Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 6 (2025)

The Renal Protective Effect of Glycyrrhiza Glabra Against Renal Injury Induced by CCl_4 in Male Rats

[1]Ayesha Aquil, [2]Lubna Naz

Article Details

Keywords: Glycyrrhiza Glabra, Carbon Tetrachloride, Nephrotoxicity.

Ayesha Aquil
Lecturer, Dadabhoy Institute of Higher Education, Main Campus, Karachi.
Ayeshaaquil000@gmail.com
Lubna Naz
Associate Professor, Department of Physiology, University of Karachi.
lunaz@uok.edu.pk

ABSTRACT

Glycyrrhiza Glabra (Licorice), is a widely used medicinal plant known for its anti-inflammatory, anti-oxidative, and anti-bacterial properties. While its therapeutic effects have been explored in various systems, its potential nephroprotective role remains inconclusive. This study aimed to evaluate the effect of aqueous G. glabra root extract on carbon tetrachloride (CCl_4)-induced nephrotoxicity in male Wistar rats. Male Wistar rats 120-200g were separated into four groups (N=6). Group I remained untreated. Group II and Group III received 0.8 ml/kg of CCl4 subcutaneously for regular 21 days. Group III and additionally Group IV received 30 mg/Kg of aqueous licorice extract was administered orally via gavage daily for a period of 21 days. On the 22nd day, animals were euthanized and blood samples were collected for biochemical testing. The increase in the final weight of group I (control) and group IV (glycyrrhiza glabra treated group), whereas, a decrease in group II (CCl4 treated) and group III (CCl4 + glycyrrhiza glabra treated) has been recorded. The level of urea, creatinine, and BUN increased in groups II, III, and IV. The lipid profile test showed a decreased cholesterol level in groups II, III, and IV. The HDL is decreased and LDL is increased in groups II, III, and IV. It can be concluded that the given dosage of glycyrrhiza glabra does not show nephroprotective effects against carbon tetrachloride toxicity.

INTRODUCTION

The kidney, two bean-shaped organs located below the rib cage on each side of the spine is about the size of a fist. They are vital organs in mammals, with cellular complexes and multiple functions (Little, M. H., & McMahon, A. P., 2012). They play a pivotal role in maintaining internal homostasis by filtering metabolic waste, and play a major role in excretion. Apart from that it also regulates blood pressure by releasing the hormone, controlling the production of red blood cells, and producing an active form of vitamin D.

Therefore, Acute Kidney Injury, previously known as acute renal failure is defined as the abrupt decrease in kidney function. It is the sudden reduction in kidney normal functioning and is measured by GFR (Goyal, 2023). It is also found to be closely linked with Chronic Kidney Disease (Hsu, 2016), where underlying CKD is the risk factor for acute kidney disease because both decrease glomerular filtration rate and increase proteinuria. Epidemiologically, In low and middle-income countries most common cause of AKI is infection and hypovolemic shock. AKI and AKD can lead to CKD and cardiovascular death as long-term outcomes (Kellum, 2021). AKI is among the primary cause of death and disabilities around the world. In the United States of America around 1% of all hospital admission have acute kidney injury (Goyal, 2023). AKI is considered to be the most impactful clinical disease since it affects the management of patients in terms of treatment. Acute kidney injury if left treated leads to chronic kidney disease which later undergoes a process of developing glomerular sclerosis, renal interstitial fibrosis, and finally to kidney failure (Hsu, 2016). Amain cause of AKI is drug toxicity (Sales and Foresto, 2020). For animal studies, liver, and kidney toxicity is caused by carbon tetrachloride (National Center for Biotechnology Information, 2023).

However, non-communicable diseases become the most common cause of early deaths and years of life lost. Chronic kidney diseases are becoming a major health problem publicly throughout the world (Hasan. M et al., 2018). CKD is a progressive disease that affects more than 10% of the world's population (Kovesdy, 2022). It is among the most common non-communicable diseases (Couser et al., 2011). It is been recognized that CKD and acute kidney injury are linked with each other and likely to endorse one another (Hsu, 2016). The prevalence of it is not well reported in the South Asian region (Hasan. M et al., 2018). CKD is progressively increasing in Pakistan due to the lack of a proper healthcare system, and health education, and the increasing prevalence of CKD-related risk factors such as diabetes and CVD (Ullah et al 2015). The mortality rate of CKD increased by 41.5% between 1990 to 2017

(Kovesdy, 2022). CKD is also characterized by elevated triglycerides, reduced HDL, and a slight reduction in LDL level (Visconti et al., 2016). With the advancement in the field of medicine, it is apparent that the use of alternative remedies is becoming more common. It is estimated that one-third of Americans used alternative remedies like acupuncture, relaxation techniques, reflexology, folk remedies, therapeutic touch, and herbal medications. Around 80% of the world's population uses herbal medicine for treatment. Chinese herbal medicines are considered an alternative and complementary source to treat CKD. Even in ancient China, abnormalities of the kidneys were considered to disrupt the body. There are hundreds of Chinese herbs used to treat CKD including Glycyrrhiza glabra. (Li, X. and Wang, H. 2005).

Furthermore, Glycyrrhiza glabra is commonly known as licorice is an herbaceous perennial legume belonging to the family Fabaceae. Glycyrrhiza glabra has been used as an herbal drug since ancient times (Nazari et al., 2017) and consists of pharmacological benefits of anti-inflammatory, anti-viral, anti-cancer, anti-asthmatic, anti-diabetic, anti-atherogenic, and anti-microbial activities (Hasan et al., 2021). The biological effect of licorice is still not clear (Aksoy et al., 2012). Glycyrrhiza glabra can reduce body weight, total cholesterol, triglyceride, and LDL level (Murray, M., 2020). The increased blood HDL and decreased LDL are helpful in other diseases (Awad, 2017). It is also found effective in the reduction of serum urea, creatinine, and uric acid level and also decrease tubular necrosis caused by induced toxicity (Aksoy et al., 2012).

Thus, several studies have shown that Glycyrrhiza glabra has the capacity to decrease cholesterol, triglyceride, and LDL while increasing HDL levels, suggesting its use in managing dyslipidemia (Murray, 2020; Awad, 2017). Moreover, Aksoy et al. (2012) indicated that extracts of licorice have significantly reduced serum creatinine, BUN, and urea. It also improved historical renal results in rat models of nephrotoxicity. However, there are contradictory findings as well. Excessive use of glycyrrhizin has been associated with side effects like pseudoaldosteronism, hypertension, and metabolic disturbances (Wahab et al., 2021). Although, licorice may be seen to possess therapeutic value but its effects on kidney is still not fully understood and sometimes debated. However, considering the CKD burden and the limitations of existing therapies, there is a need for safe, effective, and affordable nephroprotective agents. This study was conducted to explore the nephroprotective action of Glycyrrhiza glabra root extract in carbon tetrachloride (CCl_4) induces nephrotoxicity in male Wistar rats. The study evaluates the therapeutic efficacy of licorice extract by analyzing changes in serum renal

biomarkers and lipid profiles.

MATERIALS AND METHODS

This study employed a quantitative, in vivo experimental research design using a completely randomized controlled trial (RCT) model to investigate the nephroprotective potential of Glycyrrhiza glabra in a rat model of carbon tetrachloride (CCl_4)-induced renal toxicity. Comparative analysis was performed between control, toxin-exposed, and treatment groups.

Therefore, thirty healthy male Wister Albino rats were selected weighing 160-200g and maintained under routine laboratory conditions. The animals were provided with easy access to a standard pellet diet and clean water. After one week of acclimatization they were randomly divided into 4 groups (n=6), group I was the untreated control group, group II received only CCl_4 (0.8 mL/kg body weight in a 1:1 combination with olive oil, injected subcutaneously for 21 days); group III received both CCl_4 and Glycyrrhiza glabra aqueous extraction (30mL/kg); and group IV received only Glycyrrhiza glabra extract with same dosage.

However, on day 22 all animals were fasted overnight and killed under light ether anaesthesia. Blood samples were collected from carotid artery puncture, allowed to clot, and centrifuges at 3000 rpm for 15 minutes to separate serum. Kidneys were dissected out with care, washed in cold saline and fixed in 10% formalin for histopathological examination.

The aqueous extract of Glycyrrhiza glabra was prepared by boiling 100 g of dried, powdered roots in 1 L of distilled water at 60 °C for 2 hours. The mixture was filtered, and the extract was concentrated using a rotary evaporator and stored at 4 °C in an amber glass container.

However, renal function biomarkers, including serum creatinine, urea, and blood urea nitrogen (BUN), were analyzed using commercial diagnostic kits (Jaffe's method for creatinine and Berthelot method for urea) according to the manufacturer's protocols. BUN values were calculated using standard conversion formulas. Lipid profile parameters, including total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), were measured using enzymatic colorimetric endpoint assays. LDL-C was calculated using the Friedewald equation.

All data were presented as mean , standard error of the mean (SEM). Statistical analysis was performed using SPSS version [Insert version], and group comparisons were made using independent t-tests. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The final body weight of rats in the control group and the Glycyrrhiza glabra-treated group increased as compared with the initial body weight. Whereas, the body's final weight decreased in the groups treated with CCl_4 and licorice+CCl_4 as compared to their initial weights.

However, final body weight of group II was reduced ($P<0.05$) as compared to the group I i-e control. The final weight of group III (CCl_4+licorice) remains the same ($P>0.05$) in comparison to group I but increased ($P>0.05$) as compared to group II. The final weight of group IV decreased ($P>0.05$) as compared to groups I and III. It also decreased ($P<0.05$) compared to group II (Table 1).

In group II, III, and IV, the weight of both right and left kidneys as well as relative weight of each kidney, exhibits an increase as compared to group I. (Table 1)

TABLE 1. COMPARISON OF INITIAL AND FINAL BODY WEIGHT

	Control	CCl_4 Treated[1]	CCl_4+Licorice[1,2] Treated	Licorice[1,2,3] Treated
Initial Body Weight	150.6 ± 0.33	160 ± 3.60	163.8 ± 23.98	144.6 ± 7.37
Final Body Weight	157.33 ± 5.36	142 ± 3.51^{x}	157.6 ± $28.5^{y,x}$	152.3 ± $3.1^{y,x,y}$
Left Kidney Weight	0.40 ± 0.04	0.49 ± 0.03^{y}	0.56 ± $0.03^{x,x}$	0.75 ± $0.03^{x,x,x}$
Right Kidney Weight	0.40 ± 0.02	0.47 ± 0.23^{y}	0.54 ± $0.03^{y,y}$	0.73 ± $0.04^{x,x,x}$
Relative Weight of Left Kidney	0.25 ±0.02	0.33 ± 0.03^{y}	0.41 ± $0.05^{x,y}$	0.49 ± $0.01^{x,x,x}$
The relative weight of the Right Kidney	0.24 ± 0.01	0.32 ± 0.02^{y}	0.34 ± $0.05^{y,y}$	0.47 ± $0.03^{x,x,x}$

Statistical values are mean ± S.E.M, n=, significance is represented with symbols x= $P < 0.05$ significant, y= $P > 0.05$ non-significant. 1= compared with group I, 2= compared with group II,

3= compared with group III.

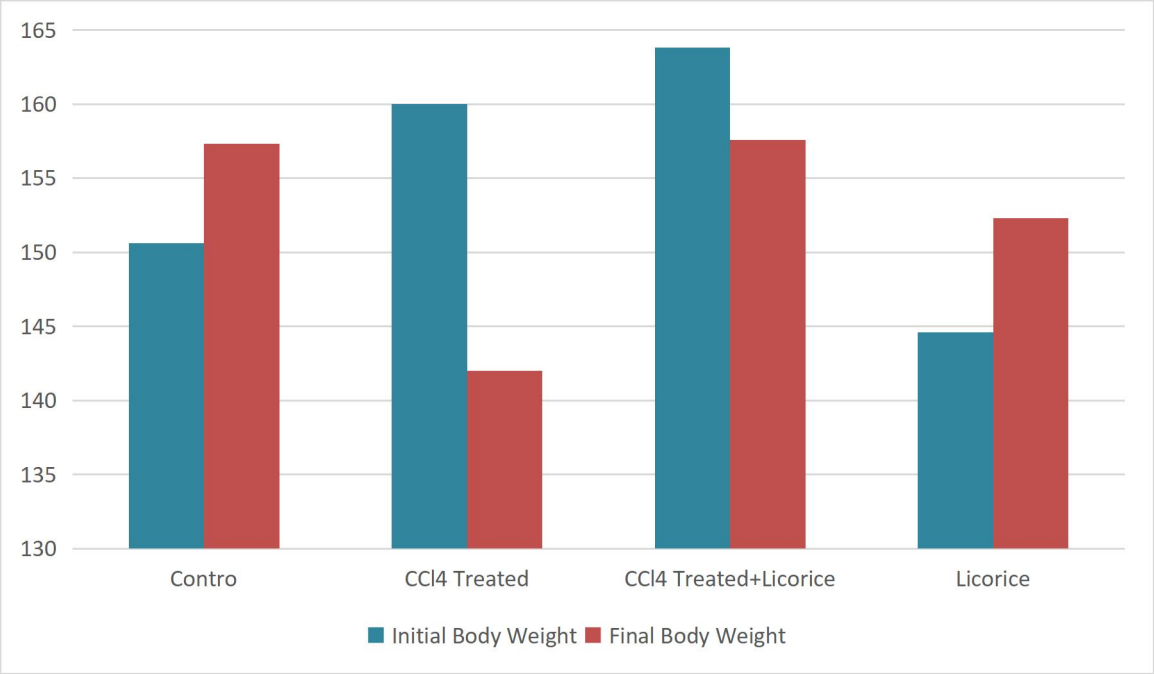


FIGURE 1: COMPARISON OF BODY WEIGHT IN CONTROL, CCL_4, GLYCYRRHIZA GLABRA + CCL_4 & GLYCYRRHIZA GLABRA TREATED GROUPS

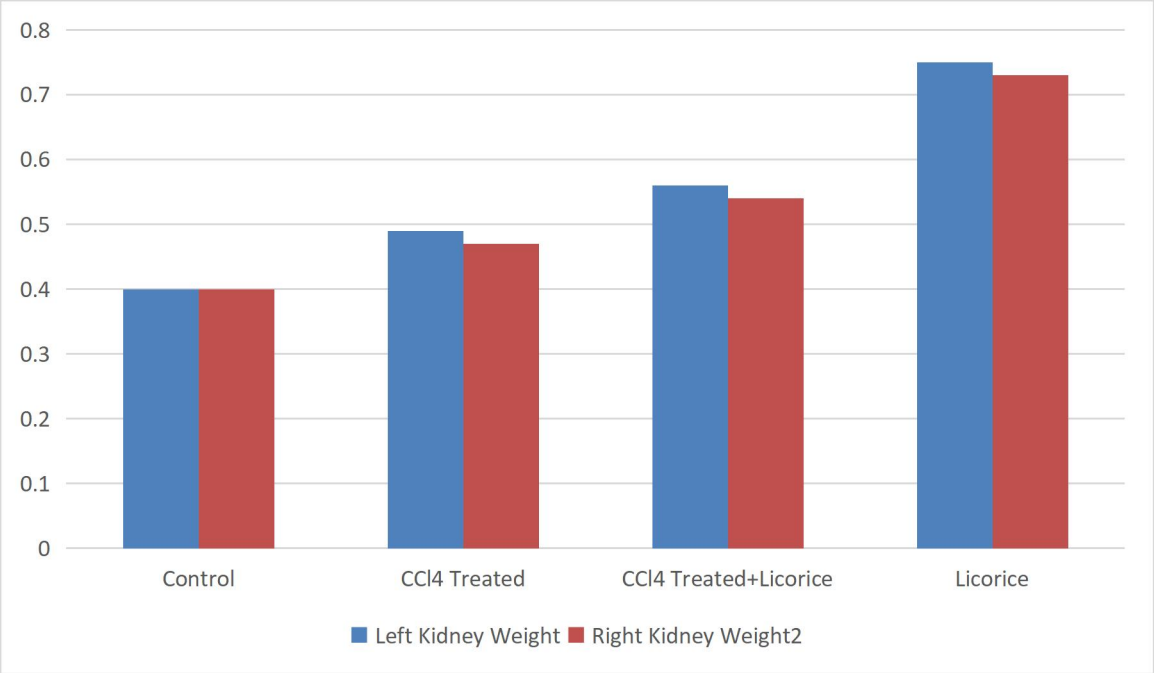


FIGURE 2: COMPARISON OF KIDNEY WEIGHT IN CONTROL, CCL_4, GLYCYRRHIZA GLABRA + CCL_4 & GLYCYRRHIZA GLABRA TREATED GROUPS

EFFECT OF CARBON TETRACHLORIDE AND GLYCYRRHIZA GLABRA ON KIDNEY FUNCTION TEST

The serum urea level increases in CCl4-induced, CCl4-induced+licorice-treated, and licorice-treated groups in relation to the control group. The serum urea level of the CCl4-induced group elevates ($P<0.05$) in contrast to the control. Secondly, the serum urea level of the CCl4-induced+ licorice-treated group increased ($P>0.05$) as compared to the control and decreased ($P>0.05$) when compared with the CCl4-induced group. In licorice treated group, serum urea increased as compared to the control ($P<0.05$) and CCl4-induced+licorice treated group ($P>0.05$). (Table 2)

Therefore, the BUN level of the CCl4-induced group significantly increased compared with the control group. The BUN level of the CCl4-induced+licorice treated group increased ($P>0.05$) as compared to the control group and decreased ($P<0.05$) from the CCl4-treated group. In Licorice Treated Groups, The BUN level significantly increased from the control group, remains equal ($P>0.05$) to CCl_4 induced group, and increased ($P>0.05$) from CCl_4 induced+licorice treated group comparatively. (Table 2)

However, the blood creatinine level of CCl_4 treated increased ($P>0.05$) from the control group.

In the CCl4-treated+Glycyrrhiza glabra-treated group, serum creatinine increased non-significantly ($P>0.05$) from control group and significantly ($P<0.05$) from CCl_4 treated group. (Table 2). Furthermore, the cholesterol level was reduced ($P<0.05$) in the CCl_4-induced group in comparison to the other groups. The cholesterol level was highest in control group in comparison with the other groups. The level of cholesterol increases significantly ($P<0.05$) in CCl4-induced+licorice-treated rats and licorice-treated rats in comparison to only CCl4-treated rats. The elevated ($P<0.05$) level of cholesterol was observed in licorice treated group in comparison to group I, II, and III. (Table 2)

However, the HDL-C level decreased ($P<0.05$) in CCl_4 induced group in comparison with control group. The HDL level in CCl_4 induced+ licorice treated group was also reduced ($P>0.05$) in relation to control group but increased ($P>0.05$) as compared with CCl_4 treated group. On the other hand, when the licorice administered group is compared with the control group, HDL level significantly decreased ($P>0.05$) but it was increased ($P>0.05$) in comparison to CCl_4-treated, and CCl_4 +licorice-treated groups. (Table 2)

Although, the LDL level raised ($P<0.05$) in CCl_4 treated group as compared with the control group. In the CCl_4 induced + licorice-treated group, the LDL level is increased ($P>0.05$)

compared with the control and CCl_4 +licorice treated group. The LDL-C level of licorice treated group increased significantly ($P<0.05$) as compared to the control group. It was noticed that LDL level was also increased ($P>0.05$) in licorice treated group from the CCl_4 Treated, CCl_4 Treated+ Glycyrrhiza glabra treated group. (Table 2)

TABLE 4.2: COMPARISON OF SERUM BIOMARKER OF KIDNEY FUNCTION TEST AND LIPID PROFILE

	Control	**CCl_4 Treated[1]**	**CCl_4+Licorice Treated [1,2]**	**Licorice Treated [1,2,3]**
Urea (mg/dl)	18.5 ± 2	27 ± 5^{x}	$25.2 \pm 6.58^{y,y}$	$26 \pm 2^{x,y,y}$
BUN (mg/dl)	8.64 ± 0.93	12.77 ± 2.04^{x}	$11.77 \pm 3.44^{y,x}$	$12.77 \pm 0.93^{x,y,y}$
Creatinine (mg/dl)	0.31 ± 0.09	0.5 ± 0.2^{y}	$0.4 \pm 0.15^{y,x}$	$0.5 \pm 0.1^{x,y,y}$
Cholesterol	138 ± 2.64	111 ± 6.55^{x}	$122.6 \pm 4.92^{x,x}$	$128.3 \pm 3.51^{x,x,x}$
HDL-C	57 ± 2	24.3 ± 3.51^{x}	$25.8 \pm 3.27^{y,y}$	$27.6 \pm 2.51^{y,y,y}$
LDL-C	40 ± 2	66 ± 5.56^{x}	$69.8 \pm 3.42^{y,y}$	$72.6 \pm 4.04^{x,y,y}$

Statistical values are mean ± S.E.M, n=, significance is represented with symbols x= $P < 0.05$ significant, y= $P > 0.05$ non-significant. 1= compared with group I, 2= compared with group II, 3= compared with group III. BUN (Blood Urea Nitrogen).

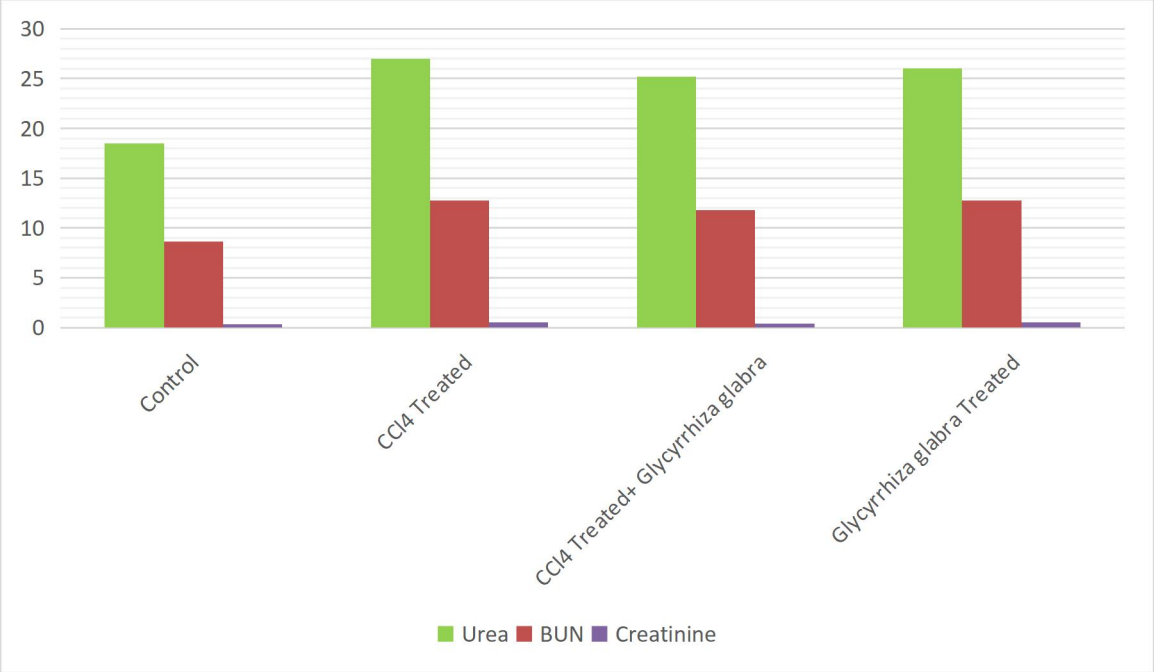


FIGURE 3: COMPARISON OF SERUM BIOMARKER UREA, BUN, CREATININE IN CONTROL, CCL_4 TREATED, CCL_4 TREATED+GLYCYRRHIZA GLABRA, AND GLYCYRRHIZA GLABRA TREATED GROUPS

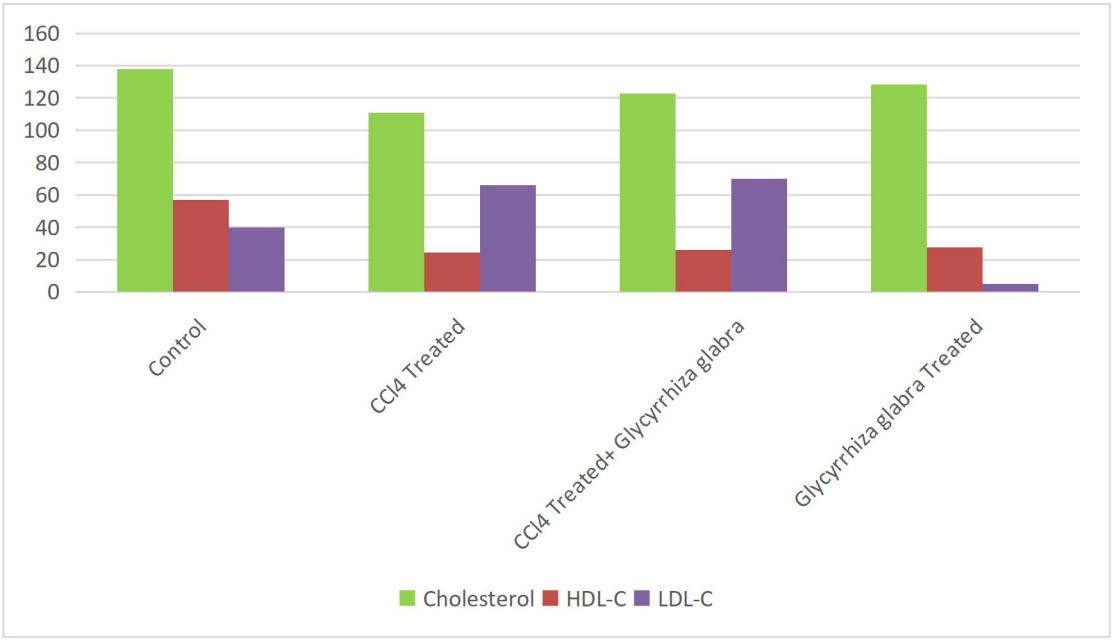


FIGURE 4: COMPARISON OF LIPID PROFILE CHOLESTEROL, HDL-C AND LDL-C IN CONTROL, CCL_4 TREATED, CCL_4 TREATED+GLYCYRRHIZA GLABRA, AND GLYCYRRHIZA GLABRA TREATED GROUPS

DISCUSSION

The kidney is a vital organ that not only filters the blood but also regulates body homeostasis to maintain the normal functioning of the body. Water and many electrolytes are conserved by the kidney to maintain the negative body balance to positive body balance (Finco, D.R., 1997). The non-communicable disease (NCDs) surpasses communicable disease as the primary cause of morbidity and premature mortality globally. The four common NCDs are CVDs, cancer, diabetes, and chronic respiratory disease. Kidney disease is an important determent of poor health in diabetes and CVD (Couser, W.G. et al., 2011). For the past few years, kidney damage and loss of its functioning is of great concern due to the elevated burden of public health. Among the kidney diseases, chronic kidney diseases are of the greatest prevalence at more than 10% (Kovesdy, C.P., 2022). It is independent of age, sex, and ethnicity (Eckardt, K. et al., 2013). Among several renal abnormalities, chronic kidney disease is common. CKD is defined as a glomerular filtration rate lower than 60ml/min/1.73 for three months. CKD is a progressive disease that affects more than 10% of the general population of the world and is of high prevalence among the elderly, women, and people with high blood pressure and diabetes (Kovesdy, 2022).

Oxidative stress is lethal for cells because of disturbance in the oxidant balance due to the production of ROS and RNS (Daenen, K. et al., 2018). Oxidative stress plays a crucial role in the pathophysiology of several renal diseases. Carbon tetrachloride induces cellular damage by releasing free radicals that cause oxidative stress. CCl_4 is a toxic substance and can cause severe damage to living organisms. In mammals, organs like kidneys, lungs, brains, and spleen were highly affected by CCl_4 toxicity. The free radical released by CCl_4 causes nephrotoxicity by lipid peroxidation and the build-up of dysfunctional proteins leading to kidney failure (Habashy, N.H. et al., 2021).

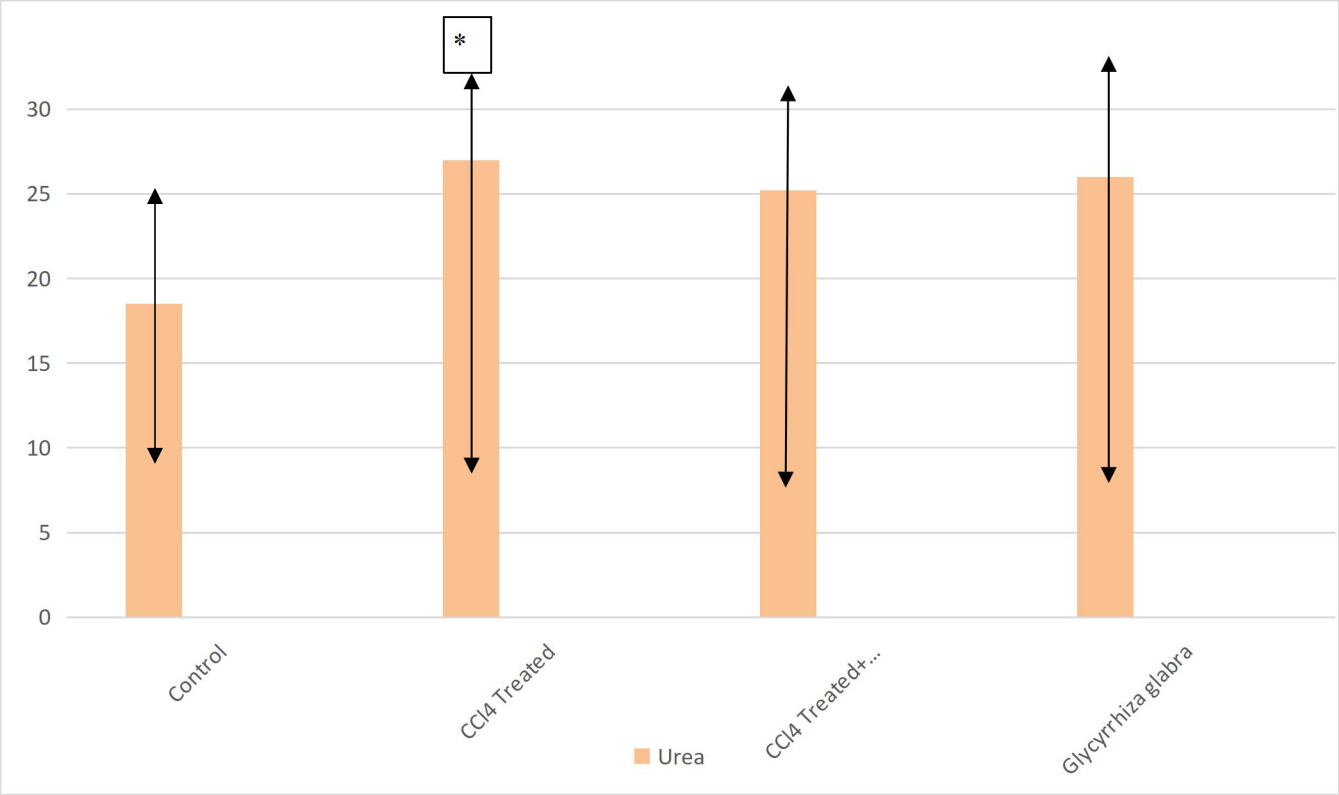
The use of herbal medication increased in the last three decades up to 80% of people in the world prefer using herbs as a treatment for primary healthcare purposes (Ekor, M. 2014). Different studies have proven that herbal extracts and pure chemicals obtained from plants protect the organs against carbon tetrachloride toxicity by altering the anti-oxidant enzyme level. Glycyrrhizin glabra, known as licorice, has been used since ancient times to treat several diseases mostly related to respiratory, skin, and reproductive issues (Hasan et al., 2021). The licorice roots are also known to contain therapeutic effects such as anti-inflammatory, anti-cancer, and anti-oxidant effects. Several studies suggested the use of licorice against

hepatotoxicity and also on other organs (Murray, M. 2020).

However, studies have proved the beneficial impact of glycyrrhiza glabra on different organs but the effect of the herb on the kidney was not fully explained. We aim to examine the therapeutic effect of glycyrrhiza glabra on the kidney against the nephrotoxicity induced by carbon tetrachloride in male rats. The biomarker for nephropathy, serum urea, creatinine, and BUN level as well as the lipid profile were estimated. The glycyrrhiza glabra is proven to possess anti-obesity characteristics. According to a study, the consumption of licorice for two months causes 1.2% and 2.8% of weight loss in men and women respectively (Murray, M. 2020). The difference in initial and final body weights is also observed. The control group gains weight. The final body weight drops ($P<0.05$) in CCl_4 toxicity induced group in contrast to the control. In the carbon tetrachloride treated+licorice treated group, the body weight remains the same ($P>0.05$) as compared to the control group but increased ($P<0.05$) from the CCl_4 treated group. The body weight of the licorice group decreases non-significantly ($P>0.05$) from the control and CCl_4 treated+licorice group but increased significantly ($P<0.05$) from CCl_4 treated group (Table 1). The exact reason for the increase in body weight is not clear. However, it can be taken into account that licorice lead to mineralocorticoid excess that increases the cortisol level by inhibiting the 11β-HSD which further stimulates the appetite, especially for fatty and carb-rich foods. It results in the deposition of fats in the visceral and truncal region thereby increasing body weight (Sonita, B. et al., 2008).

Creatine and urea are the metabolic waste products that are needed to be removed by the body. After the administration of CCl_4, the level of blood urea nitrogen (BUN) and creatinine increases because of slowed kidney functioning (Table 4.2). Many studies proved that carbon tetrachloride is known to significantly increase serum urea and creatinine level and induce nephrotoxicity (Khan et al., 2009).. The use of licorice proved to be beneficial against nephrotoxicity as it decreases the serum urea, creatinine, and uric acid (Aksoy et al., 2012).

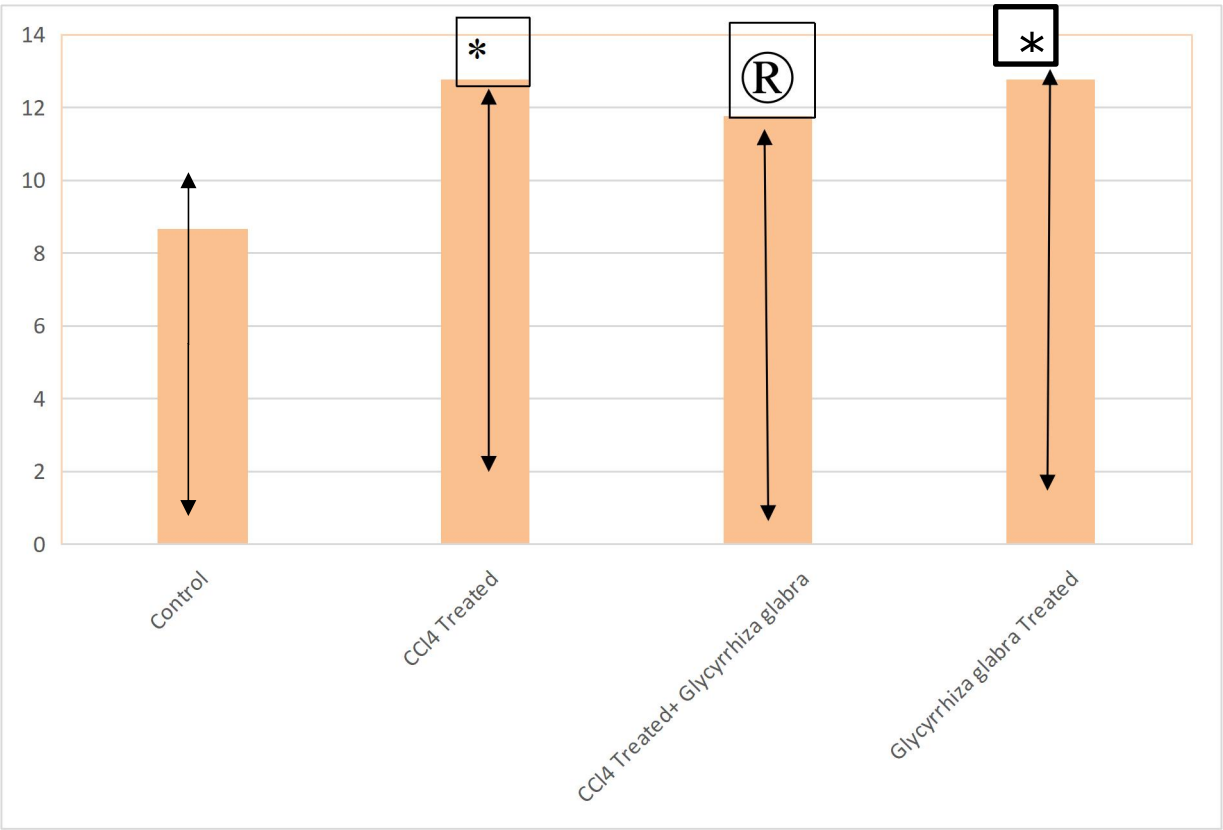
The serum urea level in CCl_4 treated group rose ($P<0.05$) as compared to the control group. In the CCl_4+licorice treated group; urea level increased significantly ($P>0.05$) from control group but decreased ($P>0.05$) when compared with only CCl_4 treated group. In licorice administered group, the serum urea level was found to be elevated ($P<0.05$) from the control group, decreased ($P>0.05$) from CCl_4 treated group, and increased ($P>0.05$) from CCl_4 treated+licorice treated group (Table 2).



* = P-Value showed statistical significance when compared with control group

FIGURE 5: COMPARISON OF SERUM UREA IN CONTROL, CCL_4 TREATED, CCL_4 TREATED+GLYCYRRHIZA GLABRA, AND GLYCYRRHIZA GLABRA TREATED GROUPS

The BUN level in CCl_4 treated group is noticeably high ($P<0.05$) from the control group. In the CCl_4 treated+licorice group, the BUN level is also elevated ($P>0.05$) as compared to the control group but less ($P<0.05$) than CCl_4 treated group. The level of BUN is also monitored in the licorice-treated group and compared with group I, II, and III. It was found that the BUN level in licorice treated group increased ($P<0.05$) from the control group. The BUN level in licorice treated group was non-significantly similar ($P>0.05$) to the BUN level of CCl_4 treated group. The level of BUN in licorice treated group was increased ($P>0.05$) as compared to the CCl_4 +licorice-treated group. (Table 2)

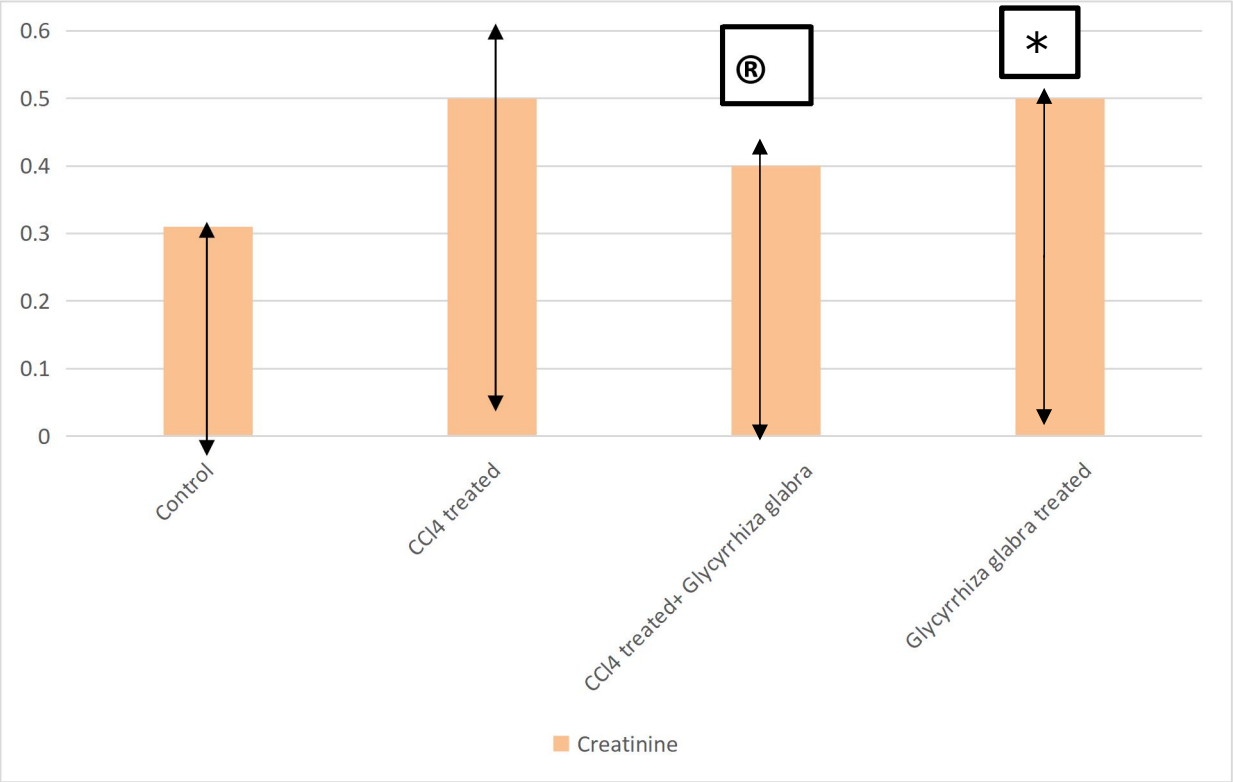


* = P-value showed statistical significance when compared with control group

® = P-value showed statistical significance when compared with the CCl4-treated group

FIGURE 6: COMPARISON OF BUN IN CONTROL, CCL_4 TREATED, CCL_4 TREATED+LICORICE TREATED, AND LICORICE TREATED GROUPS

The level of creatinine was also monitored in all the groups. The serum creatinine level was found to be elevated (P>0.05) in the CCl4-induced toxicity group than the control group. In the CCl4-induced+licorice treated group, serum creatinine was increased (P>0.05) in relation to control group and slightly reduced (P<0.05) from CCl_4 treated group. In a licorice treated group, when compared with the control serum creatinine level was found to be elevated (P<0.05), in comparison with CCl_4 treated group it remains equal (P>0.05), and elevated (P>0.05) from CCl_4 +licorice treated group. (Table 2).



* = P-value showed statistical significance when compared with control group

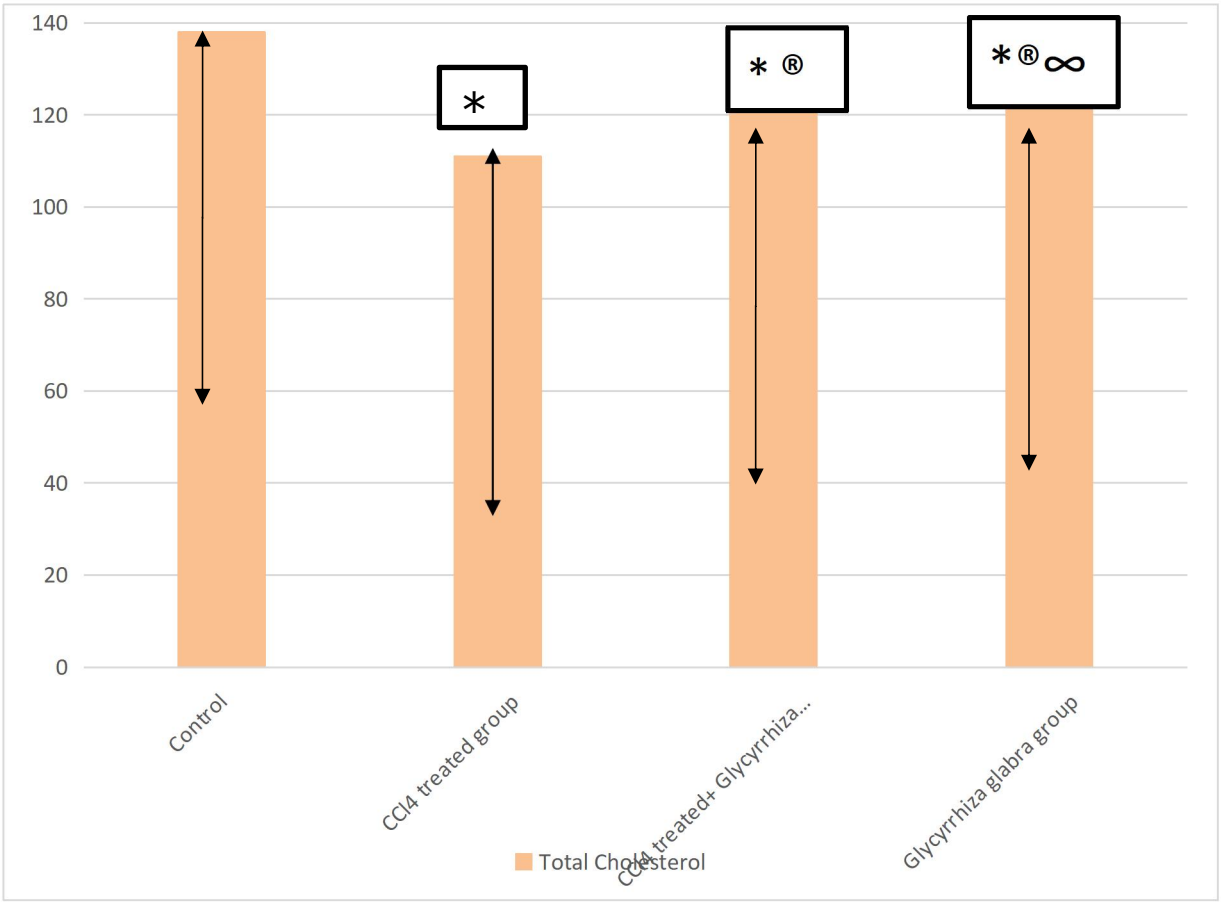
® = P-value showed statistical significance when compared with the CCl4-treated group

FIGURE 7: COMPARISON OF SERUM CREATININE IN CONTROL, CCL_4 TREATED, CCL_4 TREATED+LICORICE, AND LICORICE TREATED GROUPS

The increased level of serum urea, creatinine, and BUN in the CCl_4 treated group is because of carbon tetrachloride-induced toxicity that causes nephrotoxicity by lipid peroxidation, accumulation of proteins, and the main reason for plasma membrane damage that further leads to chronic renal injuries (Khan et al., 2009). Due to these changes the kidney losses its physiological abilities and failed to remove urea and creatinine from the blood. The elevated level of urea and creatinine in the licorice administered group is possible because glycyrrhizin can mimic the activity of the hormone aldosterone and cause pseudo-hyperaldosteronism, which can further cause hypokalemia, sodium reabsorption, fluid retention, electrolyte imbalance and increase blood volume. The hypokalemia and electrolytic imbalance may indirectly affect serum urea and creatinine by increasing the blood pressure that put strain on kidney and reduces its functioning (Sonita, B. et al., 2008). Although glycyrrhiza glabra increases the serum urea and creatinine, it is still less toxic that the carbon tetrachloride for the kidney which is why the level of urea, BUN, and creatinine in CCl_4 treated+licorice treated is

lower than CCl_4 treated group but remain higher than the control group.

Therefore, the lipid panel test that includes total cholesterol, HDL-C, and LDL-c was also conducted to observe the effect of glycyrrhiza glabra on lipid metabolism. However, the lipid panel test is not the biomarker for kidney disease but it is important to determine cardiovascular diseases and CVDs are the major risk factor of chronic kidney disease. The licorice has an anti-atherosclerotic and anti-hypercholesterolemic characteristic and reduces blood pressure, total cholesterol, and low-density lipoproteins and increases high-density lipoprotein cholesterol (Murray, M., 2020). The cholesterol level was decreased ($P<0.05$) in CCl_4 treated group as compared to control group. The cholesterol level in CCl_4+licorice treated group reduced significantly ($P<0.05$) from the control group and increased ($P<0.05$) from CCl_4 treated group when compared. In licorice treated group the cholesterol level decreased ($P<0.05$) in contrast to control group. In licorice treated group the cholesterol level was significantly increased ($P<0.05$) from CCl_4 treated group and CCl_4+licorice treated group. (Table 2)



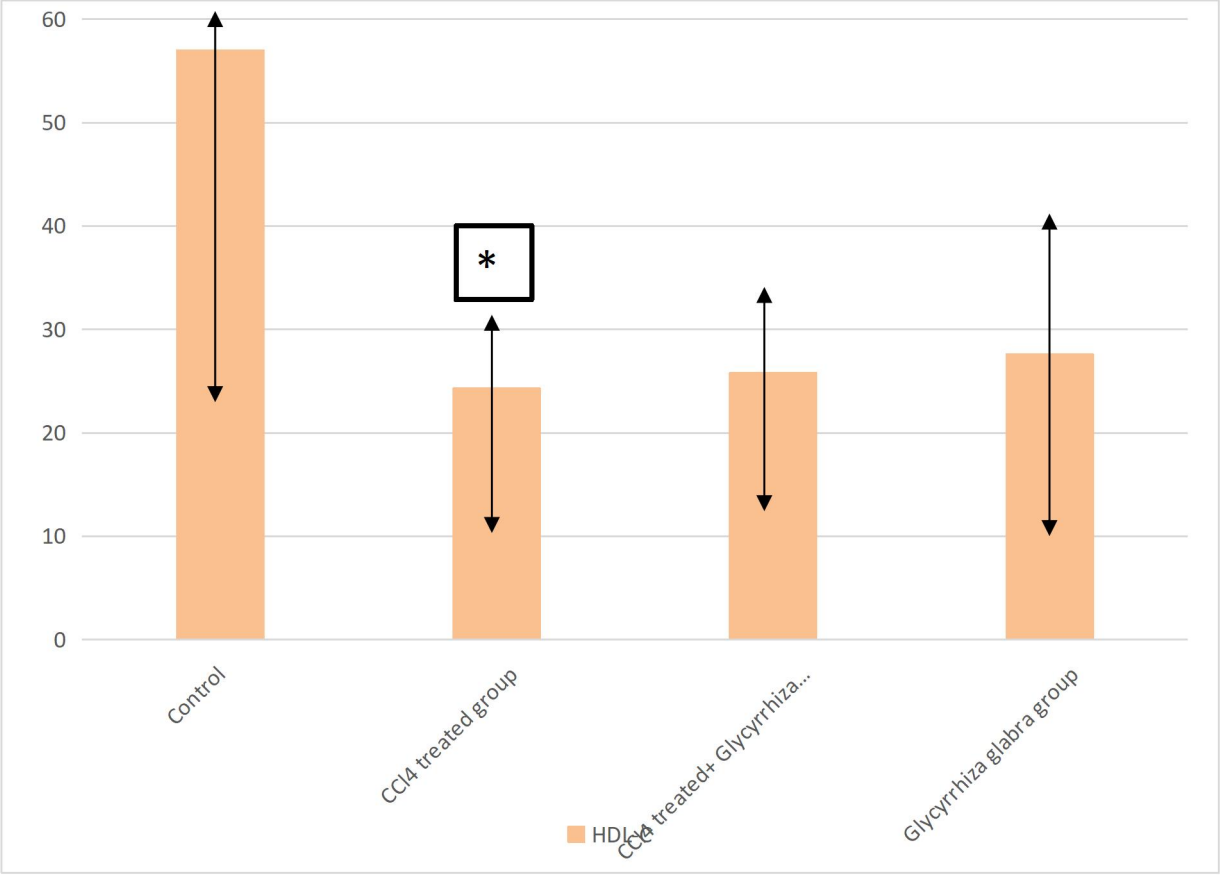
* = P-value showed statistical significance when compared with control group

® = P-value showed statistical significance when compared with CCl4-treated group

∞ = P-value showed statistical significance when compared with CCl_4 Treated+Glycyrrhiza glabra treated group

FIGURE 8: COMPARISON OF TOTAL CHOLESTEROL IN CONTROL, CCL_4 TREATED, CCL_4 TREATED+GLYCYRRHIZA GLABRA, AND GLYCYRRHIZA GLABRA TREATED GROUPS

The HDL level in CCl_4 treated group decreases significantly ($P<0.05$) as compared to the control group. In CCl_4 +licorice treated group the level of HDL decreased as compared to control group and showed increased results in comparison with CCl_4+licorice treated group. The level of HDL decreased in the licorice treated group as compared to the control group but increased in contrast to the CCl_4 treated and CCl_4+licorice treated groups. (Table 2)

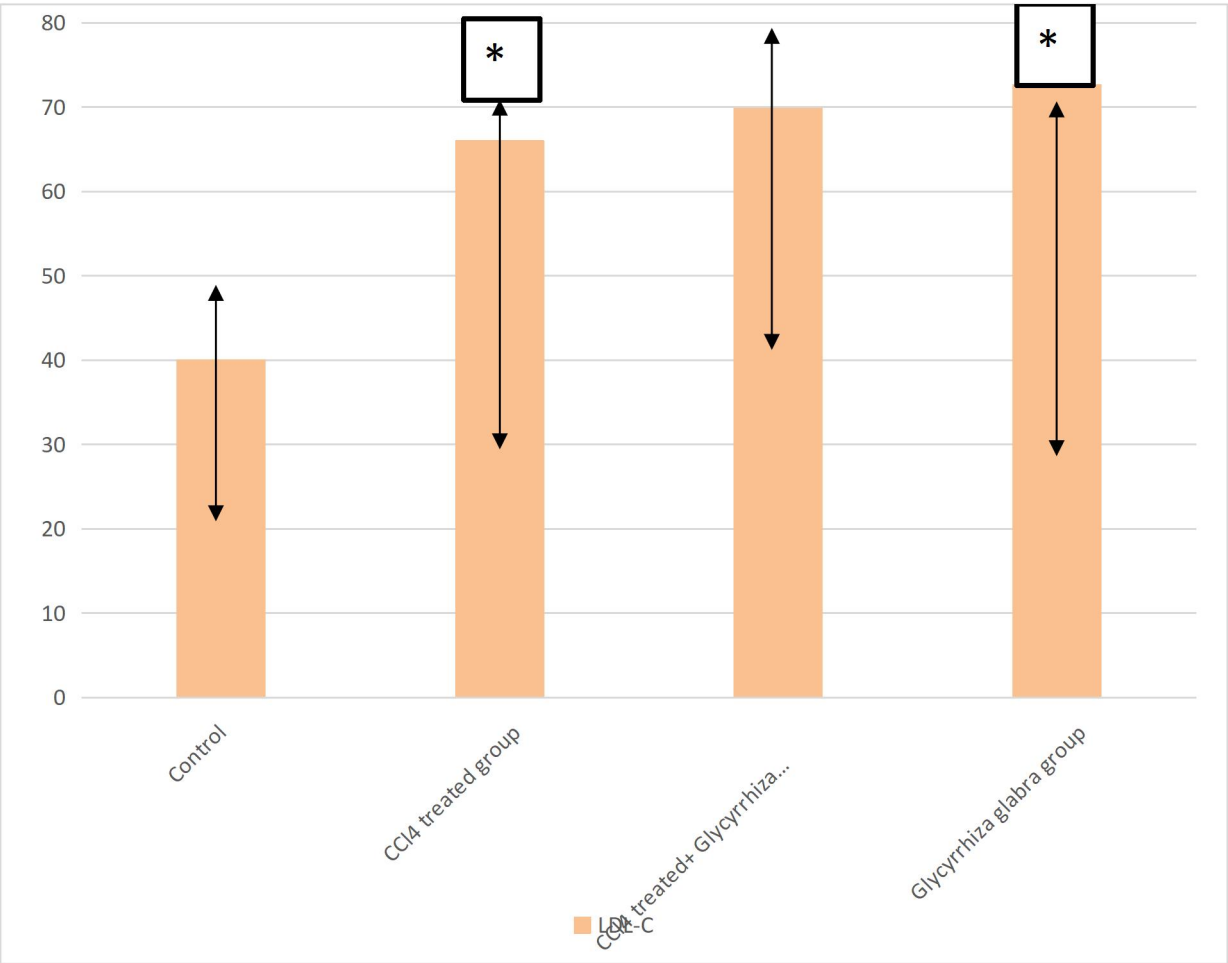


* = Significant P value when compared with the control group

FIGURE 9: COMPARISON OF HDL-C IN CONTROL, CCL_4 TREATED, CCL_4 TREATED+GLYCYRRHIZA GLABRA, AND GLYCYRRHIZA GLABRA TREATED GROUPS

The LDL level was reported to be increased in group II, III, and IV as compared to group I.

The licorice treated group showed a significant increase (P<0.05) in LDL level as compared to the control group and a non-significant increase (P>0.05) in LDL level of licorice treated group in comparison with the CCl_4 treated group and CCl_4+licorice treated group. (Table 2)



* = Significant P value when compared with the control group

FIGURE 10: COMPARISON OF LDL-C IN CONTROL, CCL_4 TREATED, CCL_4 TREATED+GLYCYRRHIZA GLABRA, AND GLYCYRRHIZA GLABRA TREATED GROUPS

There is a decrease in the cholesterol level but also reduced high-density lipoproteins and increased low-density lipoproteins that are considered to be good and bad cholesterol respectively. The results obtained were not fully according to our expectations and deviated from other studies as well. The licorice contains chemical glycyrrhizin that cause hypertension, hypokalemia and abnormal cortisol level. These changes can causes elevated cholesterol and LDL and decreased HDL that will lead to CVD and stroke (Sigurjosdottir, H.A et al., 2001).

The purpose of the study was to observe the therapeutic effect of licorice on renal damage in male Wister rats produced by carbon tetrachloride. The target was to analyze the effect of licorice root on body weight, biomarkers for the kidney including serum urea, BUN, and creatinine levels as well as lipid panel including cholesterol, HDL-C, and LDL-C to evaluate the risk of development of CVD, that is considered to be a potential risk factor of CKD. The results were not obtained according to the expectation as it does not report any major therapeutic effect of licorice on the renal injury at the given dosage. There are also contradictions in a lot of research regarding the use of glycyrrhiza glabra. The old studies suggested the positive impacts of licorice however, recent studies are also reporting the negative impact of glycyrrhiza glabra on the body system (Omar, H.R. et al., 2012). If there are positive effects reported then there are also some cases which report six patients were treated in ICU due to licorice toxicity, all showing symptoms of arterial hypertension, hypokalemia, and metabolic acidosis (Bangert K. et al., 2021). Therefore, a lot of research is required to prove the efficacy and safe dose of licorice before consuming it.

CONCLUSION

Renal diseases are one of the rapidly increasing non-communicable diseases in the world. It is common in developed countries due to an increase in cardiovascular diseases and diabetics which are considered to be the major risk factor of CKD. In developing countries, the prevalence of CKD is low because most of the cases are not reported and people can't afford the treatments. It can be concluded that the administration of glycyrrhiza glabra at this dosage does not show effectiveness against nephrotoxicity or the duration of dosage needs to be reduced. It can be lucrative if taken in low quantity for a short period. The glycyrrhiza or glycyrrhizic acid can be dangerous for long-term consumption as it causes swelling, reduce potassium and other electrolytes, causes arrhythmias, and water and sodium retention. However, more research is needed to be done on the effectiveness of glycyrrhiza glabra on kidney and lipid levels.

REFERENCES

Ullah, K., Butt, G., Masroor, I., Kanwal, K., & Kifayat, F. (2015). Epidemiology of chronic kidney disease in a Pakistani population. Saudi Journal of Kidney Diseases and Transplantation, 26(6), 1307. https://doi.org/10.4103/1319-2442.168694

Kovesdy, C. P. (2022). Epidemiology of chronic kidney disease: an update 2022. Kidney International Suppliments, 12(1), 7–11. https://doi.org/10.1016/j.kisu.2021.11.003

Couser, W. G., Remuzzi, G., Mendis, S., & Tonelli, M. (2011b). The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. Kidney International, 80(12), 1258–1270. https://doi.org/10.1038/ki.2011.368

Saeed, Z. A., & Hussain, S. A. (2012, June 1). Chronic kidney disease in Pakistan: an under-recognized public health problem. International Society of Nephrology. https://doi.org/10.1038/ki.2012.47

Omar, H. R., Komarova, I., Elghonemi, M. O., Fathy, A., Rashad, R., Abdelmalak, H. D., Yerramadha, M. R., Ali, Y., Helal, E., & Camporesi, E. M. (2012). Licorice abuse: time to send a warning message. Therapeutic Advances in Endocrinology and Metabolism, 3(4), 125–138. https://doi.org/10.1177/2042018812454322

Sontia, B., Mooney, J., Gaudet, L. and Touyz, R.M. (2008), Pseudohyperaldosteronism, Liquorice, and Hypertension. The Journal of Clinical Hypertension, 10: 153-157. https://doi.org/10.1111/j.1751-7176.2008.07470.x

Bangert K, Kluger MA, Kluge S, Janneck M. Life-Threatening Complications of Excessive Licorice Consumption. Dtsch Arztebl Int. 2021 Dec 27;118(51-52):890-891. doi: 10.3238/arztebl.m2021.0390. PMID: 35321787; PMCID: PMC8962506.

Visconti, L. M., Benvenga, S., Lacquaniti, A., Cernaro, V., Bruzzese, A., Conti, G., Buemi, M., & Santoro, D. (2016). Lipid disorders in patients with renal failure: Role in cardiovascular events and progression of chronic kidney disease. Journal of Clinical & Translational Endocrinology, 6, 8–14. https://doi.org/10.1016/j.jcte.2016.08.002

Li, X., & Wang, H. (2005). Chinese Herbal Medicine in the Treatment of Chronic Kidney Disease. Advances in Chronic Kidney Disease, 12(3), 276–281. https://doi.org/10.1016/j.ackd.2005.03.007

Nazari, S., Rameshrad, M., & Hosseinzadeh, H. (2017). Toxicological Effects of Glycyrrhiza glabra (Licorice): A Review. Phytotherapy Research, 31(11), 1635–1650. https://doi.org/10.1002/ptr.5893

Hasan, M. K., Ara, I., Mondal, M. S. A., & Kabir, Y. (2021b). Phytochemistry, pharmacological activity, and potential health benefits of Glycyrrhiza glabra. Heliyon, 7(6), e07240. https://doi.org/10.1016/j.heliyon.2021.e07240

Hasan, M., Sutradhar, I., Gupta, R., & Sarker, M. (2018). Prevalence of chronic kidney disease in South Asia: a systematic review. BMC Nephrology, 19(1). https://doi.org/10.1186/s12882-018-1072-5

Aksoy, N., Dogan, Y., Iriadam, M., Bitiren, M., Uzer, E., Ozgonul, A., & Aksoy, S. (2012). Protective and Therapeutic Effects of Licorice in Rats With Acute Tubular Necrosis. Journal of Renal Nutrition, 22(3), 336–343. https://doi.org/10.1053/j.jrn.2011.07.002

Murray, M. (2020). Glycyrrhiza glabra (Licorice). In Elsevier eBooks (pp. 641-647.e3). https://doi.org/10.1016/b978-0-323-43044-9.00085-6

Little, M. H., & McMahon, A. P. (2012, May 1). Mammalian Kidney Development: Principles, Progress, and Projections. Cold Spring Harbor Perspectives in Biology. https://doi.org/10.1101/cshperspect.a008300

Goyal, A. (2023, February 19). Acute Kidney Injury. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK441896/

Hsu, R. K., & Hsu, C. (2016). The Role of Acute Kidney Injury in Chronic Kidney Disease. Seminars in Nephrology, 36(4), 283–292. https://doi.org/10.1016/j.semnephrol.2016.05.005

Kellum, J. A., Romagnani, P., Ashuntantang, G., Lippi, G., Zarbock, A., & Anders, H. (2021). Acute kidney injury. Nature Reviews Disease Primers, 7(1). https://doi.org/10.1038/s41572-021-00284-z

Sales, G. F., & Foresto, R. D. (2020). Drug-induced nephrotoxicity. Revista Da Associacao Medica Brasileira, 66(suppl 1), s82–s90. https://doi.org/10.1590/1806-9282.66.s1.82

Awad, A. (2017). Assessment of Licorice (Glycyrrhiza glabra l.) Aqueous Extract on Lipid Profile in Hypercholestermic Rats. Journal of Agricultural Chemistry and Biotechnology, 8(2), 21-26. doi: 10.21608/jacb.2017.38422

Wahab, S., Annadurai, S., Abullais, S. S., Das, G., Ahmad, Ws., Ahmad, M. F., Kandasamy, G., Vasudevan, R., Ali, M. S., & Amir, M. (2021). Glycyrrhiza glabra (Licorice): A Comprehensive Review on Its Phytochemistry, Biological Activities, Clinical Evidence and Toxicology. Plants, 10(12), 2751. https://doi.org/10.3390/plants10122751

Finco, D. R. (1997). Kidney Function. In Elsevier eBooks (pp. 441–484). https://doi.org/10.1016/b978-012396305-5/50018-x

Eckardt, K., Coresh, J., Devuyst, O., Johnson, R. J., Köttgen, A., Levey, A. S., & Levin, A. (2013). Evolving importance of kidney disease: from subspecialty to global health burden. The Lancet, 382(9887), 158–169. https://doi.org/10.1016/s0140-6736(13)60439-0

Daenen, K., Andries, A., Mekahli, D., Van Schepdael, A., Jouret, F., & Bammens, B. (2018). Oxidative stress in chronic kidney disease. Pediatric Nephrology, 34(6), 975–991.

https://doi.org/10.1007/s00467-018-4005-4

Habashy, N. H., Kodous, A. S., & Abu-Serie, M. M. (2021). Targeting ROS/NF-κB signaling pathway by the seedless black Vitis vinifera polyphenols in CCl4-intoxicated kidney, lung, brain, and spleen in rats. Scientific Reports, 11(1). https://doi.org/10.1038/s41598-021-96008-0

Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in Pharmacology, 4. https://doi.org/10.3389/fphar.2013.00177

Khan, M. S., Rizvi, W. R., Khan, G. M., Khan, R. A., & Shaheen, S. (2009b). Carbon tetrachloride-induced nephrotoxicity in rats: Protective role of Digera muricata. Journal of Ethnopharmacology, 122(1), 91–99. https://doi.org/10.1016/j.jep.2008.12.006

Sigurjónsdóttir, H. Á., Franzson, L., Manhem, K., Ragnarsson, J., Sigurdsson, G., & Wallerstedt, S. (2001). Liquorice-induced rise in blood pressure: a linear dose-response relationship. Journal of human hypertension, 15(8), 549-552.