



# The Effect of Different Levels of *Matricaria chamomilla* on Functional Status of the Liver in Rats Injected with Carbon Tetrachloride

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## ABSTRACT

Chamomile is a member of the *Asteraceae* family and has been well established as a medicinal plant. There are many different species of chamomile, the two most common being German chamomile (*Matricaria chamomilla*) and Roman chamomile (*Chamaemelum nobile*). The use of chamomile has been associated with calming and anti-inflammatory properties. The plant's healing properties come from its flowers, which contain volatile oils including bisabolol, bisabolol oxides A and B, and matricin as well as flavonoids. This study aims to elucidate the possible therapeutic applications of different levels of *M. chamomilla* on the functional status of liver, in rats injected with carbon tetrachloride ( $CCl_4$ ), a known hepatotoxin. Thirty-six male albino rats were treated with subcutaneous injection of  $CCl_4$  in paraffin oil 50% V/V (2ml/kg b. wt.) twice a week for 2 weeks to induce chronic liver damage. After injection of  $CCl_4$ , blood samples were obtained using the retro-orbital method to confirm liver damage and estimate liver function. Rats given  $CCl_4$  prior to feeding *M. chamomilla* demonstrated decrease in serum lipoprotein HDL, LDL, VLDL fraction levels, indicating liver damage. Rats given  $CCl_4$  and fed on 5% *M. chamomilla* showed the most significant increase in organ weight as compared to all levels of treatment suggesting that *M. chamomilla* can reduce liver damage. Rats given  $CCl_4$  then fed on a combination of all levels of *M. chamomilla* showed a decrease of AST, ALT and ALP enzyme levels in the serum, suggesting a reduction in liver damage. This study showed that proceeding liver damage using  $CCl_4$  and administering 5% *M. chamomilla*, liver function improved significantly as shown with a reduction in serum AST, ALT and ALP, and use of *M. chamomilla* should be considered in combination therapy for treating liver disease or damage.

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## INTRODUCTION

*Matricaria chamomilla* is a plant species from the *Asteraceae* family which has medicinal properties. It has been referred to as the star among medicinal species (Singh, 2011). Various functions of the plant has been established which include cosmetic, nutritional and medicinal properties. It has been used for a variety of different ailments including nervousness, anxiety, and headaches. Its anti-inflammatory properties as well as its relaxing and soothing functionalities make it an excellent candidate for use in a variety of medical conditions. Many studies in the literature have researched the use of chamomile to treat a variety of illness's including small bowel inflammation, intestinal mucositis and rheumatoid arthritis. However, its use in treating liver damage is not as well studied.

Most of the studies pertaining to drug induced toxicity employ liver as a target organ. This is because, liver is

responsible for the antioxidative properties of the body as well as metabolism, secretion and storage of substances making it one of the most vital organs of the body (Madrigal-Santillán *et al.*, 2014). Drug and alcohol abuse can cause liver damage and may require transplantation in extreme cases. The use of plant based products has been studied to treat liver disease but little is known about how the use of such products occurs (Guan and He, 2015).  $CCl_4$  is well known hepatotoxin and its role of in induction of liver damage and subsequent oxidative stress has been reported elsewhere (Bhattacharjee and Sil, 2007; Aksoy and Sozbilir, 2012; Mughal *et al.*, 2019).

This investigation aims to study the possible therapy of different levels of *M. chamomilla* on functional status of liver in rats injected with carbon tetrachloride ( $CCl_4$ ), a well-known liver toxin which has been used widely in research to induce liver damage. Some studies have shown that the use of *M. chamomilla* can reduce enzymes linked to liver damage (Srivastava *et al.*, 2010). This study examines the serum enzyme levels of  $CCl_4$  treated rats, and also to identify the ideal concentration of *M. chamomilla* in treating liver damage. Using this data a more personalized approach in treating liver damage with

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Group 2C fed with basal diet plus 15% *M. chamomilla*, Group 2D received basal diet plus 20% *M. chamomilla* and Group 2E fed with basal diet plus 25% *M. chamomilla*.

#### Induction of liver intoxication in rats

Thirty-six male albino rats were treated with subcutaneous injection of CCl<sub>4</sub> in paraffin oil 50% V/V (2ml/kg B. Wt.) twice a week for 2 weeks to induce chronic damage of the liver according to the method described by Jayasekhar *et al.* (1997). After the injection of CCl<sub>4</sub>, blood samples were obtained by retro-orbital method to ensure occurrence of liver injury and to estimate liver function.

#### Blood sampling

At the end of the experiment period (28 days) rats were sacrificed by ether and anesthesia. Blood samples were obtained by retro-orbital method in a clean dry centrifuge tube. They were left to clot by standing at room temperature for 20 min, and then centrifuged at 1500 r.p.m for 15 min. Serum samples were collected by a dry clean syringe, poured in Wisserman tubes and then kept frozen in a refrigerator at -10°C for biochemical analysis. Rats were thereafter opened, liver, spleen, heart, lungs and kidneys removed and washed in saline solution, then dried and weighed. Relative weights of mentioned organs were calculated using the following formula:

$$\text{Relative organ weight} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

For fixation prior to histopathological investigation, organs were kept in formalin solution (10% V/V) according to methods described by Drury and Wallington (1967).

#### Biological evaluation

During the period of the experiment, all the rats were weighed once a week and the consumed diets were recorded everyday (daily food intake). At the end of the experiment, biological evaluation of the experimental diets was carried out by determination of body weight gain (%) (BWG%) and food efficiency ratio (FER), according to method of Chapman *et al.*, 1959, using the following formulae:

$$\text{BWG}(\%) = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{FER} = \frac{\text{Body weight gain (g)}}{\text{Food intake (g)}}$$

Food intake was also calculated daily.

#### Determination of the activity of liver enzymes

Determination of aspartate aminotransferase (AST) enzyme was carried out by spectrophotometer using specific kits (BioMerieux) according to Reitman and Frankel (1957). Alkaline phosphatase (ALP) and alanine

aminotransferase (ALT) determination was based on colorimetric determination method of Roy (1970). Serum total bilirubin was determined colorimetrically as described by Doumas *et al.*, (1973), using spectrophotometer adjusted at 578 nm.

#### Determination of components of lipid profile

Total cholesterol was determined according to Ratliff and Hall (1973). Enzymatic colorimetric determination of triglycerides was carried out according to Jacobs and Van Denmark (1960). Determination of HDL was carried out according to the method of Fnedewaid (1972), and Gordon and Amer (1977). The determination of VLDL and LDL was carried out according to the method of Lee and Nieman (1996).

#### Statistical analysis

The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, statistical soft-ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test and  $p < 0.05$  was used to indicate significance between different groups (Snedecor and Cochran, 1967).

## RESULTS AND DISCUSSION

Liver is the vital organ widely employed for toxicity studies. Chemical induced liver damage may be attributed to the toxicity of the parent compound or to the toxic metabolic products (Kuntz and Kuntz, 2005). In the present study CCl<sub>4</sub> is utilized for inducing hepatotoxicity in rats, owing to its similarity in mechanism of action in humans (Sancheti *et al.*, 2013). Moreover, the hepatoprotective activity of *M. chamomilla* against CCl<sub>4</sub>-induced liver injury in rats have not been reported. The present investigations try to elucidate the hepatoprotective activity of *M. chamomilla* against CCl<sub>4</sub>-induced liver injury in rats.

#### Effect on food intake, body weight gain and feed efficiency ratio

Data presented in Table II shows the effect of different levels of *M. chamomilla* on food intake (FI), body weight gain % (BWG%) and feed efficiency ratio (FER) in CCl<sub>4</sub> - intoxicated rats. It could be observed for rats intoxicated with CCl<sub>4</sub> (C+ve) group that FI was significantly reduced (15.05±0.038 g/day) compared to the C-ve normal rats (17.19±0.041 g/day). All rats treated with CCl<sub>4</sub> and fed on all tested concentration (5%, 10%, 15%, 20% ,25%) of *M. chamomilla* had a significant increase in FI. Rats given CCl<sub>4</sub> and fed on 10% *M. chamomilla* showed the most significant increase in FI compared to all levels of *M.*

*chamomilla*, which reached to  $19.09 \pm 0.015$  g/day.

**Table II.- Effects of the different levels of *M. chamomilla* (5%, 10%, 15%, 20%, 25%) on FI (food intake), BWG% (body weight gain) and FER (feed efficiency ratio) of CCl<sub>4</sub>-intoxicated rats (n=6rats).**

Parameters / Groups	FI (g)	BWG (%)	FER
Control - ve	17.19±0.04 <sup>d</sup>	35.78±0.97 <sup>a</sup>	0.13±0.007 <sup>a</sup>
Control + ve	15.05±0.03 <sup>s</sup>	10.24±0.62 <sup>d</sup>	0.06±0.003 <sup>e</sup>
5% <i>M. chamomilla</i>	17.61±0.02 <sup>c</sup>	34.04±0.57 <sup>a</sup>	0.11±0.004 <sup>b</sup>
10% <i>M. chamomilla</i>	19.09±0.01 <sup>a</sup>	33.58±0.45 <sup>a</sup>	0.11±0.006 <sup>b</sup>
15% <i>M. chamomilla</i>	18.54±0.03 <sup>b</sup>	24.88±0.64 <sup>b</sup>	0.09±0.003 <sup>c</sup>
20% <i>M. chamomilla</i>	16.46±0.02 <sup>c</sup>	14.37±0.41 <sup>c</sup>	0.08±0.004 <sup>c</sup>
25% <i>M. chamomilla</i>	16.19±0.01 <sup>f</sup>	13.73±0.51 <sup>c</sup>	0.07±0.004 <sup>d</sup>

Values denote arithmetic means ± Standard error of the mean. Means with different letters (a, b, c, d) in the same column differ significantly at  $p < 0.05$  using one way ANOVA test, while those with similar letters are non-significant.

Concerning body weight gain, the C -ve group was  $35.78 \pm 0.97\%$  but in C+ve group there was significant reduction at  $10.24 \pm 0.62\%$ . A significant increase in BWG% in CCl<sub>4</sub>-intoxicated rats and fed on all levels of *M. chamomilla* was observed compared to C +ve group. There were non-significant changes between rats fed on 5% and 10% *M. chamomilla* which were  $34.04 \pm 0.57$  and  $33.58 \pm 0.45\%$ , respectively. There were also significant changes between rats fed on levels 20% and 25% for BWG% which were  $14.37 \pm 0.41$  and  $13.73 \pm 0.53\%$ , respectively.

Regarding FER, it was found that in rats injected with CCl<sub>4</sub> without treatment (C+ve group), FER was  $0.06 \pm 0.003$  while in normal rats (C-ve) it was  $0.13 \pm 0.007$ . These results denote that there was a significant decrease

in FER of rats poisoned by CCl<sub>4</sub> as compared to normal rats. Rats intoxicated by CCl<sub>4</sub> and fed on formulas 5% and 10% did not show a significant increase in FER at  $0.11 \pm 0.004$  and  $0.11 \pm 0.006$ , respectively. Similarly FER of groups fed with 15% and 20% of *M. chamomilla* showed values of  $0.09 \pm 0.006$  and  $0.08 \pm 0.004$  respectively, and the differences were non-significant. Meanwhile, rats treated with CCl<sub>4</sub> and fed on 25% *M. chamomilla* showed significant increase in FER compared to C+ve rats which were  $0.07 \pm 0.004$  and  $0.06 \pm 0.003$ , respectively.

Dickerson and Lee (1988) reported that many patients with acute or chronic liver disease are ill, and commonly lose weight. Moreover, Clevely and Richmond (1998) concluded that *M. chamomilla* is medicinally valuable and for treatment of liver disorders.

#### Effects on relative organs weight

Data listed in Table III shows the effect of feeding at different plant concentration on relative organs weight of CCl<sub>4</sub>-intoxicated rats. There was a significant decrease in relative liver, spleen, lungs, heart and kidneys weight of rats poisoned by CCl<sub>4</sub> as compared to the control (-ve) normal rats. For the relative weight of liver, there were non-significant differences between rats treated with CCl<sub>4</sub> and those fed on 10% and 20% concentration of *M. chamomilla*. CCl<sub>4</sub> treated rats fed on 5% concentration of *M. chamomilla* showed the most significant increase in the relative organ weight as compared to all levels of treatment, while CCl<sub>4</sub> treated rats fed on 25% concentration of *M. chamomilla* showed the lowest significant increase as compared to all levels.

Regarding relative spleen weight, there were non-significant changes between rats injected with CCl<sub>4</sub> and fed on 10% and 15% concentration of *M. chamomilla*. Meanwhile, there were non-significant changes between rats injected with CCl<sub>4</sub> and fed on 20% and 25%

**Table III.- Effects of feeding with different levels of *M. chamomilla* on relative organ weight of CCl<sub>4</sub>-intoxicated rats (n=6rats).**

Parameters / Groups	Relative organs weight (g/100 g. B.Wt.)				
	Liver	Spleen	Lungs	Heart	Kidneys
Control - ve	3.79±0.08 <sup>b</sup>	0.62±0.05 <sup>a</sup>	0.87±0.04 <sup>a</sup>	0.91±0.04 <sup>a</sup>	0.94±0.12 <sup>a</sup>
Control + ve	3.49±0.07 <sup>c</sup>	0.49±0.05 <sup>d</sup>	0.67±0.00 <sup>d</sup>	0.49±0.02 <sup>d</sup>	0.79±0.07 <sup>d</sup>
5% <i>M. chamomilla</i>	4.09±0.09 <sup>a</sup>	0.62±0.01 <sup>a</sup>	0.83±0.01 <sup>a</sup>	0.87±0.01 <sup>a</sup>	0.93±0.06 <sup>a</sup>
10% <i>M. chamomilla</i>	3.64±0.04 <sup>c</sup>	0.58±0.01 <sup>b</sup>	0.75±0.06 <sup>b</sup>	0.83±0.01 <sup>a</sup>	0.84±0.08 <sup>b</sup>
15% <i>M. chamomilla</i>	3.72±0.05 <sup>b</sup>	0.56±0.03 <sup>b</sup>	0.74±0.02 <sup>b</sup>	0.61±0.01 <sup>c</sup>	0.83±0.02 <sup>b</sup>
20% <i>M. chamomilla</i>	3.62±0.04 <sup>c</sup>	0.52±0.01 <sup>c</sup>	0.72±0.008 <sup>b</sup>	0.57±0.02 <sup>c</sup>	0.73±0.007 <sup>c</sup>
25% <i>M. chamomilla</i>	3.59±0.02 <sup>d</sup>	0.51±0.01 <sup>c</sup>	0.69±0.01 <sup>c</sup>	0.77±0.01 <sup>b</sup>	0.71±0.009 <sup>c</sup>

Values denote arithmetic means ± Standard error of the mean. Means with different letters (a, b, c, d) in the same column differ significantly at  $p \leq 0.05$  using one way ANOVA test, while those with similar letters are non-significant.



concentration of *M. chamomilla*, which were  $0.52 \pm 0.019$  and  $0.51 \pm 0.011$  g/100g B.Wt., respectively. Rats poisoned by  $\text{CCl}_4$  and fed 5% concentration of *M. chamomilla* showed the highest significant increase in the mentioned relative organ weight which was  $0.62 \pm 0.018$  g/100g B.Wt., showing non-significant changes as compared to control (-ve) normal rats.

Concerning relative lungs weight, there were non-significant changes between rats injected with  $\text{CCl}_4$  then fed on levels 10%, 15% and 20% of *M. chamomilla*. Rats fed on 5% *M. chamomilla* showed the highest significant increase in lung weight which was  $0.83 \pm 0.014$  g/100g B.Wt. Meanwhile, it did not reflect any significant change as compared to control (-ve) normal rats which was  $0.87 \pm 0.41$  g/100g B.Wt.

Relative heart weight showed no significant changes between rats treated with  $\text{CCl}_4$  and then fed on 5%, and 10% concentration levels of *M. chamomilla*. At the same time, there were also non-significant differences between rats given  $\text{CCl}_4$  then fed on levels 15% and 20%. The highest significant value in mentioned organs were noticed in  $\text{CCl}_4$  treated rats fed on 5% and 10% *M. chamomilla* as compared to control (+ve) group.

Kidneys relative weight showed non-significant differences between rats given  $\text{CCl}_4$  then fed on 10% and 15% concentration of *M. chamomilla*. Also, there were non-significant changes between rats injured by  $\text{CCl}_4$  then fed on 20% and 25% of *M. chamomilla*.  $\text{CCl}_4$  treated rats fed on 5% concentration of *M. chamomilla* showed the highest significant increase among the mentioned relative weights which was  $0.93 \pm 0.064$  g/100g B.Wt., and showed non-significant change as compared to that of (C-ve) normal rats. From the present findings, it can be concluded that carbon tetrachloride  $\text{CCl}_4$  decreased liver weight and induced the atrophy, however, a diet consisting of 5% and 10% concentration of *M. chamomilla* can reduce severity of this, and showing an increased organ weight, indicating reduced atrophy from liver damage caused by  $\text{CCl}_4$ . The decrease in liver weight following  $\text{CCl}_4$  treatment may be attributed to progressive liver damage due to  $\text{CCl}_4$  induced toxicity (Aksoy and Sozibilir, 2012).

#### Biochemical analysis

Tables IV, V and VI shows the effect of different concentration levels of *M. chamomilla* on liver enzymes, total protein and total bilirubin, total cholesterol and triglycerides, and lipoprotein fractions in  $\text{CCl}_4$ -intoxicated rats. Table IV shows the effect of different levels of *M. chamomilla* on serum liver enzymes including aspartate amino transaminase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) enzymes in  $\text{CCl}_4$ -

intoxicated rats. It is reported in the literature that these enzyme markers are used in diagnosis of liver disorders, the concentration of which increases during liver injury or cell damage (Chani and Jasim, 2016). Their increase in the serum has been attributed to the leakage of these enzymes from damaged hepatocytes (Yang *et al.*, 2008).

**Table IV.- Effects of different levels of *M. chamomilla* (5%, 10%, 15%, 20%, 25%) on serum levels of aspartate amino transaminase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) enzymes of  $\text{CCl}_4$ -intoxicated rats (n = 6 rats).**

Parameters / Groups	AST (U/L)*	ALT (U/L)*	ALP (U/L)*
Control - ve	65.6 ± 1.8 <sup>e</sup>	36.5 ± 1.6 <sup>e</sup>	84.5 ± 1.9 <sup>e</sup>
Control + ve	130.6 ± 2.1 <sup>a</sup>	70.5 ± 2.4 <sup>a</sup>	159.4 ± 2.7 <sup>a</sup>
5% <i>M. chamomilla</i>	124.6 ± 2.3 <sup>b</sup>	65.5 ± 2.8 <sup>b</sup>	154.7 ± 2.5 <sup>b</sup>
10% <i>M. chamomilla</i>	118.3 ± 2.4 <sup>c</sup>	46.7 ± 2.2 <sup>c</sup>	146.3 ± 2.8 <sup>c</sup>
15% <i>M. chamomilla</i>	114.8 ± 2.1 <sup>c</sup>	40.5 ± 2.6 <sup>c</sup>	139.5 ± 2.2 <sup>c</sup>
20% <i>M. chamomilla</i>	113.5 ± 1.6 <sup>c</sup>	35.5 ± 1.9 <sup>c</sup>	135.5 ± 1.2 <sup>c</sup>
25% <i>M. chamomilla</i>	94.7 ± 2.4 <sup>d</sup>	30.3 ± 2.7 <sup>d</sup>	114.2 ± 2.9 <sup>d</sup>

(U/L)\*, unit per liter. Values denote arithmetic means ± standard error of the mean. Means with different letters (a, b, c, d) in the same column differ significantly at  $p \leq 0.05$  using one way ANOVA test, while those with similar letters are non-significant.

It is clear from the Table IV that in rats intoxicated with  $\text{CCl}_4$  without treatment, the serum levels of AST, ALT and ALP enzymes were  $130.6 \pm 2.1$ ,  $70.5 \pm 2.4$  and  $159.4 \pm 2.7$  U/L, respectively. In (C -ve) normal rats, the serum levels of the mentioned enzymes were  $65.6 \pm 1.8$ ,  $36.5 \pm 1.6$  and  $84.5 \pm 1.9$  U/L, respectively. In this study there was a significant increase of AST, ALT and ALP enzymes in the serum of rats poisoned by  $\text{CCl}_4$  as compared to (C -ve) normal rats (Aksoy and Sozibilir, 2012). Rats given  $\text{CCl}_4$  then fed on different concentration (5%, 10%, 15%, and 20%) of *M. chamomilla* had a significant decrease in serum levels of AST, ALT and ALP activities. Rats given  $\text{CCl}_4$  then fed on a combination of all levels of treatment showed a decrease of AST, ALT and ALP enzyme levels in the serum. The decrease in serum AST, ALT and ALP enzyme levels reached to  $94.7 \pm 2.4$ ,  $30.3 \pm 2.7$  and  $114.2 \pm 2.9$  U/L, respectively. The results corroborate with some of the earlier findings (Troudi *et al.*, 2012; Sancheti *et al.*, 2013; Chain and Jasim, 2016).

In this study, treatment with  $\text{CCl}_4$  showed a positive result for liver damage while treatment with *M. chamomilla* at different concentrations rescued this phenotype. An increase in these liver enzymes indicate a pathological phenotype. However, treatment with *M. chamomilla*

**Table V.- Effects of different concentration levels (5%,10%,15%,20%,25%) of *M. chamomilla* on serum levels of total protein, total bilirubin, total cholesterol and triglycerides of CCl<sub>4</sub>-intoxicated rats (n = 6 rats).**

Parameters / Groups	Total protein (mg/dl)	Total bilirubin (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)
Control – ve	6.68 ± 1.3 <sup>a</sup>	0.66 ± 0.01 <sup>b</sup>	88.98 ± 1.4 <sup>d</sup>	43.35 ± 1.5 <sup>d</sup>
Control + ve	4.65 ± 1.6 <sup>b</sup>	0.99 ± 0.01 <sup>a</sup>	105.95 ± 1.6 <sup>a</sup>	56.60 ± 1.9 <sup>a</sup>
5% <i>M. chamomilla</i>	6.54 ± 1.8 <sup>a</sup>	0.83 ± 0.01 <sup>b</sup>	101.97 ± 1.8 <sup>b</sup>	52.60 ± 1.4 <sup>b</sup>
10% <i>M. chamomilla</i>	6.55 ± 1.2 <sup>a</sup>	0.80 ± 0.01 <sup>b</sup>	98.90 ± 1.2 <sup>c</sup>	49.50 ± 1.2 <sup>c</sup>
15% <i>M. chamomilla</i>	6.53 ± 1.3 <sup>a</sup>	0.75 ± 0.01 <sup>b</sup>	102.26 ± 1.3 <sup>b</sup>	54.30 ± 1.4 <sup>b</sup>
20% <i>M. chamomilla</i>	6.54 ± 1.5 <sup>a</sup>	0.70 ± 0.01 <sup>b</sup>	95.90 ± 1.5 <sup>c</sup>	46.50 ± 1.3 <sup>c</sup>
25% <i>M. chamomilla</i>	6.53 ± 1.1 <sup>a</sup>	0.69 ± 0.01 <sup>b</sup>	90.45 ± 1.1 <sup>d</sup>	40.50 ± 1.4 <sup>d</sup>

Values denote arithmetic means ± Standard error of the mean. Means with different letters (a, b, c, d) in the same column different significantly at  $p \leq 0.05$  using one way ANOVA test, while those with similar letters are non-significant.

at increasing concentrations reduced the levels of these enzymes which are a positive result for treating liver damage and should be considered for patients suffering from this disease. The present results corroborate with the findings of Aksoy and Sozbilir (2012). Whereby, ALT and AST enzymes were significantly increased in the CCl<sub>4</sub> group indicating liver damage, but simultaneous treatment with *M. chamomilla* reduced oxidative stress and antioxidant activity.

#### Effects on total protein and total bilirubin

Table V shows the effects of different concentration levels of *M. chamomilla* on serum levels of total protein, total bilirubin, total cholesterol and triglycerides of CCl<sub>4</sub>-intoxicated rats. Serum levels of total protein and total bilirubin in C +ve group were 4.65±1.6 and 0.99±0.012 mg/dl, respectively. While in the C –ve normal rats were 6.68±1.3 and 0.66±0.011 mg/dl, respectively. These results revealed a significant decrease in total protein but significant increase in total bilirubin in the serum of rats intoxicated by CCl<sub>4</sub> as compared to C –ve normal rats. The result is in consistent with earlier findings, demonstrating increased serum total bilirubin and decreased serum total protein in CCl<sub>4</sub>-induced hepatotoxicity in rats (Sancheti *et al.*, 2013). In CCl<sub>4</sub> treated rats fed on all levels of *M. chamomilla*, there was a significant increase in the serum level of total protein, but a significant decrease in serum level of total bilirubin. However, between groups there was a not significant change, which suggests that concentrations of *M. chamomilla* does not affect the total protein and bilirubin levels and is not effective for this purpose. As increased bilirubin levels are an indicator of liver damage it may be necessary to use higher concentrations of *M. chamomilla* to achieve a significant decrease in bilirubin levels, so as to aid in the treatment of liver damage. Thus, enhancements in serum ALT, AST and total bilirubin is an

indicative of CCl<sub>4</sub>-induced hepatic injury in rats (Drotman and Lawhorn, 1978). *M. chamomilla* caused significant decreased the levels of serum ALT, AST and total bilirubin, suggesting its protective function against CCl<sub>4</sub> –induced liver damage.

In rats injected with CCl<sub>4</sub> and without treatment (C +ve), levels of total cholesterol and triglycerides were 105.95±1.6 and 56.60±1.9 mg/dl, compared to 88.98±1.4 and 53.35±1.5 mg/dl in normal rats (C –ve). The obtained results showed that there was a significant increase in serum levels of total cholesterol and triglycerides in rats poisoned by CCl<sub>4</sub> as compared to normal rats. In CCl<sub>4</sub> treated rats fed on all levels of treatment, there was a significant decrease in serum levels of total cholesterol and triglycerides as compared to C +ve group. The result corroborate with earlier findings (Nargesi *et al.*, 2018). The lowest levels of cholesterol and triglycerides were observed in the group treated with 25% of *M. chamomilla*.

There were non-significant differences between 5% and 15% *M. chamomilla* which were 101.97±1.8 and 102.26±1.3 mg/dl for total cholesterol and were 52.60±1.4 and 54.30±1.4 mg/dl, respectively for triglycerides. Also, there were non-significant differences between 10% and 20% *M. chamomilla* which showed 98.90±1.2 and 95.90±1.5 mg/dl for total cholesterol and 49.50±1.2 and 46.50±1.3 mg/dl, respectively for triglycerides. Meanwhile, 25% *M. chamomilla* showed the highest significant decrease both in total cholesterol and triglycerides as compared to all levels of treatment, and revealed non-significant differences compared with (C –ve) normal rats as regards both parameters. Similar results were observed by Tsi and Tan (1996).

Table VI shows the effects of different concentration levels of *M. chamomilla* on the serum levels of lipoprotein fraction (HDLc, LDLc and VLDLc) in CCl<sub>4</sub>-intoxicated rats. There was a significant increase in HDLc, LDLc

and VLDLc lipoprotein fractions in the serum of rats poisoned by  $\text{CCl}_4$  and without treatment as compared to the control (-ve) normal rats. In C +ve rat serum levels of HDLc, LDLc and VLDLc were  $75.69 \pm 1.2$ ,  $18.64 \pm 1.4$  and  $11.32 \pm 1.6$  mg/dl, respectively. In normal rats (C -ve) the serum levels of lipoprotein fractions were  $63.96 \pm 1.1$ ,  $16.35 \pm 1.2$  and  $8.67 \pm 1.1$  mg/dl, respectively. Rats injected with  $\text{CCl}_4$  and fed on 10% and 20% *M. chamomilla* had a significant decrease in high density lipoprotein (HDL-c) while those fed on all levels had a significant decrease in low and very low density lipoprotein (LDLc and VLDLc) as compared to control +ve group. The result is consistent with previous studies (Nargesi *et al.*, 2018). With regard to Lipoprotein fraction HDL, there was no significant differences between C +ve rats treated with  $\text{CCl}_4$  and fed on 5% and 15% concentration of *M. chamomilla* compared to control (+ve) group which were  $74.75 \pm 1.3$ ,  $73.70 \pm 1.7$  and  $75.69 \pm 1.2$  mg/dl, respectively. Also, rats injured by  $\text{CCl}_4$  and fed on 5%, 10% and 20% *M. chamomilla* showed lipoprotein fraction LDLc of  $16.70 \pm 1.3$ ,  $16.90 \pm 1.3$  and  $16.50 \pm 1.4$  mg/dl, respectively. Regarding lipoprotein fraction VLDc, there were non-significant differences between rats given  $\text{CCl}_4$  then fed on 5% and 15% *M. chamomilla*, which were  $10.52 \pm 1.8$  and  $10.86 \pm 1.8$  mg/dl, respectively. Also, there were non-significant differences between rats given  $\text{CCl}_4$  and fed on 10% and 20% *M. chamomilla*, which were  $9.90 \pm 1$  and  $9.30 \pm 1.1$  mg/dl, respectively. Finally, rats treated with  $\text{CCl}_4$  prior to feeding on all levels of treatments revealed the highest decrease in serum lipoprotein HDL, LDL, VLDL fraction levels which reached to  $68.85 \pm 1.6$ ,  $13.50 \pm 1.3$  and  $8.10 \pm 1.2$  mg/dl, respectively. Similar results were obtained by

**Table VI.- Effects of different concentration levels (5%,10%,15%,20%,25%) of *M. chamomilla* on the serum levels of lipoprotein fractions (HDLc, LDLc and VLDLc) in  $\text{CCl}_4$ -intoxicated rats (n=6 rats).**

Parameters / Groups	Lipoprotein fractions (mg/dl)		
	HDLc	LDLc	VLDLc
Control - ve	$63.96 \pm 1.1^e$	$16.35 \pm 1.2^c$	$8.67 \pm 1.1^d$
Control + ve	$75.99 \pm 1.2^a$	$18.64 \pm 1.4^a$	$11.32 \pm 1.6^a$
5% <i>M. chamomilla</i>	$74.75 \pm 1.3^b$	$16.70 \pm 1.3^c$	$10.52 \pm 1.8^b$
10% <i>M. chamomilla</i>	$72.10 \pm 1.3^c$	$16.90 \pm 1.3^c$	$9.90 \pm 1.1^c$
15% <i>M. chamomilla</i>	$73.70 \pm 1.7^b$	$17.70 \pm 1.3^b$	$10.86 \pm 1.8^b$
20% <i>M. chamomilla</i>	$70.10 \pm 1.2^c$	$16.50 \pm 1.4^c$	$9.30 \pm 1.1^c$
25% <i>M. chamomilla</i>	$68.85 \pm 1.6^d$	$13.50 \pm 1.3^d$	$8.10 \pm 1.2^d$

Values denote arithmetic means  $\pm$  Standard error of the mean. Means with different letters (a, b, c, d) in the same column different significantly at  $p \leq 0.05$  using one way ANOVA test, while those with similar letters are non-significant.

Williams *et al.*, 2014. It has been reported elsewhere that *M. chamomilla* reduces intestinal absorption of lipids (Tavakol *et al.*, 2014).

Thus *M. chamomilla* seem to be potent hepatoprotective agent in maintaining healthy liver, elucidating its possible therapeutic applications. However, further in-depth studies on histological aspects are warranted to confirm their pharmacotherapeutic significance.

## CONCLUSION

This study showed that different concentration levels of *M. chamomilla* in the diet of rats suffering from liver damage aided in their recovery and decreased pathological levels of enzymes and cholesterol in the body. However, the use of *M. chamomilla* to decrease bilirubin levels was not significant, indicating that a higher concentration of *M. chamomilla* in the diet may be required in some cases. The only limitation in the present study is the histological examination of liver sections of *M. chamomilla* administered mice. Further studies on histological properties may expose positive results on degenerative changes of liver tissues. Overall findings indicate that appropriate tuning of doses of *M. chamomilla* is required for different treatment purposes. This study proves that plant materials possess the ability to aid in improving liver function primarily by reducing serum liver enzymes. The use of *M. chamomilla* would be beneficial for patients with liver disorders but 5-10% concentration would be the most effective in this case.

### Statement of conflict of interest

The authors declare no conflict of interest.

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