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Study of ameliorative effect of *Allium cepa* essential oil and purple cabbage (*Brassica Oleracea Var. Capitata F. Rubra*) leaves extract on CCl_4 induced nephrotoxicity in rats

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Abstract: In human body kidney pair acts as an essential excretory organ which keeps up the acid/base equalization. The onion and cabbage compounds play vital role as nephroprotective, because toxicity induced free radicals are neutralized by antioxidant substances present in onion (*Allium Cepa*) and cabbage (*Brassica oleracea*). This research helps to find which compound has better nephroprotective activity. In this study 25 albino rats were used as models for studying carbon tetrachloride induced nephrotoxicity. The rats were divided into 5 groups. Control group received no treatment, 2nd group got only CCl_4, 3rd group received CCl_4 and purple cabbage leaves extract, 4th group received CCl_4 and onion oil (5 ml/kg) and 5th group received CCl_4 and both onion essential oil and purple cabbage leaves extract. At 15th day, rats were euthanized, blood and kidney samples of all groups were collected for histopathology of kidney and serum testing, includes BUN, creatinine, albumin and electrolytes levels. CCl_4 administration altered creatinine, albumin protein and blood urea nitrogen level of serum. It also changed the electrolytes' concentration in treated group animals as compared to control group rats. The extract of purple cabbage leaves and onion essential oil showed a protective effect against nephrotoxicity among treated groups of rats.

Keywords: Nephroprotective, carbon tetrachloride, onion essential oil, purple cabbage.

INTRODUCTION

Kidney is fit for the production of glucose by using glycerol, lactate and glutamine in human. The accomplishment of hormone like catecholamines, insulin and others regulate the assembly of glucose in paired kidney (Gerich, 2010). In the renal cortex, renal gluconeogenesis (GNG) takes place. The blood vessels in kidneys are positioned inside microscopic structural and funtional unit of kidney i.e. nephron. The function of nephron is to clean the blood by eliminating waste yields. When blood reaches towards nephrons, they are able to recognize the blood features by properly regulating the blood concentration as well as blood volume through filtration and reabsorption process (Boron, 2006).

CCl_4 doesn't occur naturally, it is a transparent liquefied compound with odorous smell which can be perceived at low levels. CCl_4 was first ever used for metal degreasing as cleanup, fabric-spotting, in extinguisher fluids, grain chemical and reaction medium. CCl_4 is very toxic for human health and high exposure to CCl_4 will cause liver, excretory organ and central nervous system injury and liver is particularly sensitive to CCl_4 (Adewole *et al*., 2007). CCl_4 inhalation or ingestion is also known to cause renal damage in humans (Jaramillo-Juárez *et al*., 2008). The CCl_4 caused excretory organ injury and therefore the transport capability of nephritic cells gets aggravated (Achliya *et al*., 2004).

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The harmfulness of CCl_4 depends upon development of the trichloromethyl (CCl_3) radicl. CCl_4 generates ROS in the body, in several tissues, liver, urinary organ, heart, lungs, testis, brain, and blood. Free radicals that initiate super molecule peroxidation cause damage to plasma membrane prompting numerous neurotic changes in acute and chronic excretory organ damages. The key protein related to CCl_4-incited nephrotoxicity is hemoprotein p450 that is confined within the animal tissue tube-shaped structure cells and therefore the swollen super molecule peroxidation is clear within the excretory organ brush border (Khan *et al*., 2009). The ninth leading reason behind death in Asian countries are due to renal disorder (Surekha *et al*., 2010).

The utilization of herbal drug for the aversion and treatment of different illnesses is always creating hype all through the world. Old writings recommend different therapeutic plants having nephroprotective action alongside various nephrotoxic specialties may lessen its poisonous quality. Onion is present inside the Allium hover of relatives and incorporate organosulphur mixes with cancer preventing agent, calming and antimicrobial properties. A fundamental lively component of onion is S-propenylcysteinesulphoxide. Onions possess antioxidant and antibacterial properties (Griffiths *et al*., 2002), antimutagenic (Singh *et al*., 2009) and cellular reinforcement sporting events (Dini *et al*., 2008). Onion oil contains important flavonoids that are essential for our body (Bonaccorsi *et al*., 2008). The restoratively most

noteworthy added substances of onion oil are the organosulfur containing compounds. The greatest rich flavonols in onions are quercetin 4'-O-β-D-glucoside and quercetin 3,4'-O-β-D diglucoside, which speak to over 85% of the full-scale flavonoid content (Al-Dosari, 2014).

Inside Brassicaceae family, purple cabbage speaks to a dietary source wealthy in phytochemicals, specifically (poly) phenolic, flavonoids, - carotene, lutein, and so on (Al-Dosari, 2014). It is a usually utilized vegetable – very advanced of bioactive atoms, whose impacts toward wellbeing security have been to some degree investigated, including renal assurance and cardiovascular ailments (Kim *et al*., 2008). It has hepatoprotective, neuroprotective, nephroprotective in diabetic rodents (Kataya and Hamza, 2008), mitigating and anticancer properties (Lin *et al*., 2008).

MATERIALS AND METHODS

Twenty-five female albino rats (Rattusnorvegicus) weighing about 200-300g were housed in the animal house of IMBB Department, The University of Lahore. These rats were kept under normal temperature and humidity conditions and were provided with food and water libitum.

Animal groups made for the study are arranged as follows:

- Group-I: Rats didn't receive any treatment.
- Group-II: Rats injected intra peritoneal (IP) 0.1872µl/g with olive oil and CCl_4 (1:1) for 14 days.
- Group-III: Rats orally received cabbage leaves extract 0.2802µl/g along with $CCl_4$for 14 days.
- Group-IV: Rats orally received onion oil at a volume of 2.5µl/g/day and CCl_4 for 14 days.
- Group-V: Rats received both (onion oil and purple cabbage leaves extract) the above mentioned treatments in same dosage for 14 days.

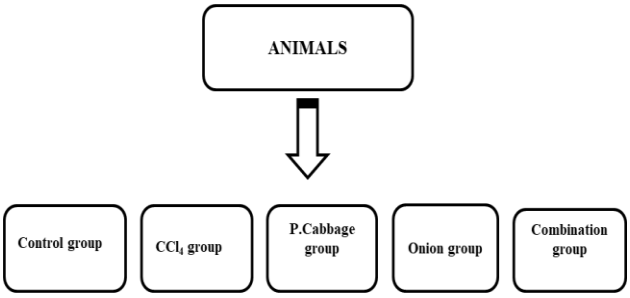


Fig. 1: Study groups: Control group with no treatment, 2nd group with only CCl_4, 3nd group with CCl_4 and onion oil, 4th group with CCl_4 and purple cabbage extract and 5th group with CCl_4, onion oil and cabbage extract.

The dosage details are given in table 1 which each group received for a period of 14 days.

After the decided period of experiment (14 Days), the rats were weighed and anesthetized with chloroform and euthanized to collect blood and renal tissue samples for further analysis and examination.

Table 1: Dosage details each group received for study period.

Treatment	CCl_4	P. cabbage	Onion Oil
Group I	NIL	NIL	NIL
Group II	0.1872µl/g	NIL	NIL
Group III	0.1872µl/g	0.2802µl/g	NIL
Group IV	0.1872µl/g	NIL	2.5 µl/g
Group V	0.1872µl/g	0.2802µl/g	2.5 µl/g

Renal histological examination was performed using Hematoxylin and Eosin staining after fixing the renal tissue with formalin. This examination was used as a tool to confirm the changes produced by toxicity of CCl_4. Post staining processing included fixation of tissue sections on slides for microscopy at different magnifications of 4x, 10x and 100x (Okolo *et al*., 2018).

ETHICAL APPROVAL

Ethical approval was granted by the University of Lahore Institutional Ethics Review Board vide letter # PZOOL02173-006.

STATISTICAL ANALYSIS

The biochemical test included Blood Urea Nitrogen (BUN), serum Albumin and Creatinine, serum electrolytes i.e., Sodium (Na^+), Potassium (K^+) and Chloride (Cl^-). All the statistical procedures were performed using IBM's SPSS (version 21.0). Data was extracted for representation as ±SEM (Standard Error of Mean). One-way ANOVA test was performed for comparison among various groups for multiple parameters. Statistical significance was laid down at $P \leq 0.05$.

RESULTS

Weight loss interventions

Body weight (BW) of experimental animals: orange bar shows the weight at 1st day of experiment while the pink bar depicts BW at day 14th in all five experimental groups.

During experimental period of 14 days, the weight of rats was observed on daily basis and fluctuation in body weight of all the groups were seen as shown in fig. 2. Body weight of control group increased at 14th day while the rest of groups those received CCl_4 abridged their weight at 14th day.

Renal morphology

At the 14th day when rats were euthanized, morphological alterations were also seen in all groups of rats except control group. No renal changes were seen in control group of rats as depicted in fig. 2.

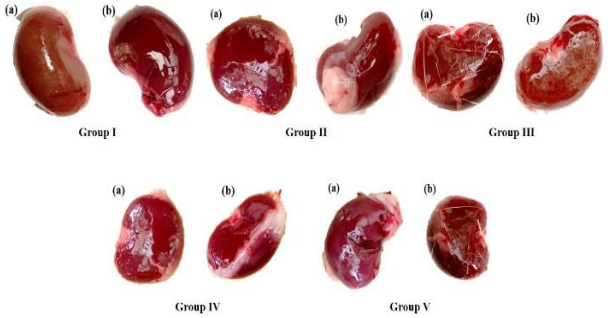


Fig. 2: Renal Morphology: (a) represents left and (b) represents right kidney in pair each inset for Group I to Group V respectively.

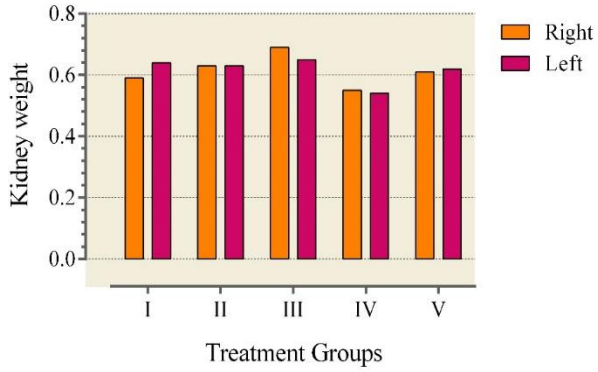


Fig. 3: Kidney weight (W) of experimental animals: orange bar shows the right (R) kidney weight at 14th day of experiment while the pink bar depicts left (L) kidney weight at day 14th in all five experimental groups.

At the day when rats were euthanized, both left and right kidneys were weighed. It was observed that the renal weight of all the treated groups increased to some extent as compared to group I which received no treatment as depicted in fig. 4.

Histopathological examination

Group I (Control Group)

During histological study, there were seen no evident pathological changes in the kidney of control group. Renal tubules maintained normal morphological features. Renal tissues also revealed no obvious pathological changes with normal tubules, glomeruli and nuclear to cytoplasmic ratio.

Renal histological examination was performed and used as a tool to examine the changes produced by toxicity of CCl_4. Renal morphology of CCl_4 treated rats showed acute venomous changes in renal tissue. The kidney parts showed momentous glomerular and tubular damage, interstitial swelling, glomerular basement thickening, bursting nuclei, tubular cell inflammation, medullary vascular blocking and normal to acute necrosis. Noticeable histological changes were also observed in renal medulla and renal cortex. Renal cortex was more severely affected. These results revealed that CCl_4 is toxic to kidney and induces morphological changes to kidney when toxicity is induced by CCl_4. Histological comparison of control and toxicity induced groups is shown in fig. 5 depicting major changes in renal tissue structure.

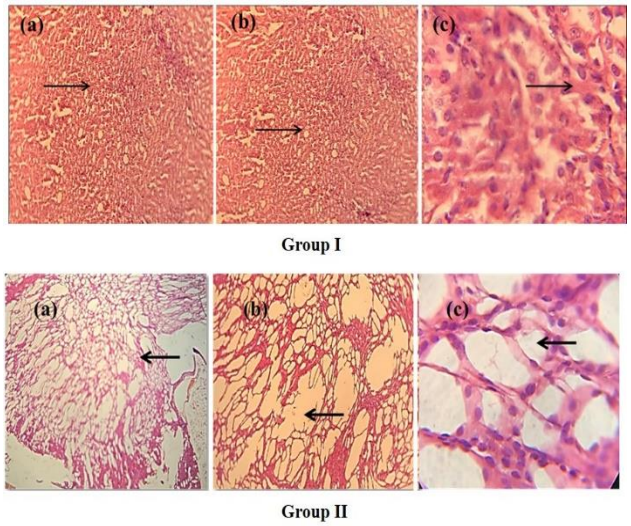


Fig. 4: Histological examination: Magnifications (a), (b) and (c) are 4x, 10x and 100x respectively in each inset for Group I to Group II. In Group I arrows represent normal tubules, glomeruli and nuclear to cytoplasmic ratio. Group II arrows depict kidney parts with tubular damage, glomerular basement thickening and bursting nuclei.

Histological exam of Group III i.e. treated with onion oil showed no morphological changes in kidney. Glomeruli were normal in this treated group's rats. This revealed that onion oil relieved tubular degeneration or interstitial inflammation as well as the effect on the medulla or renal cortex. Examination of Group IV i.e. treated with purple cabbage leaves extract showed slight morphological changes in kidney. These changes were incomparable to changes due to CCl_4. Group IV treated rats had slight lesions on renal appearance but the medulla and cortex were morphologically intact. Histological examination of Group V i.e. treatment with both compounds in combination reveals remarkable changes in epithelial tissues, again these changes were incomparable to CCl_4 treated group. In this case tubular dilation had occurred with no necrosis and diffused epithelial degeneration was found to be initiated. Histological comparison of all these treatment groups is shown in fig. 5.

Treatment groups III, IV and IV

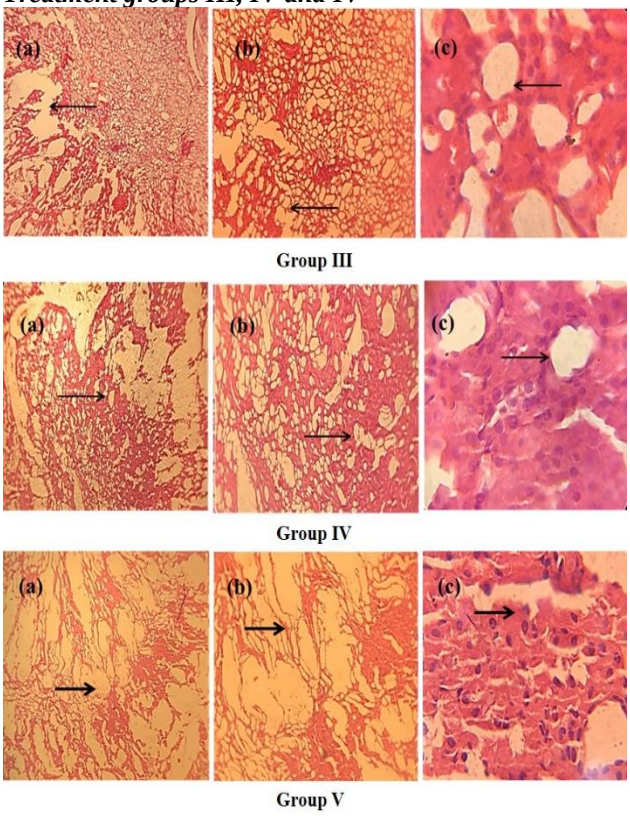


Fig. 5: Histological examination: Magnifications (a), (b) and (c) are 4x, 10x and 100x respectively in each inset for Group III to Group V. In Group III arrows represent near normal tubules, glomeruli and nuclear to cytoplasmic ratio evident of healing. Group IV arrows depict slight lesions in kidney parts with intact medulla and cortex. Group V arrows show tubular dilation and diffused epithelium.

Biochemical analysis

In case of Albumin the results showed statistically insignificant differences for group I, II, III and IV while comparing with Post Hoc analysis. On the other hand, same analysis revealed a significant difference of Group V (4.22 ± 0.09*) with all other groups. Group I had normal value of serum albumin. By administration of CCl_4 dose, the level of sreum albumin decreased in treatment Group II. In Group III having CCl_4 with Onion oil as treatment, the serum albumin level raised towards normal depicting positive effect of onion oil during CCl_4 induced toxicity. In Group IV having CCl_4 with purple cabbage leaves extract as treatment, serum albumin level reached to the same value as in control group which also indicated its nephroprotective role. In Group V having CCl_4 in combination with compounds, the albumin level significantly reached the normal value of albumin. These results predicted the most nephroprotective effect among all the groups. This trend is shown in fig. 7 while the values are given in table 2.

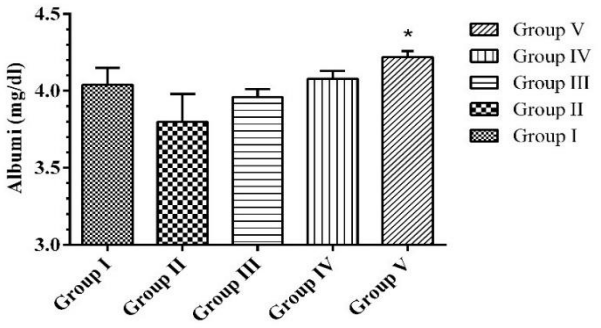


Fig. 6: Effect of CCl_4 on serum Albumin is represented graphically

Table 2: Albumin value in mg/dl

Treatment group	Albumin(mg/dl)
Normal group	4.04 ± 0.26
Treated with CCl4	3.80 ± 0.41
Onion oil & CCl4	3.96 ± 0.11
P. Cabbage & CCl4	4.08 ± 0.13
Combination	4.22 ± 0.09*

Blood urea nitrogen (BUN) levels

In case of BUN the results again showed statistically insignificant differences for group I, II, III and IV while comparing with Post Hoc analysis. BUN level of control group was not significantly disturbed by CCl_4 administration to animals. Group I and Group II showed almost the same levels but Group III and Group IV, both had increased value of BUN. It showed that purple cabbage increased BUN level in the body of rats which may lead to nephrotoxicity if the dose duration is prolonged.

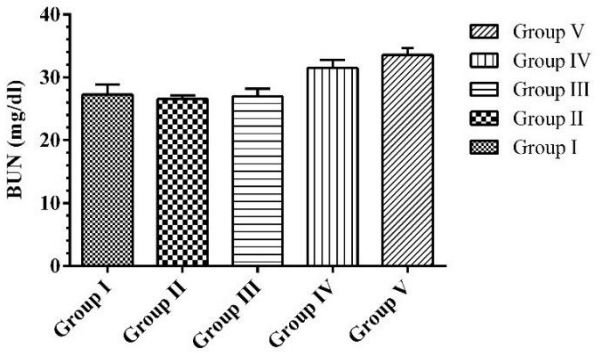


Fig. 7: Effect of CCl_4 on serum BUN is represented graphically.

Table 3: BUN value in mg/dl

Treatment group	Albumin (mg/dl)
Normal group	27.28 ± 3.49
Treated with CCl4	26.56 ± 1.35
Onion oil & CCl4	26.94 ± 2.75
P. Cabbage & CCl4	31.48 ± 2.92
Combination	33.60 ± 2.10
P- value	0.002

Serum creatinine level

In case of creatinine the results again showed statistically insignificant differences for group I, II, III and IV while comparing with Post Hoc analysis. Creatinine level was increased by CCl_4 administration as the Group II had elevated levels while Group I retained its normal range. In Group III, creatinine levels reduced to its normal value which showed nephroprotective activity of onion oil against CCl_4. Group IV and Group V both showed increased level of creatinine as compared to control group depicting no nephroprotective activity.

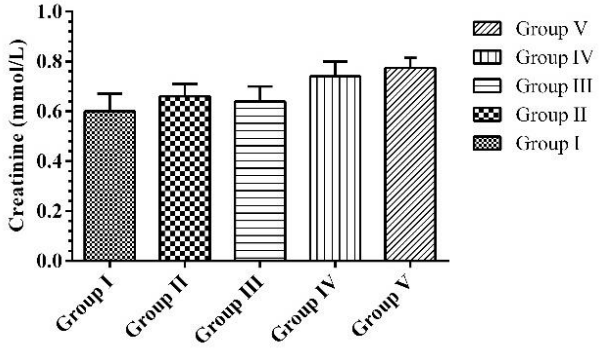


Fig. 8: Effect of CCl_4 on serum BUN is represented graphically.

Table 4: Creatinine value in mmol/L

Treatment group	Creatinine (mmol/L)
Normal group	0.60 ± 0.15
Treated with CCl4	0.66 ± 0.11
Onion oil & CCl4	0.64 ± 0.15
P. Cabbage & CCl4	0.74 ± 0.15
Combination	0.77 ± 0.09
P- value	0.322

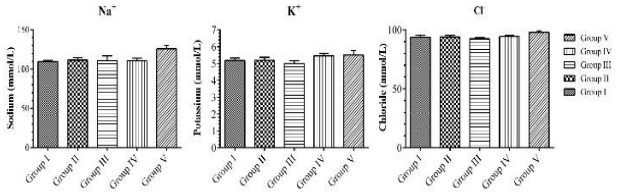
Fig. 9: Levels of Electrolytes (Na^+, K^+ and Cl^-) in serum represented graphically.

Table 5: Serum Electrolyte (Na+, K+ and Cl-) values in mmol/L

Treatment group	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
Normal group	109.60 ± 4.09	5.180 ± 0.35	93.760 ± 3.23
Treated with CCl4	111.60 ± 6.87	5.200 ± 0.39	94.000 ± 3.16
Onion oil & CCl4	110.80 ± 6.26	5.000 ± 0.38	92.280 ± 2.58
P. Cabbage & CCl4	110.40 ± 7.79	5.460 ± 0.35	94.200 ± 1.92
Combination	125.75 ± 8.77 *	5.500 ± 0.58	97.750 ± 2.98
P-value	0.14	0.343	0.106

Effect of electrolytes (sodium (Na^+), potassium (K^+), chloride (Cl^-)) in all groups

Electrolyte Na^+ in serum showed normal levels in Group I (control), Group II, Group III and Group IV. While Na^+ concentration was found to be raised in Group V. In case of K^+ electrolyte level in Group I and Group II had no obvious change in its concentration. While in Group III with onion oil treatment, a reduced value than control group was noticed. Concentrations in both groups IV and V were evidently raised in serum. In case of 3rd electrolyte i.e. Cl^- there was a marked difference of levels in Group III i.e. treated with onion oil in reference to Group I and Group II i.e. control and CCl_4 treatments respectively. While Groups IV and V both showed an increasing trend. All the levels are compared in fig. 10 while the values are shown in table 5.



DISCUSSION

Experimental study revealed a frequent decrease in serum albumin level in only CCl_4 treated group in comparison to control group where no marked changes were observed in serum albumin level. This shows that CCl_4 has its direct effect on liver due to which albumin level decreases in blood serum. The serum creatinine level of CCl_4 treated group is significantly raised in accordance to control group which leads to pyelonephritis (a urinary tract infection affecting one or both kidneys). In onion oil treated group, the increased level of creatinine was significantly dropped to a lesser content. This reveals about its nephroprotective activity towards CCl_4 which causes alteration in creatinine level of treated rats. The creatinine level of cabbage extract treated group and combination group were highly increased as compared to control group. Above consequence has shown that the *Allium cepa* (onion) essential oil is nephroprotective and decreases the level of creatinine during toxicity induced to kidney.

BUN test is used to indicate the renal failure. *Allium cepa* oil treated group efficiently normalized the level of BUN. Purple cabbage leaves extract treated group has shown little increase in the level of BUN in accordance with the control group. This shows that purple cabbage leaves extract increase the level of BUN in blood.

Electrolytes' imbalance has been observed in all treated group. This is may be due to renal damage and some other bicarbonates (Naidu *et al*., 2000). The level of sodium (Na^+) ion in control group was taken as standard value because control group rats were kept in cage without any treatment and left over natural diet and environment. The concentration of Na^+ ion in CCl_4 treated group is slightly increased in accordance to control group. This increase in concentration is due to the adverse effect of CCl_4 on rats' kidneys. In onion oil treated group, the concentration of Na^+ ion decreased down to its normal value as observed in control group. Same results were observed in purple cabbage leaves extract treated group. Both these groups

have normal Na^+ ion concentration. This result has shown that both agents individually act as nephroprotective. In combination group the level of Na^+ ion has reached to its highest value which may lead to toxicity after prolong usage (Dini *et al*., 2008). When the value of Na^+ ion become high a condition called hypernatremia occurs. High concentration of potassium (K^+) ion causes a stage called hyperkalemia and low concentration causes a stage called hypokalemia. The level of K^+ ions has shown a significant increase in CCl_4 treated group. In onion oil treated group, it reduces its level below the control group level. This is due to hypokalemia condition in which less potassium ions are stored in the body and more potassium ions move out from the body in the urine via kidneys. This result has shown that this agent is slightly nephroprotective. If the duration increased, the value will be more significantly positive. In purple cabbage leaves extract and combination treated group the level of K^+ crossed the control group values (Farrugia, 2010). The level of chloride (Cl^-) ions has shown slightly increase in CCl_4 administrative group when compared with the control group. This is because increased amount of Cl^- ions has adverse effects on homeostasis regulation in the body with fluid concentration being disturbed as well. In onion oil treated group, the concentration of Cl^- ion has shown slightly low concentration of ion when compared to control group. This is due to excess elimination of Cl^- ions via kidney through urination. If the duration of onion oil dosage is prolonged, then positive results can be obtained. In purple cabbage leaves extract treated group, the level of Cl^- ion significantly reverses to its normal value i.e. the control group value. This result shows its nephroprotective activity against the changes occurring due to CCl_4 induced toxicity. In combination group the level of Cl^- ion exceed its value as compared to control group. This shows that these both agents together may be fatal for renal functions (Chaudhary *et al*., 2019).

CONCLUSION

Based on the results it is found that carbon tetrachloride (CCl_4) administration changed serum urea and serum creatinine levels when compared with the control group while the extract of purple cabbage and onion oil showed a protective effect on CCl_4 induced nephrotoxicity when compared with the control group. *Allium cepa* essential oil and purple cabbage leaves extract both have demonstrated important nephroprotective property. It is concluded that the *Allium cepa* essential oil and purple cabbage leaves extract both defend kidneys against injury produced by CCl_4 over dose. Administration of both the agents shows therapeutic effects in association with CCl_4 induced nephrotoxicity. As it can been seen in current experimental study that *Allium cepa* essential oil and purple cabbage leaves extract provide prevention from nephrotoxicity induced by CCl_4 due to influence of the different active compounds of onion. As CCl_4 is a toxic agent and above study reveals about its nephrotoxicity in rats. As CCl_4 injected in peritoneal cavity, it may show its adverse effect on stomach and disturb its function. In present study, nephrotoxicity of CCl_4 has been briefly discussed and due to shortage of time the effect of CCl_4 on other peritoneal cavity cannot be studied. In future we can see its effect in the several aforementioned prospects.

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