

## Hepatoprotective Effect of Chamomile Capitula Extract against 2, 4-Dichlorophenoxyacetic Acid- Induced Hepatotoxicity in Rats

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**Abstract:** **Objective:** The present study was carried out to investigate the effect of oral administration of aqueous extract of Chamomile capitula for 4 weeks on hepatotoxicity induced to rats by herbicide 2, 4-Dichlorophenoxy acetic acid (2, 4 D). These effects could be explored by measuring body weight gain, feed efficiency ratio and relative weight of the liver. Serum levels of liver enzymes; aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP); albumin, total protein, total bilirubin, lactate dehydrogenase (LDH) enzyme and antioxidant enzymes; glutathione reductase (GR) and superoxide dismutase (SOD) were determined. **Methods:** Thirty six male Wistar rats were allocated into six groups as follows: - Group1: negative (normal rats) control; Group 2: positive (hepatotoxic) control given dose of 75 mg/kg b.wt., of 2, 4-D for 4 weeks; Group 3: Positive (hepatotoxic) control given dose of 150 mg/kg b.wt., of 2, 4-D for 4 weeks; Groups 4: given orally Chamomile capitula extract in a dose of 500 mg/kg b.wt., alone. Groups 5 and 6: given combination of Chamomile capitula extract with either the accumulative doses of the 75 mg/kg b.wt, or the 150 mg/kg b.wt., of 2, 4-D for 4 weeks. **Results:** The results showed that oral administration of Chamomile capitula extract to hepatotoxic rats for 28 days significantly decreased the elevated serum levels of liver enzymes (AST, ALT and ALP), total bilirubin and lactate dehydrogenase enzyme and positive groups. Levels of antioxidant enzymes glutathione reductase and superoxide dismutase were significantly increased as compared to the control positive groups. **Conclusion:** The results suggest that Chamomile capitula aqueous extract induces potent hepatoprotective and antioxidant effects in 2, 4 D - hepatotoxic rats. This study recommends that intake of Chamomile capitula extract as a herbal tea may be beneficial for patients who suffer from liver diseases and oxidative stress antioxidant enzymes.

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### 1. Introduction

Since the early 1990s, it has been argued that man-made chemicals used for agricultural, industrial or domestic purposes which can be released in the environment, enter the food chain and produce a number of disorders in animals and possibly in man (**Raseir et al., 2006**).

The 2, 4-Dichlorophenoxyacetic acid (2, 4-D) is a herbicide widely used to control the growth of broadleaf plants (**Tuschl and Schwab, 2003**). It is chemically derived from phenoxyacetic acid (**Tayeb et al., 2010**). The major uses of 2, 4-D are on cereal crops such as wheat, corn, oats, rye, barley and the cane crops (**U.S. Environmental Protection Agency, 2002**).

The Toxicity of 2, 4-Dichlorophenoxyacetic acid has been shown to produce oxidative stress and/or depleted antioxidant enzymes both *in vitro* and *in vivo* (**Tayeb et al., 2010**). Chronic toxicity of 2, 4-D in rats was manifested by decreased body weight gain, altered organ weights and hematological

parameters and other biochemical changes as explored by **Timchalk (2004)**.

The use of traditional medicine is widespread and plants still represent a large source of natural antioxidants that might serve as leads for the development of novel drugs (**Conforti et al., 2009**). The herbal based preparations were effective for the treatment of liver disorders. Therefore, several herbal medicines are experimented for their possible antioxidant and hepatoprotective effects against various chemical induced liver damages in animals. Chamomile is one of these medicinal herbs (**Merlin and Parthasarathy, 2011**).

Chamomile contains a large number of therapeutically interesting and active compound classes. The most important ones are the components of the essential oil and the flavonoids fraction. The Chamomile is used as a drug and is included in the pharmacopoeia of 26 countries. It is used mainly as an anti-inflammatory, antiseptic and antispasmodic (**Singh et al., 2011**). Traditionally, the essential oil

obtained from Chamomile flowers has been used to treat inflammations of the skin and mucosa. It is also inhaled to treat nasal catarrh, inflammation and irritation of the respiratory tract. The tea is drunk to treat flatulent nervous dyspepsia, gastritis, diarrhea, travel sickness and mild anxiety (Wang *et al.*, 2005).

There are few scientific investigations have so far been reported in literature regarding action of Chamomile on the liver. Therefore, the present study was designed to investigate hepatoprotective effect of Chamomile aqueous extract, on some serum biochemical parameters against 2, 4-Dichlorophenoxyacetic acid (2, 4-D)-induced hepatotoxicity in male rats.

## 2. Materials and Methods

### Materials

#### Chamomile capitula:

*Matricaria chamomilla* or *Matricaria recutita*, Family Asteraceae flowers were purchased from a local market, Jeddah, Kingdom of Saudi Arabia.

#### Herbicide:

The herbicide 2, 4-Dichlorophenoxyacetic acid was purchased under the brand name (2, 4-Kill<sup>®</sup>) from the local market, Jeddah, Kingdom of Saudi Arabia.

#### Animals:

Thirty six male albino rats of Wister strain weighing 230-250 grams body weight and 12 -14 weeks old were obtained from King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia.

#### Feeding of Rats:

All rats were fed on commercial rat pellets obtained from Grain Silos and Flour Mills Organization, Jeddah, KSA. These pellets are consisted of 20% crude protein, 4% fat, 3.5% crude fibers, 6% ash, 1% calcium, 0.6% phosphorus, 0.5% other salts, 20 IU/g vitamin A, 2.2 IU/g vitamin D and 70 IU/g vitamin E. These constituents were thoroughly mixed and commercially manufactured in the form of pellets.

#### Methods

##### Preparation of Chamomile capitula Extract:

Dry Chamomile flowers were weighed and grinded into a fine powder using a marble porcelain mortar and pestle. A 5% suspension (w/v) was prepared in a flask by adding boiled water. The flask was then placed on an electric shaker (200 rpm) for 4 hrs., and the temperature was maintained at 37 °C. After being shaken, the flask was brought to room temperature and the mixture was filtered through a series of Whatman filter paper to obtain an aqueous infusion. The filtered aqueous extract was freeze-dried (Freeze Dryer Alpha 1-2 LD plus, Christ,

Germany) and stored at -20 °C until used (Srivastava and Gupta, 2007).

The administrated dose of the Chamomile capitula extract was 500 mg / kg body weight daily for 4 weeks according to Kato *et al.* (2008).

#### Determination of Plant Bioactive Constituents:

Plant bioactive compounds of Chamomile capitula water extract were determined using High Performance Liquid Chromatography (HPLC, Shimadzu Corporation, Kyoto, Japan).

#### Induction of Hepatotoxicity:

Experimental hepatotoxicity was induced by administration via oral gavage of an accumulative dose of the herbicide 2, 4- Dichlorophenoxyacetic acid (one of phenoxyacetic acid herbicides) at two dosage levels 75 and 150 mg/kg body weight daily for 4 weeks according to Tayeb *et al.* (2010).

#### Experimental Design and Grouping of Rats:

The experiment was performed on thirty six male Wistar rats. The rats were randomly distributed into six equal groups of six rats each as follows: Group 1 (Control): Rats were fed on the basal diet and water was provided *ad libitum*, and kept as a negative control group (C-ve). Group 2 (2, 4-D75) and group 3 (2, 4-D150): Rats were fed on the basal diet and given accumulative dose of 75 and 150 mg/kg b.wt., of 2, 4- D respectively, to induce hepatotoxicity and kept as a Positive Control group1 (C+ve1) and Positive Control group 2 (C+ve 2). Group 4 (C500): Rats were fed on the basal diet and received oral gavage of Chamomile capitula extract in a dose of 500 mg/kg b.wt., alone. Groups 5 (Mix75) and 6 (Mix 150): Rats were fed on the basal diet and given combination of Chamomile capitula extract with either the accumulative doses of the 75 mg/kg b.wt., or the 150 mg/kg b.wt., of 2, 4-D with some hours in between. Daily feed intake (FI) per group was calculated throughout the experimental period (4 weeks); body weight gain percentage and feed efficiency ratio were calculated according to the method of Chapman *et al.* (1959). At the end of the experimental period, blood samples were collected and centrifuged at 3000 rpm for 15 minutes. Serum samples were separated and frozen at -80 °C until used for the biochemical analyses. The liver was removed by careful dissection and blotted free of adhering blood immediately after sacrificing the rats. The liver was washed with cold saline solution and dried between two filter papers then weighed. Calculation of the relative organs weight was done according to the following equation:

Organ weight

$$\text{Organ relative weight} = \frac{\text{Organ weight}}{\text{Animal final bodyweight}} \times 100$$

#### Serum Biochemical Analysis:

Biochemical analyses were estimated using spectrophotometric techniques by different methods as explained below:

The centrifuged samples were used for the assay of marker enzymes namely Aspartate aminotransaminase (AST), Alanine aminotransaminase (ALT), Alkaline phosphatase (ALP) and Lactate Dehydrogenase (LDH) by reported method **Tietz (2006)**. Albumin (ALB) and Total Bilirubin (TBIL) were measured according to the method described by **Burtis et al. (2006)**. Total Protein (TP) was estimated by the methods of (**Henry, 1974**).

#### **Estimation of Serum Oxidative Stress Markers:**

Tests of antioxidant enzymes were performed using enzyme-linked immunosorbent assay (ELISA) technique (Microplate reader, Biotech, USA). The serum samples were used to measurement the activity of antioxidant enzymes such as glutathione reductase (GR) and Superoxide dismutases (SOD) by the methods of **Carlberg and Mannervik (1985)** and **Kakkar et al. (1995)** respectively.

#### **Statistical Analysis:**

Data were expressed as means  $\pm$  standard deviation (SD). Statistical analysis of variance between mean values of different groups was performed using Kruskal-Wallis test followed by the Mann-Whitney test to determine the variance between all rat groups. Differences were considered significant at  $P<0.05$ . Statistical analysis was done

using computerized SPSS program (Statistical Package for the Social Sciences, version 16).

#### **3. Results and Discussion**

##### **Effect of Chamomile capitula extract on the initial and final body weight, body weight gain% and feed efficiency ratio in hepatotoxic rats induced by 2, 4- D.**

The present study showed that oral administration of 2, 4-D to rats at two dosage levels 75 and 150 mg/kg b.wt., induced a significant ( $P<0.05$ ) decrease in body weight gain (BWG) and feed efficiency ratio when compared to the negative control group as shown in Table 2. This decrease in body weight of rats could be attributed to the reduction in feed intake which might be due to loss of appetite of rats as a result of 2, 4 -D administration. This finding agreed with **Troudi et al. (2012)** who demonstrated that there was a positive correlation between the decrease of (BWG) and the dose of 2, 4 -D herbicide administered to rats. Oral administration of Chamomile capitula extract when given together with 2, 4-D in doses of 75 and 150 mg/kg b.wt., significantly ( $P<0.05$ ) increased the (BWG) as compared to rats received only 75 or 150 mg/kg b.wt., of 2, 4-D. This result could be explained by the high content of flavonoids (63.3%) and total phenolic compounds (23.2 %) in Chamomile which have an antioxidant effect against the oxidative stress induced by 2, 4-D, which in turn enhance food consumption by rats given Chamomile.

**Table 1: Analytical measurements for Chamomile recutita bioactive Compounds**

Chamomile recutita bioactive Compounds	Percentage
<b>Flavonoids (apigenin 7-O-glucoside)</b>	63.3%
<b>Total phenolic compounds</b>	23.2 %
<b>Essential oil</b>	1.5%
<b>Other constituents</b>	12%

**Table 2: Effect of oral administration of Chamomile extract on body weight, body weight gain% and feed efficiency ratio against 2, 4-D-induced hepatotoxicity in rats.**

Groups	Body weight (g)		Body weight gain (%)	Feed efficiency ratio (FER)
	Initial	Final		
<b>Control (C-ve)</b>	252.66 $\pm$ 11.71 <sup>a</sup>	345 $\pm$ 3.00 <sup>a</sup>	36.54 $\pm$ 0.71 <sup>a</sup>	5.077 $\pm$ 0.11 <sup>a</sup>
<b>2,4-D75 (C+ve1)</b>	255.66 $\pm$ 4.04 <sup>a</sup>	329 $\pm$ 5.00 <sup>a,b</sup>	28.68 $\pm$ 0.34 <sup>b,c</sup>	2.210 $\pm$ 0.14 <sup>b</sup>
<b>2,4-D150 (C+ve2)</b>	259 $\pm$ 10.14 <sup>a</sup>	318 $\pm$ 1.00 <sup>b</sup>	22.78 $\pm$ 0.73 <sup>c</sup>	1.111 $\pm$ 0.13 <sup>c,d</sup>
<b>C500</b>	253.66 $\pm$ 15.14 <sup>a</sup>	345.66 $\pm$ 5.50 <sup>a</sup>	36.27 $\pm$ 0.81 <sup>a</sup>	4.343 $\pm$ 0.11 <sup>a</sup>
<b>Mix75</b>	250.66 $\pm$ 24.50 <sup>a</sup>	338 $\pm$ 16.00 <sup>a,b</sup>	34.84 $\pm$ 0.57 <sup>a</sup>	3.366 $\pm$ 0.11 <sup>a,b</sup>
<b>Mix150</b>	258.33 $\pm$ 14.36 <sup>a</sup>	334.33 $\pm$ 9.86 <sup>a,b</sup>	29.42 $\pm$ 0.30 <sup>a</sup>	2.924 $\pm$ 0.13 <sup>a,b</sup>

-Values are presented as mean  $\pm$  standard deviations.

-Values with different superscript letters within a column are significantly different at  $P<0.05$ , while those with similar or partially similar are non significant.

### **Effect of Chamomile capitula extract on liver absolute and relative weight in hepatotoxic rats induced by 2, 4-D.**

Results obtained in the present study showed that oral administration of 2, 4-D at the two dosage levels 75 or 150 mg/kg b.wt., induced a significant ( $P<0.05$ ) increase in the relative liver weight when compared to the negative control group as shown in Table 3. This result was consistent with **Tayeb et al. (2010)** who demonstrated that exposure of rats to 15, 75 and 150 mg/kg b.wt., of 2, 4-D increased the liver weight. This increase might be due to edema inflammatory changes seen in liver tissues (**Undeğer et al., 2000**), induction of enzyme cytochrome-P

450(**Amacher et al., 1998**) as well as enlargement of the liver which may be related to the increase in most of the major cellular components in response to the degenerative toxic effects of 2, 4-D (**Bucher, 1964**). A significant ( $P<0.05$ ) decrease was shown in relative liver weight only in Mix150 group compared to rats orally given 2, 4-D<sub>150</sub>. This recovery could be due to the high content of flavonoids (63.3%) and total phenolic compounds (23.2 %) in Chamomile. These bioactive compounds were reported to act as free radical scavengers, intercepting those radicals involved in 2, 4-D metabolism by microsomal enzymes such as cytochrome-P 450 (**Gupta and Misra, 2006**).

**Table 3: Absolute and relative liver weight of 2, 4-D at two doses and Chamomile aqueous extract after 4 weeks**

Groups	Absolute liver weight (g)	Relative liver weight (g/100 g body weight)
<b>Control (C-ve)</b>	9.77± 0.12 <sup>a,b</sup>	2.848± 0.07 <sup>c</sup>
<b>2,4-D75 (C+ve1)</b>	10.99± 0.47 <sup>a,b</sup>	3.265± 0.04 <sup>b</sup>
<b>2,4-D150 (C+ve2)</b>	11.79± 1.27 <sup>a</sup>	3.752± 0.13 <sup>a</sup>
<b>C500</b>	9.59± 0.98 <sup>b</sup>	2.777± 0.27 <sup>c</sup>
<b>Mix75</b>	9.93± 0.71 <sup>a,b</sup>	2.964± 0.17 <sup>b,c</sup>
<b>Mix150</b>	10.14± 0.48 <sup>a,b</sup>	3.129± 0.02 <sup>b,c</sup>

-Values are presented as mean ± standard deviations.

-Values with different superscript letters within a column are significantly different at  $P<0.05$ , while those with similar or partially similar are non significant.

### **Effect of Chamomile capitula extract on serum liver enzymes AST, ALT and ALP in hepatotoxic rats induced by 2, 4-D.**

Administration of 2, 4-D at 75 and 150 mg/kg b.wt., significantly ( $P<0.05$ ) increased AST and ALT levels compared to negative control rats as recorded in Table 4. An extensive liver injury was induced by 2, 4-D due to the increase in lipid peroxidation causing membrane damage. The present results agreed with **Troudi et al. (2012)** who demonstrated that there were increases in the level of ALT and AST transaminases of the 2, 4-D-intoxicated groups. This could be due to that ALT and AST enzymes are the most sensitive biomarkers directly correlated with the extent of hepatic damage and toxicity. In fact, these enzymes increased and secreted into blood when hepatocellular injury occurred as mentioned by **Kalender et al. (2005)**. Concerning ALP results showed a significant ( $P<0.05$ ) increase in (C+ve2) group, when compared to negative control rats. This result was in agreement with **Kalender et al. (2005)** who reported that exposure of rats to diazinon insecticide resulted in an increase in ALP level. This

increase could be due to the degenerative changes and damage in liver tissues. Coadministration of Chamomile extract with two dosage 75 and 150 mg/kg b.wt., resulted in a significant ( $P<0.05$ ) decrease in ALT and AST levels compared to corresponding positive control groups. Our results agreed with that of **Gupta and Misra (2006)**, who reported that the administration of Chamomile extract reduced the elevated serum levels of ALT and AST induced by paracetamol. This reduction could be attributed to the protective effect of Chamomile extract and the maintenance of the functional integrity of hepatic cells. Serum ALP analysis showed no significant change at the Chamomile mixed with 2, 4-D when compared to rats orally given 2, 4-D at both tested doses. This result agreed with that obtained by **Gupta and Misra (2006)**, who reported that the administration of Chamomile extract reduced the enhanced level of serum ALP. This reduction could be attributed to the protective effect of Chamomile extract and the maintenance of the functional integrity of hepatic cells.

**Table 4: Effects of 2, 4-D at two doses and Chamomile aqueous extract on serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzymes of rats compared to control.**

Groups	AST (U/l)	ALT (U/l)	ALP (U/l)
<b>Control (C-ve)</b>	163.50± 7.50 <sup>d</sup>	56.50± 5.00 <sup>c</sup>	154.50± 6.94 <sup>b</sup>
<b>2,4-D75 (C+ve1)</b>	194.00± 2.00 <sup>b</sup>	65.00± 3.00 <sup>b</sup>	171.50± 1.50 <sup>a,b</sup>
<b>2,4-D150 (C+ve2)</b>	215.50± 0.50 <sup>a</sup>	74.33± 1.52 <sup>a</sup>	199.50± 2.35 <sup>a</sup>
<b>C500</b>	163.00± 6.00 <sup>d</sup>	56.00± 3.00 <sup>c</sup>	152.66± 4.93 <sup>b</sup>
<b>Mix75</b>	167.33± 8.50 <sup>c,d</sup>	59.00± 1.00 <sup>c</sup>	165.50± 2.42 <sup>a,b</sup>
<b>Mix150</b>	178.50± 5.50 <sup>c</sup>	65.00± 1.00 <sup>b</sup>	177.00± 14.73 <sup>a,b</sup>

-Values are presented as mean ± standard deviations.

-Values with different superscript letters within a column are significantly different at  $P<0.05$ , while those with similar or partially similar are non significant.

#### **Effect of Chamomile capitula extract on serum LDH, ALB, TP, and TBIL in hepatotoxic rats induced by 2, 4-D.**

Regarding other biomarkers of liver toxicity, the present results as seen in Table 5 showed that serum levels of ALB and TP were significantly ( $P<0.05$ ) decreased after administration of 2, 4-D at two dosage levels when compared to the negative control group. Similar results were demonstrated by **Tayeb et al. (2010)** who reported that there were significant decreases in serum ALB and TP after 2, 4-D administration to rats. This is because albumin is synthesized by the liver and often transports or binds to drugs or chemicals such as 2, 4-D that may negatively influence total protein and albumin metabolism. Combined administration of Chamomile capitula extract with 2, 4-D in a dose 75 mg/kg b.wt., significantly ( $P<0.05$ ) increased serum ALB and TP levels when compared to rats orally given 2, 4-D<sub>75</sub>. This result was consistent with **Gupta and Misra (2006)** who reported that there was a significant increase in TP after Chamomile treatment against paracetamol intoxication. In addition, **Kumar et al. (2012)** reported that the extract of Chamomile capitula induced a hepatoprotective effect and increases serum ALB levels. The hepatoprotective activity of Chamomile may be due to normalization of impaired membrane function activity. It is clear from Table 5 that there was a significant ( $P <0.05$ ) increase only in LDH level in rats orally given the large dose of 2, 4-D when compared to the negative

control group .This finding may be due to the release of isozymes from destroyed liver . This result was in agreement with **Tripathi and Shukla (1990)** and **Vander and Hunsaker (2003)**. It was noticed that combined administration of Chamomile with 2, 4-D at a dose150 mg/kg b.wt., showed a significant ( $P<0.05$ ) decrease in LDH when compared to rats given 2, 4-D at a large dose. The same results were obtained by **Chandrasekhar et al. (2012)** who reported a significant decrease in LDH when rats treated with Chamomile. The reduction in LDH could be attributed to the protective effect of Chamomile capitula extract and maintenance of the functional integrity of hepatic cells (**Gupta and Misra, 2006**). Concerning serum TBIL analysis, there was a significant ( $P <0.05$ ) increase in the level of TBIL in both groups given 2, 4-D at 75 and 150 mg/kg b.wt., compared to the negative control group. These results are nearly similar to those mentioned by **Nakbi et al. (2010)** and **Troudi et al. (2012)** who reported that TBIL level increased after 2, 4-D administration. These elevated levels indicated an extensive liver damage. The combined administration of Chamomile capitula extract with both doses of 2, 4-D induced a significant ( $P<0.05$ ) decrease in TBIL level when compared to rats at both tested doses. This result agreed with **Gupta and Misra (2006)** who reported that treatment with Chamomile extract reduced the paracetamol-enhanced level of serum bilirubin which seems to offer the protection and to maintain the functional integrity of hepatic cells.

**Table 5: Effects of 2, 4-D at two doses and Chamomile aqueous extract on serum lactate dehydrogenase (LDH), albumin (ALB), total protein (TP) and total bilirubin (TBIL) of rats compared to control.**

Groups	LDH (U/l)	ALB (g/l)	TP (g/l)	TBIL (μmol/l)
<b>Control (C-ve)</b>	2650.66± 13.01 <sup>c</sup>	12.50± 0.50 <sup>a</sup>	74.00± 5.00 <sup>a</sup>	1.16± 0.02 <sup>c</sup>
<b>2,4-D75 (C+ve1)</b>	2813.00± 30.65 <sup>c</sup>	10.86± 0.32 <sup>b</sup>	65.50± 0.50 <sup>b</sup>	2.33± 0.11 <sup>a</sup>
<b>2,4-D150(C+ve2)</b>	3709.00± 47.18 <sup>a</sup>	10.20± 0.26 <sup>b</sup>	62.50± 0.50 <sup>b</sup>	2.50± 0.05 <sup>a</sup>
<b>C500</b>	2668.00± 28.58 <sup>c</sup>	12.66± 0.57 <sup>a</sup>	74.00± 3.00 <sup>a</sup>	1.17± 0.02 <sup>c</sup>
<b>Mix75</b>	2771.66± 38.89 <sup>c</sup>	11.86± 0.32 <sup>a</sup>	72.00± 1.00 <sup>a</sup>	2.00± 0.05 <sup>b</sup>

<b>Mix150</b>	3414.33± 68.95 <sup>b</sup>	10.86± 0.32 <sup>b</sup>	68.33± 1.15 <sup>a,b</sup>	2.16± 0.2 <sup>b</sup>
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-Values are presented as mean ± standard deviations.

-Values with different superscript letters within a column are significantly different at  $P<0.05$ , while those with similar or partially similar are non significant.

#### Effect of Chamomile capitula extract on serum GR and SOD in hepatotoxic rats induced by 2, 4-D.

Data recorded in Table 6 showed the significant ( $P <0.05$ ) decrease of the antioxidant enzyme activities in the liver after 2, 4-D (75 and 150 mg/kg b.wt.) intoxication as compared to the control negative group, proved the failure of antioxidant defense system to overcome the influx of reactive oxygen species generated by 2, 4-D exposure (Tayeb *et al.*, 2010) and Troudi *et al.* (2012). In addition, the decrease in SOD activity might be due to the loss of copper and zinc which are essential for the enzyme activity (Karthikeyan *et al.*, 2007). Our result was in agreement with the previous study of Tayeb *et al.* (2010) and Troudi *et al.* (2012) who reported that exposure to 2, 4-D decreased the GR and SOD activity in the liver. Combined administration of Chamomile extract with 2, 4-D 150 mg/kg b.wt.,

produced significant ( $P<0.05$ ) increases in GR and SOD activities when compared to corresponding (c ve+) group. On the other hand, Co-administration of Chamomile extract with 2, 4-D 75 mg/kg b.wt., caused a significant ( $p<0.05$ ) increase in GR only compared to corresponding (cve+) group. These result agreed with Gupta *et al.* (2006), who reported that the administration of Chamomile extract increased the antioxidant enzymes activity in Chamomile treated group compared to CCL<sub>4</sub> control group. This may be because Chamomile extract has an antioxidant activity that affording the hepatoprotective effect against 2, 4-D toxicity via inducing cell membrane stabilization, hepatic cell regeneration and activation of antioxidant enzymes.

It must be noted that there was no increase in all parameters under investigation in groups administrated Chamomile capitula extract only. This may be due to short term of administration.

**Table 6: Effects of 2, 4-D at two doses and Chamomile aqueous extract after 4 weeks on serum antioxidant enzymes Glutathion reductase (GR) and Superoxide dismutase (SOD) of rats compared to control.**

Groups	GR (U/ml)	SOD (U/ml)
<b>Control (C-ve)</b>	79.85±1.15 <sup>a</sup>	94.15±0.85 <sup>a</sup>
<b>2,4-D75 (C+ve1)</b>	62.46±1.41 <sup>c</sup>	91.80±0.65 <sup>b</sup>
<b>2,4-D150 (C+ve2)</b>	57.65±0.25 <sup>d</sup>	89.83±0.60 <sup>c</sup>
<b>C500</b>	81.03±1.00 <sup>a</sup>	93.55±0.39 <sup>a</sup>
<b>Mix75</b>	72.96±1.10 <sup>b</sup>	92.51±0.30 <sup>a,b</sup>
<b>Mix150</b>	67.10±0.93 <sup>b</sup>	91.35±0.95 <sup>b</sup>

-Values are presented as mean ± standard deviations.

-Values with different superscript letters within a column are significantly different at  $P<0.05$ , while those with similar or partially similar are non significant.

In conclusion the results of this study denote that oral administration of Chamomile capitula aqueous extract to 2, 4-D -intoxicated rats improves body weight and feed efficiency ratio. Chamomile capitula treatment also produces hepatoprotective and antioxidant effects against hepatotoxicity induced by 2, 4-D herbicide. The hepatoprotective activity of Chamomile capitula may be attributed to its antioxidant effect which maintains the functional integrity of hepatic cells. The current study recommends that intake of Chamomile capitula flowers as herbal tea may be beneficial for patients who suffer from liver disorders and oxidative stress.

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