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Effect of *Juglansregia* and *Nasturtum officinalis* on biochemical parameters following toxicity of kidney by CCl4 in Wistar rats

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ABSTRACT

Background: Juglans regia contain compounds such as phenolic acids and flavonoids. Experimental studies demonstrated the Juglans regia were used in traditional medicine and has many beneficial effects. Use of Nasturtum officinalis has been reported to prevent cellular damage and elevate the level of antioxidant in the body. *Nasturtum officinalis* extract had Strong antioxidant properties and beneficial effects on reducing blood lipids. The purpose of this research is to determine the effect of *Juglans regia* and *Nasturtum officinalis* on biochemical parameters following toxicity of kidney by CCl4 in Wistar rats.

Materials and Methods: Sixty four Wistar rats were used and divided into 8 groups of eight animals each. Wistar rats were received intraperitoneally 50% CCl4 in olive oil. *Juglans regia* leaves and *Nasturtum officinalis* were orally administered before or after CCl4 in treatment groups twice a week for 31 days. After twenty one day, the blood samples were collected of all rats and serum levels of biochemical parameters were measured.

Results: In treatment groups, serum BUN, Bun, Alb, creatinine, Blood Urea, Uric acid levels were significantly decreased in comparison to their positive group. Also at the end of experiment, serum ALP, AST and ALT activity decreased significantly in treatment groups when compared with Positive group.

Conclusion: This study suggests that CCl4 induced liver and kidney damage in rats can be ameliorated by administration of extract of J. regia and N. officinalis.

Keywords: liver toxicity, kidney toxicity, Biochemical parameters, Juglans regia, Nasturtum officinalis

INTRODUCTION

The increase production of Reactive Oxygen Species (ROS) is cause by non-enzymatic reaction in the body and leads to various diseases (1). Different ROS such as hydrogen peroxide, singlet oxygen are unpaired electron and they are extremely reactive molecules (2). They could damage to the biological molecules including DNA, lipids and proteins (1). Furthermore, structural change of proteins leading to the loss of enzyme activity and they have not appropriate function against the free radicals (3). Oxidative stress created when the capacity of the enzymatic system is insufficient to neutralize the free radicals (4). With normal aging the oxidative damage increase to the cell membrane and cause degenerative disease such as cancer, heart and autoimmune disease (3). Recent studies have demonstrated it has been documented the ROS is implicated in many diseases from connective tissue to carcinogenesis (4). It has been shown that carbon tetrachloride (CCl4) cause triglyceride accumulation in the liver and could create the liver steatosis (5). CCl4 can disrupted the lipid metabolism and lead the increase the fat droplets in the liver cells (5). Further, it has been documented that CCl4 leads to production of free radicals that cause different pathological changes in the renal parenchyma such as cell membrane damage, nephrotoxicity and enhance lipid peroxidation (6). Antioxidant has enormous potentials to

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support the different cells from functional damage of toxic radical reaction, drug and oxidative stress (7). They have a key role in prevention of some disease such as cancer, aging, neurological disorder and cataracts (8). Recently, there has been increasing interest in the use of medicinal plants (9). Some medical plants used in many researches to protect the tissue against the oxidative stress and degenerative disease (10).

Traditionally, Juglans regia (J. regia) was used to treat diabetes, fever, skin diseases and rheumatic pains (11). J. regia leaves contained compounds such as phenolic acids and flavonoids, and the most important flavonoids present in the glands of the galactoside and quercetin pentoside derivatives, quercetin arabinoside, quercetin xyloside and quercetin umramnoside (11). N. officinalis is full of several protecting vitamins and can be used in the treatment of metabolic and chronic diseases (12). It also prevented cellular damage and increased the level of antioxidants (13). Further, N. officinalis is favorable for the evaluation of possible anti-inflammatory effects, antioxidant and antinociceptive properties (13). Since the combination of these two plants has been less studied in blood parameters this study conducted to determine the effects of Juglans regia and Nasturtum officinalis hydroalcoholic extract on blood parameters following toxicity of liver and kidney by CCl4 in Wistar rats.

MATERIAL AND METHODS

Sample Preparation and Extraction Procedure

The *J. regia* leaves and aerial parts of N. officinalis were obtained during June 2016 from Yasuj region, Yasuj, Iran. The authentication of the *J. regia* and N. officinalis plants were confirmed by a taxonomist in the Botany Department of Yasuj University and voucher specimens were 2-12 and 2-13 HMRC respectively. The plants washed thoroughly under running tap water, dried outside in the shade for 5 days and then ground into the fine powder using an electric mixer. The powdered of each plants material (500 g) was soaked in 70% ethanol at room temperature for 24 hours, a procedure repeated twice separately. The mixture was filtered using Whatman No. 1 filter paper. The solvents was removed in a rotary evaporator in vacuum at 57°C and dried for subsequent use. The gained extracts were kept at -20°C in freezer till the time of the experiment.

Animals

Sixty four Wistar rats (6 weeks age, 200-250 g) were procured from the animal house of the Yasuj University of Medical Sciences (YUMS). All experiments were performed according to the local ethical committee in YUMS, for use and care of animals. The animals were maintained under the standard conditions based on ad libitum at room temperature 20±5°C with a regular 12: 12 h light/darkcycle. The animals were divided randomly into eight groups of eight animals. Rats in Group I (negative control) received 0.5 ml/kg body weight of distilled water orally daily and 0.5 ml/kg olive oil by intraperitoneal injection twice a week. Animals in groups 2, 3, 4, 5, 6, 7 and 8 received 1 ml/kg body weight of olive oil and CCl4 solution twice a week and ratio 1: 1, by intraperitoneal injection. Group 2 (positive control) received 1 ml/kg body weight of olive oil and CCl4 solution twice a week and ratio 1: 1 by intraperitoneal injection. Group 3 and 4 treated with hydroalcoholic extract of J. regia leaves at a dose of 200 and 400 mg/kg gavage daily respectively. Group 5 and 6 treated with hydroalcoholic extract of N. officinalis leaves at a dose of 250 and 500 mg/kg gavage daily respectively. Group 7 treated with hydroalcoholic extract of J. regia leaves and N. officinalis leaves at a dose of 200 and 250 mg/kg gavage daily respectively. Group 7 treated with 50 mg/kg body weight Silymarin standard drug dissolved in normal saline by gavage daily. All groups treated for 31 days.

Serum Biochemistry

At the end of experimental, the animals were fasted overnight and sacrificed by cervical dislocation after collection of blood samples. Samples were transferred to Hospital's Laboratory for measuring BUN, albumin, Blood Urea, total protein, bilirubin total, bilirubin direct, creatinine, Uric acid and activity levels of AST, ALT and ALP. Blood samples were separated with high speed centrifuge at 3500 rev/minute for 10 minutes and serum was separated by Pasteur pipette for analysis of the biochemical assays.

Statistical Analysis

All data are expressed as mean ± SEM, One-way ANOVA was used for data analysis, followed by the Tukey test for post hoc analysis. A P-value<0.05 was considered to be statistically significant.

Table 1: *Effect of J. regia leaves and N. officinalis on serum activities of ALP, ALT, AST, BUN, Alb, creatinine, blood urea and uric acid on different groups*

Factors / Groups	ALP(IU/L)	AST(IU/L)	ALT(IU/L)	BUN (mg/dl)	Alb (mg/dl)	Creatinine (mg/dl)	Blood Urea (mg/dl)	Uric acid (mg/dL)
Negative Control	109.50±7.2 [a]	272.66±8.33[a]	686.00±17.28[a]	25.34±2.78[a]	4.10±1.38[a]	0.63±0.13[a]	24.61±1.95[a]	1.o6 ± 0.12[a]
Positive Control	162.50±15.75 [b]	454.66±9.41 [b]	957.16±20.65[b]	29.66±3.45 [b]	2.01±0.9 [c]	0.93±0.16 [b]	39.96±1.62 [b]	2.11 ± 0.78 [b]
Treatment 1	141.38±9.82[a]	421.33±6.23[b]	748.16±16.15[a]	27.16±2.83[a]	3.26±1.21[a]	0.74±0.15[a]	34.01±2.71 [b]	1.87 ± 0.86 [b]
Treatment 2	121.52±10.66[a]	344.83±8.49[a]	729.33±14.39[a]	26.83±3.37[a]	3.86±1.47[a]	0.69±0.12[a]	26.78±1.45[a]	1.57 ± 0.62[a]
Treatment 3	147.16±13.91[b]	350.16±10.07[a]	719.16±9.28[a]	28.02±3.21 [b]	3.11±1.59[a]	0.82±0.13[b]	33.20±1.21 [b]	1.91 ± 0.55 [b]
Treatment 4	120.73±12.05 [a]	306.33±8.82[a]	674.66±12.44[a]	26.16±2.93[a]	3.45±1.88[a]	0.71±0.10[a]	27.68±1.45[a]	1.26 ± 0.79[a]
Treatment 5	111.07±9.27 [a]	275.66±7.95[a]	759.16±8.75[a]	23.42±2.05[a]	3.77±1.25[a]	0.65±0.14[a]	25.70±1.90[a]	1.01 ± 0.33[a]
Treatment 6	118.33±12.63 [a]	288.50±8.06[a]	727.33±10.67[a]	21.00±1.77[a]	3.46±1.31[a]	0.73±0.11[a]	26.05±2.31[a]	1.39 ± 0.68[a]

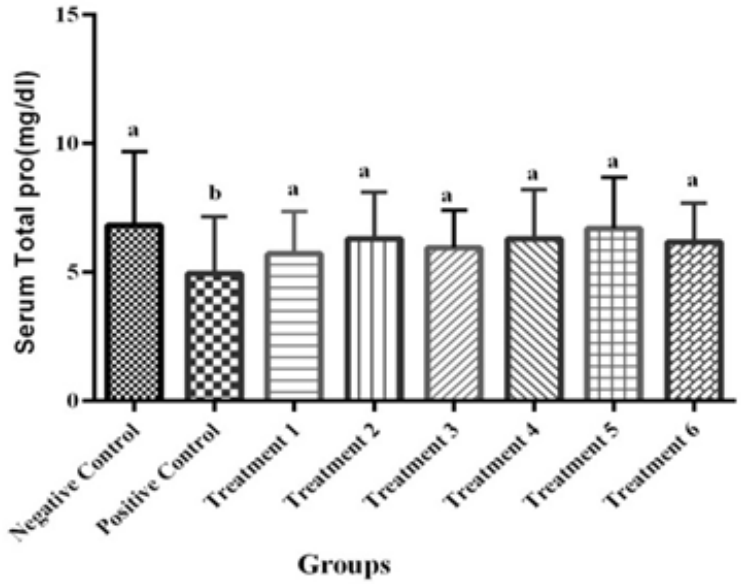

Figure 1: *The effect of J. regia leaves and N. officinalis on total protein levels in different groups (p <0.05). The vertical bars are showing mean values of bilirubin total. Lines above the bars indicate standard error (SEM). The word (b) indicates that P < 0.05 when comparing positive control group to negative control group. The word (a) indicates that P < 0.05 when comparing treated groups to positive control group*

RESULTS

Effects of *J. regia* leaves and *N. officinalis* on serum ALT, AST, ALT, Bun, Alb, creatinine, Blood Urea, Uric acid in all groups from various treatment groups are shown in **Table 1**. In Positive group, serum BUN, Bun, creatinine, Blood Urea, Uric acid, ALP, AST and ALT activity were significantly increased in comparison to their Negative group. At the end of experiment, serum ALP, AST and ALT activity decreased significantly in treatment groups of 2, 3, 4 and 5 when compared with Positive group ($p<0.05$) (**Table 1**).

In addition, **Table 1** shows changes in blood Albumin levels after 31 days. Albumin were decreased significantly in the positive control group (Group II) and brought to the near level as the negative control group in treatment groups ($p<0.05$). The hydroalcoholic extract of *J. regia and N. officinalis* had a significant effect on lowering blood Albumin ($p<0.05$) (**Table 1**).

The positive control group exhibited, the total protein level showed a significant decrease as compared to the negative group ($p<0.05$). In addition, the present findings indicate that administration of administrating 200 and 400 mg/kg BW of *J. regia* leaves and 250 and 500 mg/kg bw of *N. officinalis* and silymarin (50 mg/kg bw) lead to significant increase in serum total protein levels in treatment groups as compared with Positive group ($p<0.05$) (**Figure 1**).

The positive control group exhibited, the bilirubin direct level showed a very highly significant increase as compared to the negative group ($p<0.05$). The oral administration of J. regia and N. officinalis produced marked improvement of the bilirubin direct level of the treatment groups as compared with positive group ($p<0.05$) (**Figure 2**).

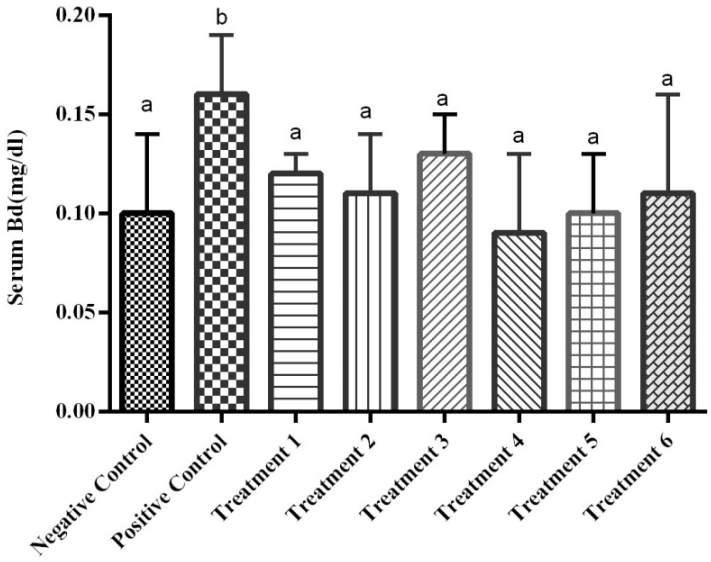

Figure 2: *The effect of J. regia leaves and N. officinalis on bilirubin direct level in different groups (p <0.05). The vertical bars are showing mean values of bilirubin total. Lines above the bars indicate standard error (SEM). The word (b) indicates that P < 0.05 when comparing positive control group to negative control group. The word (a) indicates that P < 0.05 when comparing treated groups to positive control group*

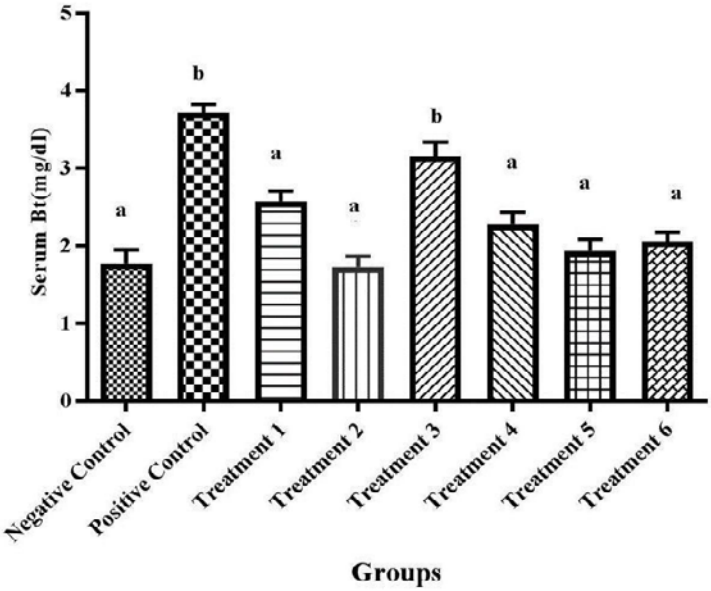

Figure 3: *The effect of J. regia leaves and N. officinalis on bilirubin total levelsin different groups (p <0.05). The vertical bars are showingmean values of bilirubin total. Lines above thebars indicate standard error (SEM). The word (b) indicates that P < 0.05 when comparing positive control group to negative control group. The word (a) indicates that P < 0.05when comparing treatment groups 1, 2, 4, 5, 6 and 7 to positive control group*

Also the positive control group exhibited, the bilirubin total level showed a very highly significant increase as compared to the negative group (p<0.05). The oral administration of J. regia and N. officinalis produced marked improvement of the bilirubin total level of the treatment groups as compared with positive group (p<0.05) (**Figure 3**).

DISCUSSION

In the present study the CCl4 treatment caused severe acute liver damage in rats, as evidenced by increased serum AST, ALT, BUN, Bun, Creatinine, Blood Urea, Uric acid and a decreased serum Alb level. Measurement of the activities of

serum marker enzymes, like AST, ALT and ALP, can make assessment of liver function (14). In this study, ALP, AST and ALT activities increased in the Positive group that was treated with CCl4 alone, indicating damage to the liver tissue by CCl4 induced hepatotoxicity ($p<0.05$).

It has been shown that among the different species, the highest activity of catalase and Peroxidase activity was observed in *N. officinalis* (15). The antioxidant potential of plant extracts is related with their phenolic content. Phenolic and flavonoid compounds in plants, have potent antioxidant activity, due to they have a functional group with antioxidant and scavenging activities (16). The hepatotoxic effects of CCl_4 are mostly caused by peroxidation of lipids and the presence of the free radicals (17). In the recent work, the hepatotoxicity was demonstrated by significant increases in the activity of AST, ALT and ALP enzyme markers in CCl_4 treated animals compared to normal group. Determination of ALP activity in plasma is a sign of hepatocyte function (18). In toxic group that treated with CCL4, the ALP was significantly increased due to damage to liver tissue. However, AST, ALP and ALT activities in the treated with *J. regia* leaves and *N. officinalis* groups were lower than that of the Positive group. This finding suggests that *J. regia* leaves and *N. officinalis* work to protect and repair liver tissue from injury. These results confirm those of previous reports (19, 20). Moreover, our findings suggest that dosages may be an important factor for curative effects of J. regia leaves and N. officinalis. In another study, oral administration of the hydroalcoholic extract of the N. officinalis leaf reduced the serum ALT and AST levels compared with high fat diet groups (20).

An obvious sign of hepatic injury is leakage of cellular enzyme into plasma (21). When the liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into blood stream. Their estimation in the serum is a useful quantitative marker for the extent and type of hepatocellular damage (22).

Decrease in serum bilirubin after treatment with the extract in liver damage indicated the effectiveness of the extracts in normal functional status of the liver. This is in agreement with the report by Ahmed *et al.* (23). Hypoalbuminemia and decline in total protein content can be deemed as a useful index of severity of hepatocellular damage. The lowered levels of Albumin and total protein recorded in the serum as well as in the liver of CCl4-treated rats reveal the severity of hepatopathy (24). From the result of this study, and other findings about antioxidant properties of silymarin, it can be firmly claimed that the antioxidant activity of silymarin leads to improve the change observed in sham rats (25).

Administration of hydroalcoholic extract of J. regia leaves increases the HDL and insulin and decreases cholesterol, triglycerides and LDL (16). The J. regia extract reduces systolic and diastolic blood pressure. It also increased plasma renin levels and significantly reduced the ratio of aldosterone to rennin (17). In another study, the effect of hydroalcoholic extract of N. officinalis leaves on the lipid profile in rats in diabetic rats has been investigated. The results showed that oral administration of hydroalcoholic extract of N. officinalis leaves (500 mg / kg) for 10 days reduced serum TC, TG and LDL-C levels respectively, the serum C-HDL level increased (20). Oral administration of the hydroalcoholic extract of the N. officinalis leaf reduced the serum ALT and AST levels compared with high fat diet groups. Based on these findings, the N. officinalis has a potential for cardiovascular protection and can be used to treat it (18-20).

CONCLUSION

According to the results suggest that the CCl4 induced liver damage in rats can be ameliorated by administration of hydroalcoholicextract of J. regialeaves and N. officinalisand improve biochemical factors near to normal.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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