagel, 1983 and Feher *et al*., 1986)**. Excess of free radicals damages essential macromolecules of the cell, leading to abnormal gene expression, disturbance in receptor activity, proliferation or cell dynamics, immunity perturbation, mutagenesis, and protein deposition and damages all components of the cell, including proteins, lipids, and DNA **(Kehrer,1993)**. To scavenge and neutralize these free radicals, the cells are endowed with the antioxidant defense system of enzymes such as superoxide dismutase (SOD), catalase (CAT) defense and glutathione peroxidase (GPx). But an imbalance between reactive oxygen metabolites and antioxidant mechanisms of the cells, leading to excessive production of free radicals, creates a condition termed as oxidativestress **(Schroeder, 1984)**. Protective potential of graviola was further confirmed by histopathological assessment in the present work. The protective action against TCA induced alternations in liver tissue may be due to the phytochemical in graviola. Earlier reports have demonstrated that graviola has a number of biological activities such as antioxidant and anti-cancer properties on multi-drug resistant cancer cell lines **(Vieira *et al*., 2010; Heinrich *et al*., 1992; Antoun *et al*., 1993; Baskar *et al*., 2007 and Luna *et al*., 2006)**. Graviola also expresses anti-inflammatory effects, promotes apoptosis (programmed cell death) and cytotoxicity on cancer cells that may result from the presence of alkaloids, essential oils and acetogenins **(De Sousa *et al*., 2010; Leboeuf *et al*., 1982; Kossouoh *et al*., 2007 and Chang *et al*., 2003)**. These acetogenins demonstrated to be selective and toxic against various types of cancer cells without harming normal and healthy host cells **(Ekaprasasti *et al*., 2012; Gajalakshmi *et al*., 2012 and Ragasa *et al*., 2012)**. More than 100 annonaceous acetogenins have been isolated from leaves, barks, seeds, roots and fruits of A. muricata **(Moghadamtousi *et al*., 2015).** Annonaceous acetogenin is known to have a potent anticancer activity **(Moghadamtousi *et al*., 2014 & Najmuddin and Romli, 2016)**. The mechanism of the acetogenin cytotoxic action is the inhibition of the mitochondrial complex I **(Lannuzel *et al*., 2003)**. **Torres *et al*. (2012)** demonstrated that A. muricata extracts suppressed phosphorylation of the key molecules involved in the extracellular signal-regulated kinase (ERK) and the phosphatidylinositol 3'kinase (PI3 K/Akt) pathway which play a crucial role in the proliferation and survival of pancreatic cancer cells. Also, plant extract inhibited the expression of glucose transporter and glycolytic enzymes, all of which lead to the reduction of glucose uptake and ATP production by pancreatic cancer cells **(Torres *et al*., 2012).** Acetogenins are potent inhibitors of NADH ubiquinone oxidoreductase, which is an essential enzyme in complex I of the electron transport system (ETS) which eventually leads to oxidative phosphorylation in mitochondria **(Kedari and Khan, 2014).** An antioxidant is a compound capable of inhibiting molecular oxidation and therefore of protecting biological molecules from reactive oxygen species or free radicals. Antioxidants can be synthesized by the body or obtained from a diet containing fruit, such as soursop **(Gordillo *et al*., 2012)**. *A. muricata* extract restores the activity of enzymes such as glutathione (GHS), catalase (CAT), nitric oxide (NO), superoxide dismutase (SOD), malondialdehyde (MDA the biomarker of lipid peroxidation that can cause defect in endothelial cells, fibroblast and collagen metabolism necessary for wound healing) and prostaglandin E2 (PGE-2) that reduces cellular ROS, also the extract protects the gastric tissue from hemorrhagic lesion associated with attenuation of leukocyte infiltration and submucosal edema **(Moghadamtousi *et al*., 2015).** A study by **Yang *et al.* (2015)** demonstrated that crude leaf extract of *A. muricata* showed in vitro inhibition of prostate cancer proliferation and more effect on tumor growth-inhibition than flavonoids-enriched extract and suggested that the effectivity of crude extract is probably due to a synergistic interaction between flavonoids and acetogenins. Additionally, *A. muricata* extract treatment reduced malondialdehyde (MDA) formation in colon tissue, confirming its protective effect against oxidative stress **(Torres *et al*., 2012)**.

Also, **Hamizah *et al.* (2012)** reported that the ethanolic extract of *A. muricata* leaves showed greater anti-tumor activity in murine models than curcumin, a known natural chemopreventive. Aqueous extract of commercial powder capsules containing leaf and stem of *A. muricata* also showed anti-tumorigenic and anti-metastatic activities on pancreatic tumors in murine models **(Torres *et al*., 2012).** Breast tumor in rats was reduced by treatment for 5 weeks with *A. muricata* fruit extract **(Dai *et al*., 2011).** The mechanism of action suggests the inhibition of multiple signaling pathways that regulated metabolism, metastasis, induction of necrosis and cell cycle arrest has been shown in cytotoxic mechanism **(Torres *et al*., 2012 and Dai *et al*., 2011).** Antitumor activity was also reported for two acetogenin isolates of *A. muricata* **(Ko *et al*., 2011 and Wang *et al*., 2002)**. The antioxidant properties of phenolic and flavonoid compounds are mediated by the following mechanisms: scavenging radical species such as ROS/reactive nitrogen species (RNS, suppressing ROS/RNS formation by inhibiting some enzymes or chelating trace metals involved in free radical production and up regulating or protecting antioxidant defense **(Cotelle, 2001)**. Furthermore, **Lukmanul *et al*. (2008)** reported that medicinal plants containing active chemical constituents with high antioxidant property play an important role in the prevention of various degenerative diseases. The above mentioning data collectively may be explain the absent of abnormal chromatin mitotic feature and most pathological lesions in liver tissue of TCA intoxicated mice treated with aqueous extract of graviola fruit pulp or aqueous DS of graviola capsules. In consistent with other previous studies this report suggests that the affectivity of aqueous extract of graviola fruit pulp or aqueous DS of graviola are probably due to a synergistic interaction between flavonoids and acetogenins and the antioxidant effect of *A. muricata* extract can play an important role in the hepatoprotection. Therefore these findings substantiated the traditional use of *A. muricata* against liver toxicity and showed the potential hepatoprotective activity

Conclusion:

The pharmacognostical parameters in the present study provide some pharmacological and therapeutical informations about extracts of the graviola fruit pulp and DS of graviola capsules which can use in future investigations and applications. The present study indicated some quality control parameters which assist in standardization for quality and purity of graviola DS which sold in the Libyan market as a cancer cure and demonstrated presence of important phyochemical constituents in the graviola fruit pulp extract and DS of graviola capsules which induced hepatoprotective against toxic agent. Also, the present results demonstrated that *A. muricata* play an important role in the protection against TCA induced hepatotoxicity which may be attributes to synergistic effect of its phytochemical contents. However further studies concerned with aqueous extract of graviola fruit pulp or DS of graviola capsules with different doses and durations are still required.

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