PROTECTIVE EFFECTS OF ANTIOXIDANTS ON THE EXPERIMENTAL LIVER AND KIDNEY TOXICITY IN MICE

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Aim: Liver and kidney are exposed to a lot of oxidant substances that are both from exogen and endogen sources. The aim of this study was to investigate the antioxidant effects of C vitamine, Melatonine (MLT) and N-acetylcystein (NAC) in carbon tetrachlorur (CCl_4) induced oxidative stress in mouse.

Methods: The study involved 6 groups; control group (Group 1), CCl_4 group (Group 2), CCl_4 + C vitamine group (Group 3), CCl_4 + MLT group (Group 4), CCl_4 + NAC group (Group 5) and combined (CCl_4 + C vitamine + MLT + NAC) group (Group 6) with each group containing 10 mice. Starting from the 4th day of the study 0,4 ml/kg CCl_4 were given intraperitoneally (i.p.) to all groups except the control group. In Groups 3, 4, 5 and 6; 150 mg/kg/day C vitamine, 10 mg/kg/day MLT, 150 mg/kg/day NAC and C vitamine + MLT + NAC combination, respectively, were given for 7 days. The malondialdehyde (MDA) level and superoxide dismutase (SOD), glutatyon peroxidase (GSH-Px), catalase (CAT) and myeloperoxidase (MPO) activities were measured in the tissues of liver and kidney of the mice.

Results: The MDA and MPO levels in the tissues of liver and kidney of the toxicity group (Group 2) were significantly higher than those of the control group ($p<0.01$), but the GSH-Px and CAT activities were significantly lower than those of the control group ($p<0.01$). Compared with the toxicity group; in groups 3, 5 and 6 liver MDA and MPO levels showed a significant decrease ($p<0.05$) while GSH-Px and SOD activities in group 4 and GSH-Px activity in group 5 showed a significant increase ($p<0.05$). In kidney, MDA levels in groups 3 and 5 and MPO levels in groups 4 and 6 showed a significant decrease ($p<0.05$). SOD in group 3; GSH-Px and CAT in group 4; GSH-Px, SOD and CAT in group 5 and SOD activities in group 6 showed a significant increase.

Conclusion: The results showed that C vitamine therapy and the combined therapy were effective in preventing oxidative stress in both liver and kidney while MLT increased antioxidant enzyme activities more effectively. Furthermore, NAC was more effective in preventing oxidative stress and increasing antioxidant enzyme activities.

Key words: Liver, Kidney, Toxicity, Antioxidants

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INTRODUCTION

Drug exposure, ionizing radiations and environmental pro-oxidant pollutants induce free radical formation. Lipid peroxidation initiated by free radicals is considered to be deleterious for cell membranes and has been implicated in a number of pathological situations (1,2). Animal tissues are constantly coping with highly reactive oxygen species, such as superoxide anion, hydroxyl radicals, hydrogen peroxide, and other radicals generated during numerous metabolic reactions. The generation of small amounts of free radicals appears to have an important biological junction, but oxidative stress is caused by excess production of reactive oxygen species. Oxidative stress can produce major interrelated derangements of cellular metabolism, including alteration of protein and nucleic acid structure, increase in intracellular free calcium, damage to membrane ion transport and permeability, and destruction of the cells by lipid peroxidation. Lipid peroxidation has attracted much attention in recent years because of its association with a number of abnormal physiological processes (3,4).

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Table 1. Effects of antioxidant treatment on aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), urea, creatinine, T. Protein, albumin and globulin levels in mice after CCl_4 administration.

	Group 1 (Control)	Group 2 (CCl_4)	Group 3 (Vitamine C)	Group 4 (Melatonine)	Group 5 (NAC)	Group 6 (Combined)
AST (U/L)	65.50±17.20	824.62±158.48^{a***}	142.62±52.43^{b***}	556.75±152.31^{a***b***}	442.25±82.29^{a***b***}	132.50±57.18^{b***}
ALT (U/L)	26.40±8.50	492.42±130.95^{a***}	80.87±16.96^{b***}	310.22±114.17^{a***b***}	291.14±54.69^{a***b***}	55.33±16.12^{b***}
LDH (U/L)	779.87±155.18	1992.50±521.50^{a***}	973.75±429.98^{b***}	1432.12±589.55^{a*}	1499.25±286.06^{a*}	1101.42±159.22^{b**}
Urea (mg/dl)	47.60±7.77	40.60±12.20	44.6±16.64	45.10±11.87	46.12±19.03	42.00±9.13
Creatinine (mg/dl)	0.13±0.04	0.15±0.06	0.18±0.12	0.24±0.11	0.24±0.14	0.13±0.02
T. Protein (g/dl)	4.68±0.35	4.73±0.31	4.88±0.44	4.05±0.43^{b*}	4.26±0.65	4.55±0.75
Albumin (g/dl)	2.35±0.48	2.48±0.50	2.63±0.40	1.78±0.53	1.87±0.71	1.88±0.78

*Results are presented as mean ± S.D.M.Tukey's multiple comparison tests: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, a refer to comparison with control group and b refer to comparison with CCl_4 group.*

Carbontetrachloride (CCl_4), an industrial solvent, is a well-established hepatotoxin. It was demonstrated that liver is not the only target organ of CCl_4 and it causes free radical generation in other tissues also such as kidneys, heart, lung, testis, brain and blood in various studies by researchers (2). It has also been reported that exposure to CCl_4 induces acute and chronic renal injuries. Case control studies and various documented case reports increasingly establish that hydrocarbon solvents produce renal diseases in humans. Extensive evidence demonstrates that as a result of the metabolic activation of CCl_4, $^{\bullet}CCl_4$ and $^{\bullet}Cl$, are formed which initiate lipid peroxidation process (1-3).

To prevent the damage caused by oxygen-free radicals, tissues have developed an antioxidant defense system that includes nonezymatic antioxidants (e.g., glutathione, uric acid, bilirubin, vitamins C and E) and enzymatic activities such as that of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). SOD catalyzes dismutation of the superoxide anion (O^{-2}) into H_2O_2 and GSH-Px and CAT both detoxify H_2O_2 and convert lipid hydroperoxides to nontoxic alcohols (3).
Vitamin C is a well-known cell protective natural antioxidant. The protective effects of vitamin C are observed in oxygen-dependent pathophysiological conditions (5). Melatonin, the chief indolamine is produced by the pineal gland, has been shown to be an effective antioxidant and free radical scavenger. Melatonin, because of its small size and high lipophilicity, crosses biological membranes easily, thus reaching all compartments of the cell. There is substantial body of evidence for the protection of DNA, lipids, and proteins by melatonin against the oxidative damage, which is the result of a number of endogenous and exogenous free radical generating processes (6,7).

N-acetylcysteine (NAC) is a small molecule containing a thiol group, which has antioxidant properties, and is freely filterable with a ready access to intracellular compartments. The diversity of pharmacological applications of NAC is due, mainly to the chemical properties of the cysteinyl thiol group of its molecule, since the ability of reduced thiol groups to scavenge oxygen free radicals is well

established. Because of these properties, NAC is widely used in clinical practice as an antioxidant (6,8).

In the present study, we examined the effects of C vitamine, MLT and NAC on the lipid peroxidation and some antioxidant enzymes in the carbon tetrachlorur (CCl_4) induced oxidative stress in the mouse liver and kidney.

MATERIAL AND METHODS

The protocol for the study was approved by the Ethical Committe of Yüzüncü Yıl University Faculty of Medicine Animal Breeding and Research. Swiss albino mice (37–54 g) were used in these experiments.

The study involved 6 groups; control group (Group 1), CCl_4 group (Group 2), CCl_4 + C vitamine group (Group 3), CCl_4 + MLT group (Group 4), CCl_4 + NAC group (Group 5) and combined (CCl_4 + C vitamine + MLT + NAC) group (Group 6) with each group containing 10 mice. Starting from the 4th day of the study 0,4 ml/kg CCl_4 were given intraperitoneally (i.p.) to all groups except the control group. In Groups 3, 4, 5 and 6; 150 mg/kg/day C vitamine, 10 mg/kg/day MLT, 150 mg/kg/day NAC and C vitamine + MLT + NAC combination, respectively, were given for 7 days. The animals were killed at 8th day after starting of the study. A total of 2 ml of cardiac blood was drawn from each subject.

Biochemical analysis

Blood samples were then centrifuged at 2000 rpm for 10 min in a refrigerated centrifuge to separate serum samples from the cells. Serum AST, ALT, LDH, urea, creatinine, total protein, and albumin levels were determined in the serum by routine colorimetric methods on a Roche modular autoanalyser (Roche modular autoanalyser, Tokyo, Japan).

The liver and kidney samples taken were washed in saline in an ice bath and homogenized in the ratio 1:10 (w:v) with ice-cold 150 mM KC1 for MDA, SOD, GSH-Px, MPO and protein determination. The MDA and protein levels of homogenates were measured immediately, the rest of the homogenates were stored at -70°C until tissue GSH-Px and SOD assays were performed.

MDA levels were determined by the fluorometric method described by Wasowich et al. (9) based on thiobarbituric

Table 2. MDA, MPO, GSH-Px, SOD, and CAT levels in the liver of control, CCl_4, CCl_4 +vitamin C, CCl_4 + melatonine, CCl_4 + NAC, and CCl_4 + combined groups

	Group 1 (Control)	Group 2 (CCl_4)	Group 3 (Vitamine C)	Group 4 (Melatonine)	Group 5 (NAC)	Group 6 (Combined)
MDA (nmol/gr prot.)	312.6±73.34	609.63±67.93^{a***}	339.43±52.70^{b***}	535.2±49.79^{a***}	430.44±89.92^{a*b***}	446.42±62.54 ^{a**b***}
MPO (U/gr tissue)	4.03±1.06	6.58±1.63^{a**}	4.35±1.38^{b**}	5.79±1.31^{a*}	4.64±0.96^{b*}	3.6±0.99^{b***}
GSH-Px (U/gr prot.)	1.27±0.23	0.73±0.12^{a***}	0.8±0.21^{a***}	1.15±0.12^{b**}	1.08±0.25^{b**}	1.03±0.23
SOD (µgr/gr prot.)	23.69±5.04	16.01±6.2	17.19±3.79	30.93±7.36^{b***}	21.39±6.09	22.04±6.62
CAT (kU/gr prot.)	487.11±77.56	322.62±65.98^{a***d**}	272.58±47.32^{a***}	439.44±73.55^{b**}	369.40±39.69^{a**}	259.5±74.5^{a***}

*Results are presented as mean ± S.D.M. Tukey's multiple comparison tests: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, a refer to comparison with control group and b refer to comparison with CCl_4 group.*

Table 3. MDA, MPO, GSH-Px, SOD, and CAT levels in the kidneys of control, CCl_4, CCl_4 +vitamin C, CCl_4 + melatonine, CCl_4 + NAC, and CCl_4 + combined groups

	Group 1 (Control)	Group 2 (CCl_4)	Group 3 (Vitamine C)	Group 4 (Melatonine)	Group 5 (NAC)	Group 6 (Combined)
MDA (nmol/gr prot.)	415.98±78.65	612.24±93.66^{a***}	468.83±88.57^{b**}	587.97±44.74^{a***}	488.27±71.13^{b*}	547.99±50.19^{a*}
MPO (U/gr tissue)	3.72±1.13	7.14±2.00^{a***}	6.18±1.72^{a**}	4.48±0.82^{b**}	5.36±1.14	4.31±1.34^{b**}
GSH-Px (U/gr prot.)	1.49±0.30	0.95±0.24^{a**}	1.04±0.33^{a**}	1.34±0.25^{b*}	1.47±0.18^{b**}	1.29±0.15
SOD (µgr/gr prot.)	17.90±3.20	7.16±2.59^{a**}	21.95±5.94^{b***}	8.14±3.15^{a**}	16.02±8.17^{b*}	19.5±5.16^{b**}
CAT (kU/gr prot.)	475.26±85.93	277.27±57.06^{a**}	255.35±92.33^{a***}	434.46±103.12^{b**}	445.39±110.47^{b**}	298.73±51.17^{a**}

a- Results are presented as mean ± S.D.M. Tukey's multiple comparison tests: * $p<0.05$, ** $p<0.01$, ***$p<0.001$, a refer to comparison with control group and b refer to comparison with CCl_4 group

acid (TBA) reactivity. In brief, 50 µL of homogenat or an adequate volume of MDA working standart solution was introduced into 10 mL glass tubes containing 1 mL of distilled water. After addition of 1 mL of the solution containing 29 mmol/L TBA in acetic acid (pH of the reaction mixture, 2.4-2.6) and mixing, the samples were placed in a water bath and heated for 1 h at 95-100 ^{0}C. After the samples cooled, 25 µLof 5 mol/L HCl was added (final pH 1.6-1.7), and the reaction mixture was extracted by agitation for 5 min with 3.5 mL of n-butanol. We separated the butanol phase by centrifugation at 1500 x g for 10 min, and measured the fluorescence of the butanol extract with a Perkin- Elmer fluorometer (Model LS50B, Perkin-Elmer,UK) at wavelengths of 525 nm for excitation and 547 nm for emission.

SOD activity was measured according to Sun et al. (10). SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with 2-(4-iodophenyl)-3(4-nitrophenol)-5-phenyltetra-zolium chloride to form a red formazan dye. The SOD activity is then measured by considering the degree of inhibition of this reaction.

GSH-Px activity was measured according to Paglia and Valentina (11). GSH-Px catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH the oxidized glutathione is immediately converted into the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured.

CAT activity was measured by the method of Goth (12), in which homogenate was incubated in H_2O_2 substrate and the enzymatic reaction stopped by 1 mL addition of ammonium molybdate. The intensity of the yellow complex formed by molybdate and H_2O_2 was measured at 405 nm.

The MPO activity was measured in kidney and liver tissues using a procedure similar to that reported by Teixeira et al. (13). The samples were centrifuged at 12,000 g at 4 ^{0}C for 20 min. The supernatant was assayed in a reaction medium containing 50 mM phosphate buffer, pH 6.0 at 25 ^{0}C, o-dianisidine-2HCl (0.167 mg/mL) and H_2O_2 (0.005 %). The enzyme activitiy was determined

by the slope of the absorption curve set at 450 nm. A standart curve of MPO activity was obtained previously with a commercial enzyme batch (Sigma). The total protein content of the homogenates was determined by the method of Lowry et al. (14).

Statistical analysis

All data were represented as mean ± standard deviation (SD). Kolmogrov-Simirnov Goodness of fit test was used to control whether the distribution of parameters are normal or not. Groups of data were compared with an ANOVA followed by Tukey's multiple comparison tests. Also chi-square analysis was performed to compare the investigation of pathologically the groups. Values of $p<0.05$ were regarded as significant.

RESULTS

Serum biochemical findings of all groups have shown in table 1. The levels of AST, ALT and LDH of Group 2, Group 4, and Group 5 were higher than those controls ($p<0.001$). The results of statistical evaluation of renal and hepatic MDA, MPO, GSH-Px, CAT ve SOD activities are shown in Table 2 and 3. The MDA and MPO levels in the tissues of liver and kidney of the toxicity group (Group 2) were significantly higher than those of the control group ($p<0.01$), but the GSH-Px and CAT activities were significantly lower than those of the control group ($p<0.01$). There was no significant difference in liver SOD activity of the group 2 compared with the control group ($p>0.05$) and SOD activity of kidneys of group 2 was significantly lower than that of the control group ($p<0.01$). Compared with the toxicity group; in groups 3, 5 and 6 liver MDA and MPO levels showed a significant decrease ($p<0.05$) while GSH-Px and SOD activities in group 4 and GSH-Px activity in group 5 showed a significant increase ($p<0.05$). In kidney, MDA levels in groups 3 and 5 and MPO levels in groups 4 and 6 showed a significant decrease ($p<0.05$). SOD in group 3; GSH-Px and CAT in group 4; GSH-Px, SOD and CAT in group 5 and SOD activities in group 6 showed a significant increase.

DISCUSSION

The degree of liver damage induced by CCl_4 intoxication shows a parallelism with the serum level of liver enzymes such as AST, ALT and LDH (15). In the present study, according to the activities of the control group, a statistically significant level of increase was observed in other groups, whereas the highest increase was monitored in the CCl_4 group ($p<0.01$).

The kidneys are responsible from the elimination of unmodified drugs and metabolites. Additionally, these organs are also capable to realize diffused biotransformation reactions. Ongoing studies demonstrate that nephrotoxicity induced by chemical agents are one of the consequences of the accumulation of certain metabolites in kidneys (16). Kidney damage is one of the most prominent reasons of death due to CCl_4 intoxication. Even so, the number of published data that shows the effects of renal toxicity induced by CCl_4 is limited. However, the effects of CCl_4 on liver cells are widely investigated (17). However the pathology related with renal function failure that is stimulated by CCl_4 remains controversial. As kidneys have an affinity against CCl_4, and as they contain predominantly, cystochrome p450 in the cortex, it is very possible that CCl_4 contributes a lot to nephrotoxicity (18). Even though Rincon et al. (19) demonstrated that the effects of CCl4 on the structure and function of kidneys are dependant to the functional state of the liver. Ogawa et al. (20) suggested that the event related with the kidneys and liver are dependant to independent etiologies. CCl_4 systemically applied on rats were reported to be distributed in a higher concentration in the liver when compared to the kidneys (21). Multiple number of studies showed tissue damage in many organs, mainly in the liver induced by CCL_4 (18).

Products of lipid peroxidation may lead to change in biological membrans, therefore these changes result in serious cellular injury. An increase was observed in the formation of MDA in the hepatocytes of rats which were exposed to CCl_4. It is suggested that, reactive oxygen metabolites (ROM) play a critical role in the accumulation of neutrophils in tissues after ischemia, whereas activated neutrophils are also a potential source for ROMs. MPO plays a basic role in the production of oxidants by neutrophils. Neutrophils are an important source of free oxygen radicals and therefore, are considered as a major effector in the tissue

damage that occurs in many inflammatory disorders (6).

In our study, hepatic MDA and MPO levels of NAC and combined groups were found significantly lower than the CCl_4 group. When MDA levels in the renal tissue were monitored, we determined that a statistically significant decrease was present in the Vitamin C and NAC groups when compared with the CCL_4 group. The MDA value in the MLT group is significantly higher when it is compared with the Vitamin C group. According to the control group, MDA displayed a higher increase when compared to the CCL_4 and MLT groups ($p<0.001$). In the CCl_4 group, MDA values showed a tendency to increase, both in the liver and the renal tissues, and this finding is an expected result that is also an important marker of the toxic effects of CCl_4. However, we expected to fall in MDA values in the MLT group, but the expected deceases weren't significantly observed both in hepatic and renal MDA levels. We assumed that this observation originated from an inadequate dose of MLT.

In certain studies (6,16), an antioxidant effect was obtained when 10 mg/kg MLT was administered; as this dosage was the dose we used, however in some studies (18), it was reported that, antioxidant effect was obtained only when a dose of 25 mg/kg was administered. In the present study, both in the liver and kidney tissues, Vitamin C group MDA values were found quite close to the MDA values of the control group when compared with other groups. Accordingly, it was decided that Vitamin C was more effective in reducing oxidative damage when compared with other groups. Nevertheless, values of the MLT group were the most closest values to CCL_4 group that was found both in liver and kidney tissues. This finding indicates that the expected decrease did not occur during the oxidative damage in the MLT group. Likewise, AST and ALT values were found higher in the MLT group when compared with the other groups who received antioxidants.

Organisms may have an endogenous protective antioxidant defend system against the damages of free oxygen radicals. SOD, CAT and GSH-Px are enzymatic antioxidants that catalyze detoxification reactions of toxic oxygen metabolites (22). The mentioned damages may be limited by non-enzymatic antioxidants such as vitamin A, E and C, melatonin, glutathione and etc. CAT and GSH-Px can provide a direct defend by cleaning the hydrogen peroxide that is one of the leading hydroxyl radicals that own a potentially reactive structure (23).

In the liver and renal tissues of the present study, GSH-Px activities of the CCL_4 group was found significantly low when compared with the control group; however, GSH-Px activities of the MLT and NAC groups that belong to the two tissues were determined to increase meaningfully when compared with the CCL_4 group. However, the decrease monitored in the GSH-Px activity of the CCL group can result from a decrease in the GSH levels. Likewise, in a study performed by Ohta et al. (24), it was reported that, a significant decrease was observed in the GSH content of the liver in mice, which were injected with CCl_4.

The decrease in the liver SOD activity is possibly an expected result that occurs due to lipid peroxyl radicals and from an inactivation of their destruction products. There is a tendency that, increased MDA levels and reduced SOD activities would favorably support this hypothesis. When a decrease occurs in the SOD activity, MPO enzyme activity shall also reduce, because that the formation of hydrogen peroxide is naturally reduced. The increase in protein oxidation levels and lipid peroxides is studied in many individuals with a known liver disease induced by exposure to hepato-toxic agents (25).

In the present study, a decrease was determined in the liver tissue, even though not significant, in the CCL_4 group when compared to the control group as a result of the measurement of SOD activity both in the liver and renal tissues. A significant decrease was observed in the CCl_4 group in the renal tissues. The SOD activity of the liver tissue in the MLT group demonstrated a meaningful increase when it was compared with the CCl_4 group, while NAC in the renal tissue showed a significant increase in the CCl_4 group when it was compared with the group in which vitamin C and combine therapies were applied.

Szymonik-Lesiuk et al. (3) reported that, in the kidneys of rats exposed to CCl_4, CCl_4 would reduce SOD and CAT activities, and that oxidative stress in the

liver was induced by CCl_4 intoxication could cause a decrease in the SOD and GSH-Px activities, or that reactive intermediary products occurred during the bio-activation of CCl_4 could inactivate SOD and GSH-Px enzymes. Accordingly, in the present study, it could be considered that in both liver and kidney, a partial increase in SOD values determined in the groups which received antioxidants could be related to the application period of antioxidants, and could be observed a more significant increase in the long-term periods of application.

In the present study, CAT values related with liver and renal tissues were found significantly low in the CCl_4 group when compared to the values found in the control group. However, in the renal tissue, it was determined that CAT values of MLT and NAC groups were significantly increased when compared with the CCl_4 group. Nevertheless, variations in CAT values in all groups related with the liver and the other groups related with the kidneys did not demonstrate a significant difference when compared with the values found in the CCl_4 group.

In a study carried out by Güven et al. (26), results showed that CCL_4 caused a decrease in GSH, GSH-Px, CAT enzyme activities in the liver and kidneys in mice. In another study carried out by Tirkey et al. (1), protective effects of hesperidine in rats against the toxic effect of CCl_4 was thoroughly studied, and the results obtained demonstrated that, GSH, SOD and CAT levels in liver and renal tissues in the CCl_4 group showed a tendency to decrease. However, it is reported that it was the production of super-oxide and peroxide radicals that were activated by CCl_4 that caused a decline in SOD and CAT activities (1).

As summarized in this study:

1- NAC application alone was effective in reducing oxidative damage in both, liver and renal tissues and in increasing their antioxidant effect.

2- Even that Vitamin C reduced the oxidative stress, both in the liver and in kidneys, it was found that it also lead to a partial increase- according to the CCl_4 group- when antioxidant enzyme activities were considered. Besides, according to the CCL_4 group, a significant decrease was observed in serum AST and ALT values.

3- According to the toxicity group, in liver and kidney tissues, where MLT was administered, a decrease was observed in the oxidative stress, even though the mentioned decrease was not statistically significant, and that a meaningful increase was present in the oxidant activity.

4- We found that, both in the liver and renal tissues oxidative damage was significantly reduced in the combined group when compared with the toxicity group, and that no any significant increase was present in antioxidant enzymes when compared with the toxicity. Yet, when some of these antioxidants are administered alone, it was observed that the mentioned antioxidants increased the activity of antioxidant enzymes. Therefore, as expected, combined applications should have increased antioxidant enzyme activities just as it is in other groups, but unfortunately this did not happen. The mechanism of this event still remains unclear and we had to consider that advance studies are required in order to explain the possible mechanism of this important event.

Consequently, as observed, Vitamin C, NAC and combined therapeutic applications, administered both to the liver and renal tissues were effective in reducing oxidative damages and MLT and NAC applications had a positive and potential impact on antioxidant enzyme activities. Accordingly, we decided that, NAC application alone would play an important role in increasing the antioxidant effect and reducing the oxidative damage that formed both in liver and in renal tissues.

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