THE POTENTIAL PROTECTIVE ROLE OF CHAMOMILE EXTRACT ON RAT LIVER ULTRASTRUCTURAL CHANGES INDUCED BY 2, 4-DICHLOROPHENOXYACETIC ACID

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ABSTRACT

The current study aimed to demonstrate the hepatoprotective effect of chamomile extract and its role in relieving the ultrathin structure changes in liver tissue caused by 2, 4-Dichlorophenoxyacetic acid (2, 4-D) using electron microscopy. This experiment was performed on 12 -14 weeks old male Wistar rats divided into six groups (six animals each). The first group was kept as control. The second and third groups received orally accumulative doses of 75 and 150 mg/kg body weight (b.wt.), of 2, 4-D respectively. The forth group received orally Chamomile extract (500 mg/kg b.wt.) alone. The last two groups received Chamomile extract with either doses of 2, 4-D (75 or 150 mg/kg b.wt.). At the end of the experimental period (4 weeks), the liver was dissected and examined by electron microscope. Histopathological examination of liver sections of rats administered 2, 4-D showed differences in nuclear shapes and size, envelope and increase in heterochromatin masses. Administration of 2, 4-D showed pyknosis and changes in mitochondria, endoplasmic reticulum, Kupffer cells, increases in lysosomes and lipid droplets. Chamomile group showed the normal control ultra structure of the liver. In group treated with chamomile and 2, 4-D, there was improvements in all degenerative changes induced by 2, 4-D. Chamomile reduces the oxidative damage induced by 2, 4-D due to its antioxidant properties. It is recommended that Chamomile extract can be taken to ameliorate hepatotoxicity.

Keywords: Chamomile, 2, 4-Dichlorophenoxyacetic acid, Ultrastructural changes, Hepatoprotective, Liver, Oxidative stress, Rat.
Contribution/ Originality

This study is one of very few studies which have demonstrated chamomile role in relieving hepatocytes ultrastructural changes caused by 2, 4-Dichlorophenoxyacetic acid. The paper's primary contribution is finding that chamomile has antioxidant effect against oxidative stress. This study documents the hepatoprotective effect of chamomile against 2,4-D toxicity.

1. INTRODUCTION

It is known that manmade chemicals such as pesticides have a significant role in minimizing the damage caused by various pests. On the other hand, their toxicity may cause many harmful effects into the environment. The 2, 4-Dichlorophenoxyacetic acid (2, 4-D) is a Selective herbicide that is used in many countries. It is mainly used in agriculture on forests, crops and recreational areas. The 2, 4-D induced toxicity to animals or humans usually occur by exposure to contaminated air, water, soil, or cereal crops [1].

The herbicide 2, 4-D has many cytotoxic effects on various organs. Teratogenic, genotoxic, neurotoxic, immunosuppressive and hepatotoxic effects of 2, 4-D have been evaluated in many in vivo and in vitro studies [2]. The 2, 4-D can bind to hepatic proteins in rat and chick irreversibly. The main metabolite of 2, 4-D is 2, 4-dichlorophenoxyacetol-S-acetyl-CoA (2,4-D-CoA) that may contribute to the formation of 2,4-D-protein complex in vivo and result hepatotoxicity [2].

Many plants have been investigated for their antitoxic effects. Chamomile recutita, (family Asteraceae) popularly known as Chamomile, can be considered as one of these plants. It is native to Europe and northern and western Asia [3]. Many active compounds are present in chamomile such as essential oils and several phenolic compounds, mainly the flavonoids which act as antioxidants in addition to various acetylated derivatives [4]. Chamomile tea is one of the medicinal plants used as a traditional medicine long time ago. It has been used to treat skin diseases, rheumatic pain and hemorrhoids [5]. Moreover, it shows various pharmacological effects such as antioxidant, anti-inflammatory, anti-cancer as well as for stress and depression treatment [6]. Food and drug administration (FDA) considered chamomile to be included in the “generally regarded as safe” (GRAS) list [7]. Since the liver is the most important vital organ that is responsible for detoxification, the examination of ultrathin sections of liver of the control and treated rats with electron microscopy helped us to determine ultrastructural organelles and showed the organelle changes which cannot be observed by light microscopy.

2. MATERIALS AND METHODS

This experiment was held at King Fahd Medical Research Center, King Abdul-Aziz University, KSA on 12-14 weeks old male albino Wistar rats. Animals were divided into six groups (six animals each). The first group was kept as a control in which rats were fed on the basal diet and water was provided ad libitum. Rats in the second and third groups were given orally accumulative doses of 75 and 150 mg/kg body weight (b.wt.), of 2, 4-D respectively, to
induce hepatotoxicity according to Tayeb, et al. [8]. In the forth group rats received oral gavage of Chamomile extract (500 mg/kg b.wt.) alone [9] the extract was prepared according to Srivastava and Gupta [5]. In the last two groups rats received Chamomile extract with either doses of 2, 4-D (75 or 150 mg/kg b.wt). The experimental period lasted for 4 weeks. At the end of the experimental period, the rats were fasted over night. On the morning of the next day the rats were anesthetized by general volatile anesthesia using ether. After decapitation of the rats, the liver was removed by careful dissection and blotted free of adhering blood immediately after sacrificing the rats. The dissected liver was fixed with 5% Glutaraldehyde in 0.2M Phosphate buffer for 5–6 h at 48°C. The fixed liver was cut into approximately 1mm thickness cubes. The cubes were post fixed in 2% Osmium tetra oxide (OsO₄) solution for 2h at 48°C and then dehydrated in an ethanol series. The pieces were embedded in tab, cut into 0.5mm thickness sections using an ultra microtome (LKB, Sweden) and mounted on Nickel grids (300 mm). The sections were double stained with uranyl acetate (EM SCOPE, England) and lead citrate (Sigma, USA) then examined by transmission electron microscope (TEM) (Philips CM100, Netherlands) and photographed [10].

3. RESULTS

The electron microscopic examination of liver sections of the control group showed that most dark and pale hepatocytes were large polygonal with one or two central euchromatic nuclei and fewer nuclear heterochromatic contents with apparent more electron dense nucleoli and their associated heterochromatin in deep or pale staining cytoplasm (Fig.1a). This was occupied by well-developed ovoid mitochondria surrounded by double membrane in which the inner one extended into mitochondrial matrix to form the cristae, few polysomes, few glycogen granules, rough endoplasmic reticulum (RER), primary and secondary lysosomes. The primary lysosomes appeared with dense content and the secondary one showed only dense concentric area as shown in (Fig.1b). The dark cells’ cytoplasm contained numerous dense mitochondria, more rough endoplasmic reticulum and small nucleus enclosed by profilated nuclear envelope. The oval nucleus with high nucleus cytoplasmic ratio surrounded by contact nuclear envelope and had two types of chromatin: electron–dense heterochromatin around the nuclear envelope forming irregular clumps and nucleolus, the other electron-lucent euchromatin spreaded in the nucleoplasm (Fig.1b). In addition, hepatocytes large phagocytic Kupffer cell which had a triangular nucleus occupied the blood sinusoid.
**Fig-1.** Photograph of ultrathin section of control rat liver: (a) control negative liver, showing polygonal hepatocytes (H) with large central nucleus (N) has smooth regular outline and small amount of marginal heterochromatin. Numerous round or oval shaped mitochondria (M) uniformly distributed in the cytoplasm and rough endoplasmic reticulum (arrows) (Uranyl acetate and lead citrate, scale bar =5µm). (b) Enlarged part of the pervious photo showing: part of hepatocytes with high nucleus (N) cytoplasmic ratio and peripheral nucleolus (Nu). Cytoplasm filled with oval shaped mitochondria (M), parallel stacks of rough endoplasmic reticulum (RER) and small primary lysosomes (Ly) (Uranyl acetate and lead citrate, scale bar =2 µm). Electron Microscope Unit, King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah –RSA.

The ultrastructural lesions in hepatotoxic rats were more obvious and diverse with increase 2, 4-D dosages. The given of 2, 4-D_75_ mg/kg b.wt., induced nuclear changes in liver cells which included differences in shapes and size, irregularity and slight distention of nuclear envelope, increase in heterochromatin masses attached to inner nuclear envelop and nucleolar margination (Fig.2a). When increasing the dosage of 2, 4-D_{150} mg/kg b.wt., the hepatic nuclei showed variable degree of pyknosis, disaggregation and fragmentation of RER cisternae in hepatocytes appears to be a common response of the rat liver cells to treatment (Fig.2c). The most well known classical apoptosis is characterized by early nuclear collapse and massive condensation of chromatin with polymorph mitochondria which have dense matrices.

Concerning the mitochondria, which showed the most dramatic changes, there was considerable variations in mitochondrial population (normally abundant and closely packed or fewer); in size (increased and swollen); in shape (curved and round) and internal structure (disorganization of cristae, matrix less electron dense, vacoulations of matrix). In all treated hepatocytes, there was an increase in lysosomes and lipid droplets in comparison with the control group. Kupffer cells appeared hypertrophied in blood sinusoid in liver parenchyma (Figs.2b,d).
Fig-2. Photograph of ultrathin section of intoxicated rat liver: (a) intoxicated liver (2, 4-D75 mg/kg b.wt.) showing: part of hepatic strand with dark (DH) and pale hepatocytes (PH) have irregular nuclei (N), damaged mitochondria (M) and congested blood sinusoid (S) with vacuolated Kupffer cell (KC) (Uranyl acetate and lead citrate, scale bar =5 µm). (b) Enlarged part of the pervious photo showing: dark hepatocytes (DH) with irregular distinct nuclear envelope, heterochromatin masses (short arrows), wide nuclear pores (long arrows), lipid droplets (L), swollen mitochondria with moderate cristolysis (M), primary and secondary lysosomes (Ly1 and Ly2) and fragmented rough endoplasmic reticulum (RER) (Uranyl acetate and lead citrate, scale bar =2 µm). (c) intoxicated liver (2, 4-D150 mg/kg b.wt.) showing: dark hepatocytes (DH) has severely lobulated electron dense nucleus with low nucleus cytoplasmic ratio and damaged mitochondria (M) with low electron dense matrix and congested blood sinusoid (S) with hypertrophied Kupffer cells and many lysosomes (arrows) (Uranyl acetate and lead citrate, scale bar =5 µm). (d) Enlarged part of the pervious photo showing: part from dark hepatocytes with apoptotic nucleus (N), lipid droplets (L), swollen mitochondria with advanced cristolysis (M), lysosomes (Ly), fragmented rough endoplasmic reticulum (RER) and accumulation of glycogen (g) (Uranyl acetate and lead citrate, scale bar =2 µm). Electron Microscope Unit, King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah –RSA.

Electron microscopic study of Chamomile 500 mg/kg b.wt., group showed the normal control ultra structure of the liver. The hepatocytes were adjacent to each other (Fig.3a). The hepatocyte appeared with euchromatic nuclei containing prominent nucleoli have smooth regular outline and small amount of marginal heterochromatin. The cytoplasm contained numerous mitochondria, lysosomes and abundant stacked cisternae of RER as illustrated in (Fig. 3b).
Fig-3. Photograph of ultrathin section of liver treated with Chamomile only (C500 mg/kg b.wt.) showing: (a) normal polygonal hepatocytes (H) with large central nuclei (N) in cytoplasm deep staining and normal spheroid mitochondria (M) uniformly distributed in the cytoplasm and numerous small lysosomes (arrows) (Uranyl acetate and lead citrate, scale bar = 5 µm). (b) Enlarged part of the previous photo showing: part from dark hepatocytes (DH) with nucleus (N) and clear nucleoli (Nu) have smooth regular outline and small amount of marginal heterochromatin, mitochondria (M), lysosomes (Ly) and abundant and in stacked cisternae rough endoplasmic reticulum (RER) (Uranyl acetate and lead citrate, scale bar = 2 µm). Electron Microscope Unit, King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah – KSA.

The improvement in group treated with chamomile extract and 2,4-D75 was well demonstrated where the electron microscopic picture exhibited hepatocytes with intact cell membrane, many normal intact mitochondria, euchromatic nuclei with prominent nucleoli, numerous small lysosomes, few lipid droplets (Fig.4a) normal granular endoplasmic reticulum profiles and glycogen deposits (Fig.4b). This improved picture of the hepatic tissue could be due to the amelioration effect of Chamomile extract and low dose of 2, 4-D at short periods.

The electron microscopic study of the group treated with chamomile extract and 2, 4-D150 showed partial improvements in the hepatocytes in both nucleus and mitochondria. Alterations in liver structure were noticed with a decrease in the number and volume of intracellular lipid droplets. Some hepatocytes showed few lipid droplets while others had many large lipid droplets (Fig.4c). The glycogen appeared as few scattered particles and the RER appeared as parallel arrays closely adherent to (and often encircling) the mitochondria. The nuclei appeared in irregular shape (Fig.4d) and few necrotic cytoplasmic areas. These incomplete improvements of the hepatic tissue are suggested to be due to the little amelioration effect of Chamomile extract with the high dose of 2, 4-D at the short period.
Fig-4. Photograph of ultrathin section of liver treated with Chamomile and 2,4-D (a) treated liver (2,4-D; 75 mg/kg b.wt.) + (Chamomile C500 mg/kg b.wt.) showing: adjacent hepatocytes (H) with noticeable improvement in both nucleus (N) and mitochondria (M) with numerous small lysosomes (arrows) and few lipid droplets (L) (Uranyl acetate and lead citrate, scale bar = 5 µm). (b) Enlarged part of the pervious photo showing: part of hepatocytes (H) has nearly normal nucleus (N) with little increased amount of heterochromatin, mitochondria (M), lysosomes (Ly), uniformed rough endoplasmic reticulum (RER) and moderate amount of glycogen (g) (Uranyl acetate and lead citrate, scale bar = 2 µm). (c) treated liver (2,4-D100 mg/kg b.wt.) + Chamomile C500 mg/kg b.wt. showing: hepatocytes (H) with less improvement in irregular nucleus (N), perforated dense mitochondria (M) and numerous lipid droplets (L) (Uranyl acetate and lead citrate, scale bar = 5 µm). (d) Enlarged part of the pervious photo showing: part of hepatocytes (H) characteristic by numerous dense cellular organelles specially mitochondria (M) with large number, short profiles of rough endoplasmic reticulum (RER), nucleus (N) surrounded by irregular nuclear envelop with nuclear pores (arrows) and few lipid droplet (L) (Uranyl acetate and lead citrate, scale bar = 2 µm).

4. DISCUSSION

The ultra findings demonstrated that cytological effects were found in all treated groups and their severity was dose dependent. Four weeks 2,4-D treatment, resulted in apoptosis of some hepatocytes with shrinkage of the nuclei and condensation of the heterochromatin. Some apoptotic cells showed swelling of mitochondria and vacuolations compared to control negative group. The histopathological findings of this study were coincided with other studies [11, 12] which showed that the liver sections of Gibberellic acid-intoxicatated rats revealed that hepatocytes were swollen and appeared with severe cytoplasmic vacuolization with degeneration of their nuclei.

The herbicide 2,4-D may be accompanied by an increase in the cytosolic Ca²⁺, due to oxidative stress and by the breakdown of phospholipid [13] chief to this hepatic disturbances. A previous study disclosed that liver mitochondrial dysfunction contributed to apoptosis via the
production of reactive oxygen species [14]. For interpretation of the mechanism of mitochondrial swelling reported in this study, oxidative stress had contributed to the opening of the mitochondrial permeability transition pore (PTP) which led to the formation of a high-conductance channel, in the inner mitochondrial membrane and led to mitochondrial swelling and subsequent release of cytochrome C from the intermembrane space [15]. The PTP opening appears to be associated with apoptosis or necrosis according to the presence or deficiency of adenosine triphosphate (ATP) [16].

The histopathological changes seen in the present study as regard to swelling of mitochondria were in accordance with the results of some studies which reported that inhibition of mitochondrial function together with accumulation of reactive oxygen species and lipid peroxidation, all these factors led to cell death (apoptosis) [17, 18].

After feeding with Chamomile extract noticeable or slight ameliorations of the unfavorable effects produced in the liver by 2, 4-D intoxication, which was associated with normal intact hepatocytes nucleus and nucleolus, many normal intact mitochondria, few necrotic cytoplasmic area, euchromatic nuclei and few lipid droplets. Chamomile may probably arrest the harmful effect on liver cells through protection of cells and tissues from oxidative damage by scavenging oxygen-free radicals and stimulate the regeneration of damaged tissues and cells as did green tea [19].

The appearance of hepatocytes treated with Chamomile was clarified by electron microscopic picture which showed partial improvement in the form of reappearance of cell organelles (mitochondria and cisternae of RER). The nuclei appeared euchromatic with prominent nucleoli. These results were coincided with a study [20] stated that following Gibberellic acid withdrawal, a few cells became nearly similar to those of the control group, while the majority of cells remained affected. Also, the improvement of liver cells could be considered as adaptive mechanism which occurred as a result of increased synthesis of organelles inside the cells such as mitochondria and RER probably to increase function of individual hepatocytes [12].

The increase in number of lipid droplets inside hepatocytes after 2, 4-D administration was comparable to fatty change developed in liver cells following either alcohol consumption [21] or taking drug [22] or treatment with styrene [23].

The observation of the current study demonstrated that 2, 4-D induces production of free radical that causes oxidative stress to the liver cells and its organelles particularly mitochondria and nucleus membranes. Chamomile reduces this oxidative damage by its antioxidant properties and ameliorates against the herbicide-induced hepatotoxicity.

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REFERENCES


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