

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.508.091>

Changes in the Oxidative Stress Biomarkers in Rat Liver Tissue Exposed to Cadmium and Protect with *Hibiscus sabdariffa* L. (Ro`ssle) Flower Extract

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ABSTRACT

Keywords

Cdcl2,
*Hibiscus
sabdariffa* extract,
oxidative stress
biomarkers.

Article Info

Accepted:
18 July 2016
Available Online:
10 August 2016

Cadmium chloride {Cdcl2} is highly toxic chemical agent, it involves production of reactive oxygen species {Ros} and induce oxidative stress mainly attributed to the impairment of antioxidant defense mechanisms in the body. The study was designed to evaluate the possible role of antioxidant of *Hibiscus sabdariffa* L.flower extract {HSSE} in the protection against cdcl2 – induced oxidative stress in rat liver tissue, specially changes in malondialdehyde {MDA} level, and enzymatic and non-enzymatic antioxidant. The following three experimental group were evaluated {1} control, {2} Cdcl2, Cdcl2 + HSSE. Cdcl2 caused significant decrease in reduced Glutathione {GSH} content, catalase {CAT} and superoxide dismutase {SOD} activities, as well as significant increase in malondialdehyde {MDA} level, indicate that cdcl2 induced hepatotoxicity was mediated through oxidative stress. In contrast, HSE pretreatment significantly improved Cdcl2 – induced biochemical alterations. These result indicated that HSE has a protective action against Cdcl2 hepatotoxicity and suggest that HSE may find clinical application against a variety of toxins and drugs where cellular damage is a consequence of reactive oxygen species.

Introduction

There are more than 300 species of *Hibiscus* L. *Hibiscus sabdariffa* belong to the *malvaceae* family which is an annual herb growing in tropical and subtropical countries.

The calyx is the most important economic parts of the plants which is used in food and cosmetic industries as source of natural coloring agent (EL-meleigy, 1989). More over it has different ethnomedical properties and local soft drink. It was reported to be

anticancer, antiseptic, depesia and fever (Duke, 1985), anti-inflammatory (Daffallah *et al.*, 1996), antimutagenic (Farombi *et al.*, 2005), is used in folk medicine to treat Diabetes, ulcer and jaundice (Yesiada *et al.*, 1995).

Hypolipidemic effects have been reported (Hirunpanich *et al.*, 2006). Other studies properties of *H.sabdariffa* extract include cytotoxicity and genotoxicity (Rosa *et al.*, 2007) and possess significant immuno-protective potential (OKoko *et al.*, 2012).

Preventing atherosclerosis and cardiovascular disease associates with Diabetes has been found (Farombi *et al.*, 2007). Antimicrobial activity has also been documented (Al-hashimi, 2012; Jacob *et al.*, 2011).

Previous phytochemical investigations of different part of this plant show the presence of phenolic compounds, flavonoids, protocatechuic acid, ascorbic acid and anthocyanins (Oboh *et al.*, 2012) which found to possess wide pharmacological activities especially potent free-radical scavenging and antioxidant actions (Olusola *et al.*, 2012; Liu *et al.*, 2006, 2010). Furthermore liu *et al.*, (2010) have demonstrated that HSE extract and anthocyanins were proved to be an effective hepatoprotective agent against carbon tetrachloride (Ccl4) – induced liver injury. concerning the inhibitory effect of *H.sabdariffa* flower extract, Shawagfeh and Al-Kubaisy reported that when HSE was given prior to induction of hepatotoxicity by >Cdcl2 it was able to give protection against its harmful effects. Also it was found that HSE and anthocyanin exhibited significant protection against acetaminophen-induced liver damage. Kowalcyzyk *et al.*, (2003) revealed that anthocyanins increase the resistance of hepatocytes to oxidation. Meanwhile, Delong *et al.*, (2003) concluded that phytosteroids found in HSE have immune modulating function and aid in general health during cancer therapy. Moreover, Adeno *et al.*, (2013) have indicated that the calyx extract of *H.sabdariffa* showed a beneficial effect against cis.platin – induces oxidative kidney damage.

Cadmium is one of the most dangerous occupational and environmental toxins. It is found in drinking water, atmospheric air and even in food. Cadmium is reported to be

very toxic to biological systems. The liver, kidney, heart and testes are the most important target organs when considering Cd – induced toxicity because this heavy metal accumulates in these organs, for this reason many researchers are carried out to find natural compounds that help in the protection against Cd – induced toxicity with fewer or no side effects. Therefore the objective of the present study was to determine the protective actions of *Hibiscus sabdariffa.L* dried flower extract (HSE) against Cdcl2 – induced oxidative stress in rat liver tissue.

Materials and Methods

Preparation of aqueous flower extracts

Dried flowers of *H.sabdriffa L.*(Rossle) was obtained from herbal store -Amman- Jordan. It was identified at the biological sciences department at the university of Jordan and confirmed by comparing with those of known in the herbarium of the department. The dried flowers were ground into fine powder using electric dry mill.

A total of 100 gm of the ground was soaked in 500 ml of distilled water for 24 hour at 40 c temperature. The mixture was filtered with whatman filter paper No.1. The filtrate was dried at 40 c temperature. The yield of HSE contain the following component; four major flavonoids phenolic acid (e.g. protocatechuic acid) and rich vitamin C content (Jarup *et al.*, 1998).

Appropriate concentration of the extract was then subsequently made by dilution with distilled water into 250 mg/kg body weight and administered to the animals. The 250 mg/kg extract was more effective than 500 mg/kg in hepatic function which was well comparable with standard drug silymarin (20 mg/kg) (Lin *et al.*, 2011).

Experimental Design

A total of 18 healthy adult male albino rats weighing between (160-180 g) obtained from animal house – university of Applied sciences – Amman were used. The animals were maintained under standard conditions of humidity 50%, temperature (25±1c) with a 12h light / dark cycle. All rats were allowed free access to food and water ad libitum. The rats were divided randomly into three groups of six rats each as follows:

Group I: receive as oil treated vehicle with saline control.

Group II: animal were administered CdCl₂; 4 mg/kg body weight in normal saline subcutaneously to induce liver injury.

Group III: Rats were treated with 250 mg/kg body weight of (HSE) for one week and subsequently exposed to a single injection of CdCl₂ : 12 hour after the last (HSE) / vehicle treatment.

All experimental animals were handled according to the guidelines of the institutions animals ethical committee.

All chemicals used were of analytical grade, purchased locally.

Sample Collection

The rats were sacrificed under ether anaesthesia and their livers were excised washed with ice-cold saline and blotted to dryness. Sample of liver tissue were homogenized with ice-cold (0.25 M sucrose).

Assessment of cellular macromolecule damage

The level of lipid peroxidation (LPO) served as an index of the intensity of oxidative stress.

As malondialdehyde by (MDA) was determined in the liver tissue as described by (23) after incubation at 95 °C with thiobarbituric acid to generate pink color produced, which has absorption maximum at 532 nm.

Estimation of oxidative stress

Estimation of catalase (CAT) activity in the liver homogenates were estimated by the method of (24) using a commercially available Kits obtained from calbiochem (USA) according to manufacturer's instruction.

Estimation of superoxide dismutase activity (SOD) was determined by the method of (25) using kits obtained from (calbiochem USA) according to manufacturer's instruction.

Estimation of total reduced glutathione (GSH) was determined in the liver homogenates by method of Ellman based on the development of yellow color when 5,5 dithio – bis 2-nitrobenzoic acid (DTNB) is added to the supernatant and was measured spectrophotometrically at 412 nm against reagent blank with no homogenate.

Statistical analysis

The values were expressed as mean ± standard error of the mean for six animals in each group. Differences between group were assessed by the one way analysis of variance (ANOVA) using the SPSS version 10 software, and p-value (<0.05) was regarded to be significant.

Results and Discussion

After experiment period of administration of single dose of CdCl₂, the lipid peroxidation (LPO) level in the liver homogenate of control and experimental rats is presented in Table (1). The effect of intoxication with

CdCl₂ in the absence and presence of HSE extract supplementation on oxidative stress parameters, the data show in table (1) a significant elevation in the tissue lipid peroxidation biomarker (MDA) in CdCl₂ intoxicated rats (group 2) versus normal control at (p<0.05). On other-hand, significant decrease total reduce glutathione content in liver homogenate (p<0.05) was recorded at period of treatment with CdCl₂. However the pretreatment with HSE (group 3) reversed the level of (MDA) to be near the normal control level and also resulted in a significant elevation in the content of (GSH) at (p<0.05) when compared to CdCl₂ control (group2) rat. Table (1) also present the status of antioxidant defense system. Administration of toxic dose of CdCl₂ produced marked depletion of the liver antioxidant enzyme, catalase and superoxide dismutase (group 2) animals. This depletion of antioxidant enzyme activities (CAT and SOD) was markedly prevented by administration of HSE in the CdCl₂ treated rats. The results of present study confirm that the inhibition of oxidants production and restoration of enzymatic and non-enzymatic antioxidant status within control protected against these changes in dose dependent manner, indicates its potent antioxidant effect against oxidative stress induced by CdCl₂.

Many toxicants, including chemical and drugs induced liver injuries. Oxidative stress is typically result in an imbalance of oxidative homeostasis with excess production of reactive oxygen species (ROS) and relative deficiency of endogenous antioxidants. ROS induce lipid peroxidation. The lipid peroxidation is believed to play an important role in the pathogenesis of several disease such as cancer, diabetic, inflammation and liver injury. Many natural antioxidant play significant roles in the prevention and treatment of organic disease associated with

oxidative stress.

Enormous reports suggest that oxidative stress, depletion of hepatic antioxidant system and increase in lipid peroxidation are the possible mechanisms of CdCl₂ (Beutler *et al.*, 1963).

Values are given as mean ± SEM for group of six animals each values are statically significant at (p<0.05). treat rats were compared with control. *H.sabdariffa* flower extract + CdCl₂ treated rats were compared with CdCl₂ treated rats. Cadmium, one of the most common toxic heavy metals can bind to metallothionein, but free Cd which has not combined with metallothionein, changes the enzyme activity and membrane structure by reacting with sulfhydryl group of the membrane, resulting in liver injury (Renugadevi *et al.*, 2010; Foulkes, 1982) exposure to CdCl₂ lead to a decrease in the activity of antioxidant enzymes, such as catalase and superoxide dismutase (Klassen *et al.*, 1999). Cd also found depletes glutathione and sulfhydryl protein leading to increased lipid peroxidation and enhanced intracellular oxidized states.

In the present study, the rats treated with CdCl₂ showed a significant hepatic damage, as malondialdehyde (MDA), the biomarker of lipid peroxidation, generation of free radicals probably because of the alteration in normal homeostasis of the body resulting in oxidative stress. Also show depletion in the reduced glutathione (GSH) content in liver tissue, GSH, is an early consequence of oxidative stress. Acute CdCl₂ damage could significantly decrease the expression of antioxidant enzymes in the liver cells such as catalase and superoxide dismutase (SOD). GSH-related enzymes play in detoxifying and antioxidant role in metabolizing xenobiotics through conjugation with glutathione or reduction of free radicals.

Table.1 effect of cdcl2 and flower extract of *H.sabdariffa* on lipid peroxidation (MDA) level reduced glutathione (GSH) content and antioxidant enzyme activities of hepatic cell in rats groups.

Parameters	Control	Cadmium (cdcl2)	Cdcl2+extract
MDA nmol/g wet tissue	139.87 ± 1.28	181.20 ± 1.17 *	143 ± 3.10 *
GSH nmol/g wet tissue	1.52 ± 0.17	1.02 ± 0.07 *	1.38 ± 0.13 *
CAT u/ml	118.41 ± 2.12	69.71 ± 1.33 *	109.42 ± 0.18 *
SOD u/ml	26.75 ± 1.14	14.88 ± 1.28 *	22.37 ± 0.66 *

Many plants derived natural products have the potential to be hepatoprotective against various toxic chemicals and drugs, therefore they can be used to treat acute and chronic liver disease. The challenge is to identify the most promising compounds and evaluate their protective mechanism. The phytochemical analysis of the extract from flower of *H. Sabdariffa* showed the presence of phenolic compounds, four major flavonols, protocatechuic acid, anthocyanin and vitamin C. Since the antioxidant and hepatoprotective activities of certain flavonols from plant origin have already been established (Koyu *et al.*, 2006). The relatively high polyphenol content of 16 mg/ml observed for the crude extract of *H. Sabdariffa* flowers is indicative of potential of antioxidative properties and the DPPH radical scavenging activity of HSE was more than vitamin C (Olusola *et al.*, 2012). Moreover, AL-Hashimi (2000) reported that the crude extract of HSE has total phenolic content (77-87) mg/g.

HSE co-supplementation in our study significantly restored the activities of hepatic oxidative stress markers to considerable extent (table 1). this protective action might possibly be due to its effect on preserving the cellular membrane of hepatocytes from breakage by reactive metabolites, thereby restoring the status of these markers, it has been found that HSE to have excellent scavenging ability on free radicals which was well comparable with standard drug silymarin (20mg/kg).

Shwaghfah and AL-Kubaisy have revealed that the aqueous extract of HSE showed a potent beneficial effect against Cdcl₂ induced liver damage by protecting the liver biochemical markers as well as emphasizing its antioxidant potential.

Further, dried flower extract of HS was reported to be highly effective in scavenging free radicals and thereby offering hepatoprotection against Ccl₄,paracetamol and phenobarbitone – induced hepatotoxicity in rats (AL – Hashimi, 2012) respectively.

From the data observed in the present study thus strongly confirms that the dried flower extract of HS might have scavenged detoxified the free radicals and improve the activities of hepatic oxidative stress and antioxidant status. Therefore, HSE could be useful as a hepatoprotective agent against chemicals and drugs-induced oxidative damage of liver in vivo.

Acknowledgment

Author is thankful to dean of college for infrastructure and all other essential facilities for the successful completion of this work.

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How to cite this article:

Khaled N. Al-kubaisy, Rania M. Al-Groom and Adeeb Al- Amoush. 2016. Changes in the Oxidative Stress Biomarkers in Rat Liver Tissue Exposed to Cadmium and Protect with *Hibiscus sabdariffa* L. (Ro`ssle) Flower Extract. *Int.J.Curr.Microbiol.App.Sci* 5(8): 818-824. doi: <http://dx.doi.org/10.20546/ijcmas.2016.508.091>