BIOCHEMICAL STUDIES OF POMEGRANATE BIOACTIVE COMPOUNDS AND THEIR ROLE IN OXIDATIVE STRESS REMEDY IN SOME BIOLOGICAL SYSTEMS

By

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Approval Sheet

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ABSTRACT

Mariam Thabet Sawy Ibrahim: Biochemical studies of pomegranate bioactive compounds and their role in oxidative stress remedy in some biological systems. Unpublished, Doctor of Philosophy Dissertation, university of Ain Shams, Faculty of Agriculture, Depratment of Agric. Biochemistry, 2015.

The target of this study is to compare the antioxidant activity of three species of pomegranate (early wonderful, wonderful and white sweet) in different parts of their fruits (husk, peel and juice) at different maturity stages. The antioxidant activities of ethanolic extracts were measured by two different assays (DPPH and FRAP). The husk extract of early wonderful in pre-matured stage was the higher in their antioxidant activity. Using HPLC; the major bioactive compounds were Syringic (phenolic compound) and Hisperdin (flavonoid).

The second target of this study was to evaluate the role of pomegranate extract which has higher antioxidant activity on liver damage induced by carbon tetrachloride (CCl_4) in rats. The results showed that the administration of CCl_4 increased serum alanine transaminase (ALT), aspartate transaminase (AST) and bilirubin, but decreased superoxide dismutase (SOD), induced lipid peroxidation and caused depletion of liver reduced glutathione (GSH).

DNA damage was determined by random amplified polymorphic DNA (RAPD) method, the data revealed that the molecular genetic variability among the treated rats genomes and their controls were evaluated using 3 random primers (C1, H5 and P13). Primers gave positive and detectable bands and high level of polymorphism was generated using these primers.

Histopathological examination of liver tissues of treated group with CCl_4 shows steatosis of hepatocytes (ballooning of hepatocytes), apoptosis and inflammatory cells infiltration. On the other hand, liver

histology of rat treated with pomegranate extract at three concentrations (0.5, 1.0 and 2.0) treated group shows no histopathological changes.

Generally, it could be concluded that the pomegranate husk extract is an effective antioxidant as hepatoprotective agent.

Keywords: Pomegranate husk extract; Antioxidant activity; Liver damage; Carbon tetrachloride

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Finally, words are not enough to express how I am grateful to my parents who pushed me to accomplish success in my life. The last but not the least, I would like to express my appreciation to my beloved husband for his unconditional continuous support to me**.**

CONTENTS

LIST OF TABLES

1- INTRODUCTION

The reactive oxygen species (ROS) are free radicals such as superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\cdot}OH$). The production of ROS is a normal physiological event in various organs, including liver and kidney tissues. However, the over production of ROS causes structural damage of biological macromolecules including nucleic acids, proteins and lipids that results in the formation of cytotoxic secondary products such as malondialdehyde (MDA) **(Cestmir and Jitka, 2015)**.

Endogenous protection against oxidative stress is achieved by enzymes that remove free radicals. This includes superoxide dismutase, catalase and glutathione peroxidase. Another possibility is the existence of low molecular weight agents that scavenge ROS like glutathione and α-tocopherol. There is an intimate relationship between nutrition and antioxidant defense system, as some exogenous low molecular weight antioxidant may be supplied by the diet. These two main systems of the antioxidant defense act in coordination, their levels being regulated by each other, to avoid oxidative stress events. Few years ago, a considerably large group of molecules widespread in plants has come into focus such as those present in pomegranate **(Ana *et al*., 2007)**.

Pomegranate is an important source of bioactive compounds and has been used for folk medicine for many centuries. Pomegranate juice has been demonstrated to be high in its antioxidant activity, but it was found that pomegranate peel had the highest antioxidant activity among the peel, pulp and seed **(Fatma, 2009)**. Pomegranate peel exerted diverse pharmacological functions as antioxidant activity such as hepatoprotective activity **(Murthy *et al.*, 2002)**. Pomegranate peel extract contains phenolic punicalagine, gallic acid, catechin, quercetin , rutin and other flavonols, flavones, flavonones and anthocyanidins **(Julie-Jurenka, 2008)**. These compounds are known for their properties in scavenging free radical and inhibiting lipid peroxidation *in vitro* **(Noda *et al.*, 2002)**

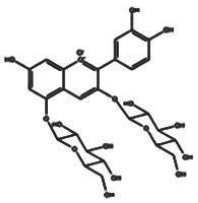
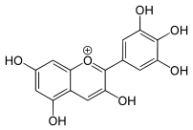
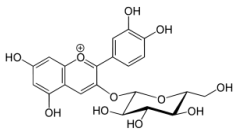
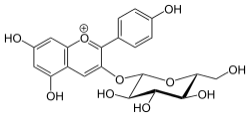
The aim of the present investigation is: studying the bioactive compounds of different pomegranate extracts; their free radical scavenging activity and their expected role to avoid oxidative stress, especially liver oxidative damage induced by CCl_4 in rats**.**

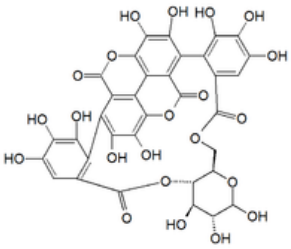
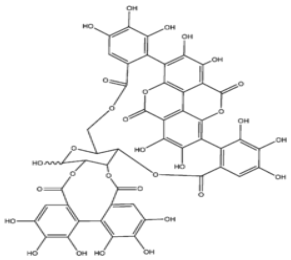
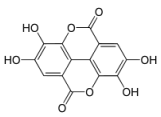
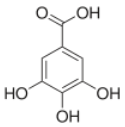
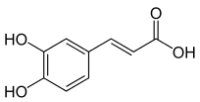
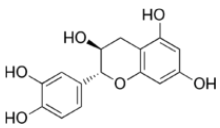
2- REVIEW OF LITERATURE

2.1. Chemical constituents of pomegranate antioxidants:

Monica *et al.*, (2013) declared that the high antioxidant capacity of pomegranate has been mostly attributed to its high levels of polyphenolic compounds, such as anthocyanin, tannins and phenols:

Table (1): chemical constituents and structures of the main antioxidants in pomegranate fruit

	Constituent	Chemical structure	Part of fruit
Anthocyanine	Cyanidin 3,5-diglucoside		Seed,peel,juice
	Delphinidin 3-glucoside		Seed,peel,juice
	Cyanidin 3-glucoside		Seed,peel,juice
	Pelargonidin 3-glucoside		Seed,peel,juice

tannins	Punicalin		Seed,peel,juice
	Punicalagin		Seed,peel,juice
	Ellagic acid		Seed,peel,juice
phenols	Gallic acid		juice
	Caffeic acid		juice
	catechin		Peel, juice

2.1.1. Pomegranate flavonoids:

Flavonoids isolated from pomegranate include flavones, flavonols, anthocyanidins and flavan-3-ols. The brilliant colors of pericarp and juice are attributed to anthocyanidins and flavan-3-ols, of which their content decrease or increase with the time of ripening.

Anthocyanidine reported in pomegranate usually present in the form of glycoside with aglycons of delphinidin, cynidin, epicatechin, epigallocatechin and their derivatives. Flavones and flavonols constitute the major flavonoids of pericarp and leaves, which frequently exist as glycosides with aglycons of luteolin, kaempferol, quercetin, epigenin and naringin **(Wang *et al.*, 2010)**.

Ali *et al.*, (2014) determined the total phenolic and flavonoid contents of different parts of pomegranate fruit (peel, flesh, seed,and whole fruit) using different solvent (methanol and ethyl acetate). The highest total phenolic and flavonoid contents was detected in peel methanol extract (103.2 mg/g dw and 132.4 mg/g dw) respectively. Using HPLC analysis the major phenolic compounds in pomegranate peel metanolic extract were investigated. Among the phenolic compounds, chlorogenic acid, rutin and coumaric acid are present predominantly in the peel extract.

2.1.2. Pomegranate Tannins and phenols:

Pomegranate contains different bioactive compounds. Hydrolysable tannins of diverse structures including ellagitannins and gallotannins constitute the most prevalent compounds presenting in various parts of pomegranate. Ellagitannins are mainly found in the pericarp, bark, seeds and flowers. For example, punicalin, punicalagin and ellagic acid are the major constituents of pericarp **(Seeram *et al.*, 2005 and Wang *et al.*, 2010).**