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# Evaluation of Hepatotoxicity of Carbon Tetrachloride and Pharmacological Intervention by Vitamin E in Balb C Mice

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**TAM, MZS, SA and KKA designed the study. TAM, MMB, MB and MAK conducted the experimentations. TAM and SA analyzed the data.**

**Key words**
**Carbon tetrachloride, Hepatotoxicity, Vitamin E, Balb C mice, Pre-treatment.**

## ABSTRACT

Present study was designed to investigate the toxic effects of carbon tetrachloride ($CCl_4$) on the liver of Balb C mice and also to investigate the protective effect of Vitamin E pre-treatment on the carbon tetrachloride-induced hepatotoxicity. Study included the estimation of the activities of the enzymes such as ALAT (alanine aminotransferase), ASAT (aspartate aminotransferase) and LDH (lactate dehydrogenase) and biochemical components like glucose, urea, lipids, cholesterol and protein contents both in the liver and blood while DNA and RNA contents only in liver. The administration of $CCl_4$ resulted in increase in plasma ALAT and decrease in LDH. Vitamin E pre-treatment abolished $CCl_4$-induced changes in the activities of these enzymes. Blood glucose content was increased while cholesterol content was decreased. Vitamin E pre-treatment abolished only $CCl_4$-induced change in blood glucose content but failed to abolish $CCl_4$-induced change in cholesterol content. Glucose, urea, lipids, cholesterol contents in liver were decreased whereas total protein contents increased. Vitamin E pre-treatment also prevented $CCl_4$-induced changes in glucose, urea, lipids and total protein contents in liver. $CCl_4$ treatment caused massive damage to the liver. This was prevented by vitamin E pre-treatment. These results show that vitamin E pre-treatment prevented the mice from $CCl_4$-induced hepatic damage, which clearly indicates its preventive effects against liver damage caused by both oxidative and non-oxidative mechanisms.

## INTRODUCTION

Carbon tetrachloride ($CCl_4$) is well-known to be extensively used hepatotoxin for decades; persuading liver injury in different experimental approaches (Basu, 2003). In microsomal compartment of liver, this hepatotoxin is rapidly transformed into trichloromethyl ($CCl_3$) radical by cytochrome P450-2EI (*CYP2EI*). This $CCl_3$ radical reacts with oxygen to form trichloromethylperoxyl ($CCl_3O_2$) radical (Sotelo *et al*., 2002; Ilavarasan *et al*., 2003). These free radicals react with polyunsaturated fatty acids to propagate a chain reaction leading to lipid peroxidation or bind covalently to lipids and proteins, resulting membranes destruction (Sheweita *et al*., 2001).

Imbalance between cellular antioxidant defences and reactive oxygen species (ROS) results in oxidative stress (OS). ROS is involved in many disease conditions such as neurodegenerative disorders, cardiovascular diseases, cancer and aging (Halliwell and Gutteridge, 1984; Lin and Beal, 2006; Nathan and Cunningham-Bussel, 2013). They can be generated by many reactions taking place in the cell and can be activated by some external factors. Their interaction with tumor could activate the signalling pathways; this will cause cellular transformationin cancer (Noreen *et al*., 2018).

Chronic exposure to $CCl_4$ can cause liver damage and could result in liver cancer (Rood *et al*., 2001). Some tissues of other organs including kidneys, heart, lungs, testes, brain and even blood are also affected by $CCl_4$ through generation of free radicals. Oxidative damage

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to these tissues has been observed in $CCl_4$ treated rats (Abraham *et al*., 1999). Oxidative mutilation is one of the essential mechanisms of $CCl_4$ hepatotoxicity which triggers apoptosis via mitochondrial initiated pathway (Ravagnan *et al*., 2002).

Carbon tetrachloride causes necrosis of cell or tissue which results in leakage of enzymes from affected tissues into blood stream (Obi *et al*., 2001). $CCl_4$ is known to cause rapid, prolonged depletion of liver glycogen in rats (Hickenbottom and Hornbrook, 1971). While, it is considered to be effective agent for gene expression (Pietrangelo, 1990).

Vitamin E is a fat-soluble antioxidant essential for the majority of metabolic processes preventing lipid peroxidation. It has a crucial role in anti-inflammatory processes, inhibition of platelet aggregation, and immune enhancement (Bansal *et al*., 2005). Subsequently, vitamin E (α-tocopherol) is a chain-breaking antioxidant to inhibit the propagation step where the alkyl radical reacts with molecular oxygen at a very high rate, giving a peroxyl radical. α-tocopherol efficiently transfers a hydrogen atom to a lipid free radical; α-tocopheroxyl radicals and stops the chain reaction (Messarah *et al*., 2013). Earlier studies had shown that dietary vitamin E intake reinforces the level of glutathione peroxidise (Abdel-Samie *et al*., 2013). In the present study, $CCl_4$ induced liver damage and hepatoprotective effects of Vitamin E pre treatment were studied in Balb C mice.

## MATERIALS AND METHODS

*Ethical statement*

All animal experimental procedures were conducted in accordance with local and international regulations. The international regulation is the Wet op de dierproeven (Article 9) of Dutch Law (International). Approval of the study was obtained from the Institutional Review Board of "The University of Azad Jammu and Kashmir", Muzaffarabad, Pakistan.

*Animals and dose preparation*

Nineteen Balb C mice were obtained with average weight of 25 g from National Institute of Health Islamabad, Pakistan. Mice were maintained in an animal house 20°C ± 2°C and 12 h light and 12 h dark conditions. They were provided with a standard mouse pellets and drinking water *ad libitum*. Lethal dose ($LD_{50}$) of $CCl_4$ was taken as 1 ml/kg b.w. These animals were placed in four groups namely I, II, III and IV. Olive oil (0.4 ml/kg) was given to group 1 as control group. Group II was given Vitamin E (5 mg/kg b.w.) dissolved in olive oil to make volume up to 0.4 ml. Group III was given Vitamin E plus $CCl_4$ (0.4 ml/kg b.w.) and Group IV was given $CCl_4$ (0.4 ml/kg b.w.). All the injections were intra-peritoneal. After 24 h of dosing, animals were anesthetized using chloroform, dissected and blood was collected directly from heart with help of disposable syringes. Blood was kept in tubes having heparin (20 µl heparin/1 ml of blood). For the isolation of plasma, blood was centrifuged at 3000 rpm for 20 min to estimate enzymes activities and biochemical components. Immediate after blood collection, livers were taken and divided into two parts, one for saline extraction and other for preparation of total lipids, cholesterol, nucleic acid and protein extract.

*Saline extract preparation*

For estimation of glucose, urea, and soluble proteins and to estimate activities of ALAT, ASAT and LDH, 250 mg liver part was homogenized with 5 ml of 0.9% saline solution using Teflon glass homogenizer.

*Lipids, nucleic acid and protein extraction*

For obtaining lipids, nucleic acid and proteins, a separate liver part was crushed in boiling ethanol and centrifuged for 20 min at 3000 rpm. Supernatant was collected in separate tube and pellet was again mixed with normal ethanol. It was kept overnight and centrifuged again at 3000 rpm. Supernatant was collected and pellet was mixed with mixture of methanol and ether (3:1). After keeping it for 24 h, it was again centrifuged for 20 mins at 3000 rpm. All collected supernatants were mixed and used for estimation of total lipids and cholesterol contents. Pellet left after extraction of lipids was dried in vacuum desiccators for 18-24 h. Nucleic acid was extracted using procedure mentioned by Shakoori and Ahmed (1973). Pellet left after extraction of nucleic acid was digested in 0.5 N NaOH for 24 h and used for estimation of total protein contents according to method described by Lowery *et al*. (1951).

*Estimation of enzyme activities*

Estimation of enzymes activities was carried out from saline extract as well as plasma. Calibration curve for ALAT and ASAT were prepared according to the procedure of Reitman and Frankel (1957) as mentioned in Merck test manual methods.

*Estimation of glucose, cholesterol and protein contents*

In blood plasma and saline extract, glucose was determined by O-toluidine method of Hartel *et al*. (1969) while cholesterol contents were estimated by mode of Zak (1957), Hamid *et al*. (2017). For estimation of plasma protein, total protein and soluble protein Lowry *et al*. (1951) method was used.

*Estimation of nucleic acid, total lipids and urea contents*

Zollner and Kirsch (1962) method was followed for total lipid estimation. Estimation of urea from both saline extract and plasma was done according to diacetylmonooxime by Natelsen *et al*. (1951) method.

Estimation of nucleic acid was determined as described by Schneider (1957). For DNA, diphenylamine method was used while orcinol method was used for RNA estimation.

## RESULTS

*Effect on liver*

*Total lipid*

Intraperitoneal administration of $CCl_4$ (0.4 ml/kg for 24 h) caused highly significant decrease in hepatic total lipid level as compared to control and vitamin E. When Vitamin E was given in combination with $CCl_4$ it caused significant increase in the level of total lipid as compared to $CCl_4$treated group (Table I).

*Urea*

Intraperitoneal administration of $CCl_4$ caused highly significant decrease in Hepatic urea level as compared to control and vitamin E. When Vitamin E was given in combination with $CCl_4$ it caused highly significant increase in its level as compared to $CCl_4$ (Table I).

*Glucose*

Intraperitoneal administration of $CCl_4$ caused high significant decline in hepatic glucose level as compared to control and vitamin E. When Vitamin E was given in combination with $CCl_4$ it caused high significant increase in its level as compared to $CCl_4$ (Table I).

*Cholesterol*

Intraperitoneal administration of $CCl_4$ caused highly significant fall in hepatic cholesterol level as compared to control and vitamin E. When Vitamin E was given in combination with $CCl_4$ it caused no significant change in its level as compared to $CCl_4$ (Table I).

*Total protein*

Intraperitoneal administration of $CCl_4$ caused significant increase in hepatic total protein level as compared to control and vitamin E. When Vitamin E was given in combination with $CCl_4$ it caused highly significant drop in its level as compared to $CCl_4$ (Table I).

**Table I.- Level of biochemical components in liver of Balb C mice.**

| S. No. | Parameters | Experimental groups | | | |
|---|---|---|---|---|---|
| | | Control | Vit E | $CCl_4$ | $CCl_4$ + Vit E |
| 1 | Total lipid (mg/g) | 64.0±2.8 | 67.8±4.4 | 47.5±2.9*, ## | 65.5±2.7^ |
| 2 | Urea (mg/g) | 13.5±1.2 | 11.3±0.8@@ | 5.8±0.2** | 10.6±0.5^^ |
| 3 | Glucose (mg/g) | 6.0±1.1 | 5.5±0.8 | 0.8±0.15** | 6.3±0.9^^ |
| 4 | Cholesterol (mg/g) | 9.9±0.7 | 7.2±0.9@ | 6.4±0.3*** | 7.8±0.2 |
| 5 | Total protein (mg/g) | 163.3±5.2 | 168.0±3.3 | 184.6±2.2**,# | 157.0±3.4^^^ |
| 6 | Soluble protein (mg/g) | 51.5±4.1 | 60.1±4.1 | 79.6±1.6***, ### | 62.1±2.8^^ |
| 7 | DNA (mg/g) | 9.9±0.8 | 8.4±0.7 | 7.6±1.2 | 9.3±0.7 |
| 8 | RNA (mg/g) | 12.8±0.8 | 8.9±0.4 | 6.4±0.5 | 9.3±0.8 |
| 9 | ALAT (IU/g) | 0.15±0.04 | 0.09±0.002 | 0.06±0.01 | 0.07±0.01 |
| 10 | ASAT (IU/g) | 35.4±5.7 | 42.3±2.2 | 27.7±6.2 | 31.3±3.0 |
| 11 | LDH (IU/g) | 2.0±0.3 | 1.8±0.1 | 1.9±0.4 | 1.3±0.2 |

ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; LDH, Lactate dehydrogenase. @, significance difference between control and vitamin E; *, significance difference between control and $CCl_4$; #, significance difference between Vitamin E and $CCl_4$; ^, significance difference between $CCl_4$ and $CCl_4$ + Vitamin E, each bar represents the mean value of six replicates and SEM. Statistical icons: *, ^=p≤ 0.05 **, ##, ^^, @@ = p ≤ 0.01, ***, ### ^^^ = p ≤ 0.001.

*Soluble protein*

Intraperitoneal administration of $CCl_4$ caused highly significant increase in hepatic soluble protein level as compared to control and vitamin E. When Vitamin E was given in combination with $CCl_4$ it caused high significant decline in its level as compared to $CCl_4$ (Table I).

*Hepatic DNA*

Intraperitoneal administration of $CCl_4$ caused no significant change in hepatic DNA level as compared

to control and vitamin E. When Vitamin E was given in combination with $CCl_4$ it also caused no significant variation in its level as compared to $CCl_4$ (Table I).

*Hepatic RNA*

Intraperitoneal administration of $CCl_4$ caused no significant change in hepatic RNA level. When Vitamin E was given in combination with $CCl_4$ it also caused no significant variation in its level as compared to $CCl_4$ (Table I).

*Hepatic enzymatic activities*

Intraperitoneal administration of $CCl_4$ and $CCl_4$+ vitamin E combination caused no significant change in Hepatic ALAT, ASAT and LDH level.

*Effect on blood*

*Total lipids*

Intraperitoneal administration of $CCl_4$ caused no significant change as compared to control and vitamin E (Table II).

*Urea*

Intraperitoneal administration of $CCl_4$ caused highly significant decrease in Plasma urea level as compared to control and vitamin E. When vitamin E was given in combination with $CCl_4$ it caused highly significant increase in its level as compared to $CCl_4$ (Table II).

*Glucose*

Intraperitoneal administration of $CCl_4$ caused highly significant increase in Plasma Glucose level as compared to control and vitamin E. When vitamin E was given in combination with $CCl_4$ it caused significant decrease in its level as compared to $CCl_4$ (Table II).

*Cholesterol*

Intraperitoneal administration of $CCl_4$ caused highly significant decrease in Plasma cholesterol level as compared to control and vitamin E. When Vitamin E was given in combination with $CCl_4$ it also caused highly significant increase in its level as compared to $CCl_4$ (Table II).

*Total protein*

Intraperitoneal administration of $CCl_4$ caused highly significant decrease in Plasma total protein level as compared to vitamin E. When Vitamin E was given in combination with $CCl_4$ it caused no significant change in its level as compared to $CCl_4$ (Table II).

*Plasma ALAT*

Intraperitoneal administration of $CCl_4$ caused highly significant increase in plasma ALAT level as compared to control and vitamin E. When vitamin E was given in combination with $CCl_4$ it caused highly significant decrease in its level as compared to $CCl_4$ (Table II).

*Plasma ASAT*

Intraperitoneal administration of $CCl_4$ and vitamin E + $CCl_4$ caused no significant change in Plasma ASAT level as compared to control and vitamin E (Table II).

**Table II.- Level of biochemical components of blood plasma of Balb C mice.**

| S. No. | Parameters | Experimental groups | | | |
|---|---|---|---|---|---|
| | | Control | Vit E | $CCl_4$ | $CCl_4$ + Vit E |
| 1 | Total lipid (mg/100 ml) | 216.7±32.4 | 183.1±3.00 | 231.8±46.1 | 197.3±4.6 |
| 2 | Urea (mg/100 ml) | 75.1±4.6 | 74.7±8.9 | 31.9±3.2***, ### | 83.5±6.6^^^ |
| 3 | Glucose (mg/100 ml) | 87.1±4.5 | 76.8±7.8 | 189.6±23.0***, ### | 90.1±33.3^^^ |
| 4 | Cholesterol (mg/100 ml) | 309.2±41.3 | 312.0±3.3 | 150.3±17.5***, ### | 236.8±10.2^^^ |
| 5 | Total protein (mg/100 ml) | 4073.5±204.8 | 5687.4±221.2@@ | 3967.2±158.4## | 4599.2±240.0 |
| 9 | ALAT (mg/100 ml) | 99.8±18.2 | 71.8±21.1 | 594.9±73.6***, ### | 61.65±12.1^^^ |
| 10 | ASAT (mg/100 ml) | 293.8±20.8 | 225.3±9.5 | 436.7±24.1**, ## | 212.8±19.4^^^ |
| 11 | LDH (mg/100 ml) | 110.7±5.8 | 97.4±6.5 | 30.3±5.5***, ### | 70.6±10.0^^^ |

For abbreviations and statistical details, see Table I.

*Plasma LDH*

Intraperitoneal administration of $CCl_4$ caused highly significant decrease in Plasma LDH level as compared to control and vitamin E. When Vitamin E was given in combination with $CCl_4$ it caused high significant increase in its level as compared to $CCl_4$ (Table II).

## DISCUSSION

Carbon Tetrachloride is used as a solvent for oils and fats, as a refrigerant and as a dry-cleaning agent. Inhalation of its vapors can depress central nervous system activity and cause degeneration of the liver and kidneys. Carbon Tetrachloride is reasonably anticipated to be a human carcinogen based on evidence of carcinogenicity in experimental animals. $CCl_4$ studies on brain demonstrated that oxidative stress was prompted in the brain at a solehepatotoxic dose (1 ml/kg b.w.) of $CCl_4$. Increased lipid peroxidation (LPO), protein carbonyls (PC) content and glutathione (GSH) reduction were noticed in the brain regions of rats treated with $CCl_4$ which was greater than that of liver. An extreme fall in the activity of glutathione-S-transferase (GST) was seen in the brain regions which was higher than that of liver. Similarly, activities of glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), NADH- and NADPH-dehydrogenase were reduced in the brain regions similar to that of liver. Higher induction of oxidative stress in the brain compared to that of liver implies vulnerability of the brain for $CCl_4$ neurotoxicity. Single hepatotoxic dose of $CCl_4$ is equally neurotoxic to rats (Ritesh *et al*., 2015). These factors indicate the necessity of studying the hepatotoxicity and neurotoxicity induced by $CCl_4$ and possible preventive options.

Vitamin E is a fat-soluble antioxidant essential for the majority of metabolic processes preventing lipid peroxidation. It has a crucial role in anti-inflammatory processes, inhibition of platelet aggregation, and immune enhancement (Bansal *et al*., 2005) Subsequently, vitamin E (α-tocopherol) is a chain-breaking antioxidant to inhibit the propagation step where the alkyl radical reacts with molecular oxygen at a very high rate, giving a peroxyl radical. α-tocopherol efficiently transfers a hydrogen atom to a lipid free radical; α-tocopheroxyl radicals and stops the chain reaction (Messarah *et al*., 2013). Earlier studies had shown that dietary vitamin E intake reinforces the level of glutathione peroxidise (Abdel-Samie *et al*., 2013). These findings make vitamin E a reasonable molecule to investigate the protective effects against liver damage caused by $CCl_4$.

Administration of a single dose of $CCl_4$ for 24 h to Balb C mice resulted in highly significant increase in plasma ALAT level, highly significant decrease in Plasma LDH level while no significant change in Plasma ASAT level as compared to control and vitamin E. When Vitamin E was given in combination with $CCl_4$ it caused highly significant decrease in ALAT level, high significant increase in LDH level as compared to $CCl_4$ (Table I).. Increase in plasma ALAT, ASAT, LDH and hepatic ALAT were also monitored in rats after $CCl_4$ administration (Lin *et al*., 1993).

Carbon tetrachloride administration in rats for 24 h is known to induce marked increase in serum ASAT and ALAT activities, primed liver lipid peroxidation, depleted sulfhydryl contents, impaired total antioxidant capabilities and induced genotoxicity. $CCl_4$ increases ALAT and ASAT level along with lipid peroxidative enzymes, for example superoxide dismutase and catalase in liver (Bhattacharjee, 2006).These may reflect damage to the liver tissues resulted in leakage of these enzymes into the blood. A highly significant increase in plasma glucose and decrease in cholesterol contents was observed after $CCl_4$ administration. When Vitamin E was given in combination with $CCl_4$ it caused highly significant decrease in glucose level while highly significant increase in cholesterol contents as compared to $CCl_4$ (Table I). Decreased level of cholesterol could be the result of liver damage because liver produces 80% cholesterol in the body (Anthea *et al*., 1993). $CCl_4$ administration induced highly significant decrease in plasma urea and plasma total proteins, while total lipids level remained unaltered. Vitamin E pre-treatment abolished the changes in plasma contents of urea and total plasma proteins. Decrease plasma level of urea and total plasma proteins may denotes the liver damage as synthesis of urea and proteins is the function of liver while amelioration of these contents by vitamin E pre-treatment demonstrate its hepatic protective effects.

Intraperitoneal administration of $CCl_4$ caused highly significant decrease in hepatic total lipid, Urea and cholesterol, high significant decrease in glucose level while highly significant increase in total proteins as compared to control and vitamin E. When Vitamin E was given in combination with $CCl_4$ it caused significant increase in the level of total lipid, total proteins, ureas and glucose level while cholesterol contents remained unaltered as compared to $CCl_4$ treated group (Table I).

Decrease in hepatic glucose contents in $CCl_4$ treated group could be result of increased consumption of glucose during toxic insult. Toxic components are subjected to P-450 metabolism (Phase I biotransformation). Its product undergoes conjugation with phase II enzymes. Conjugates like glucoronides are removed from the body. In this way, usage of glucose in glucoronidation process might have resulted low glucose level. Under stress conditions, In addition, glucose can be utilized for energy production to combat toxin induced stress conditions.

Decreased level of hepaticglucose, urea, total lipids and cholesterol contents while increased protein contents has also been observed in Wister Albino rats after $CCl_4$ treatment (Hamid, 2006). Vitamin E pre-treatment prevented $CCl_4$ induced changes in glucose, lipids and

cholesterol contents to some extent. This increase in total protein could be due to increased protein synthesis to regenerate damaged cells/tissues. Decreased level of hepatic urea contents could be due to loss of liver tissues as urea is synthesized through urea cycle mainly in liver. Increase in protein contents could be due to increased protein synthesis by liver to repair injured tissues. Since pre-treatment of vitamin E, a known antioxidant abolished some of the biochemical changes, which indicate that damaged caused by carbon tetrachloride was oxidative as well as non-oxidative. End stage renal disease patients enduring chronic dialysis treatment may be at great risk of vitamin E deficiency. This shortage results in fatigue, concentration problems, weakened immune system vision problem, irritability, major depression and anemia. It has been linked to prevention of multiple disease spectrums (Jiang and Elson, 2000).

## CONCLUSION

It was concluded that carbon tetrachlorideat given doses in mice is potential hepatotoxicant. Vitamin E has the ability to prevent the damage caused by carbon tetrachloride.

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*Statement of conflict of interests*

All authors declare there is no conflict of interest.

## REFERENCES

Abraham, P., Wilfred, G. and Catherine, 1999. Oxidative damage to the lipids and proteins in the lungs, testis and kidney of rats during carbon tetrachloride intoxication. *Clin. Chim. Acta*, **289**: 177-179. https://doi.org/10.1016/S0009-8981(99)00140-0

Abdel-Samie, H.A., Nassar, S.A. and Hussein, Y., 2013. Ameliorative potential of selenium against bisphenol A-induced hepatotoxicity in rats. Egypt. *J. Hosp. Med.,* **67**: 444–454.

Anthea, M., Hopkins, J., William, C., Laughlin, M.C., Johnson, S., Warner, M., LaHart, D. and Wright J., 1993. *Human biology and health*, 1st ed. Englewood Cliffs Publishing, New Jersey, USA, pp. 52-59.

Basu, S., 2003. Carbon tetrachloride-induced lipid peroxidation: Eicosanoid formation and their regulation by antioxidant nutrients. *Toxicology,* **189**: 113-127. https://doi.org/10.1016/S0300-483X(03)00157-4

Bansal, A.K., Bansal, M., Soni, G. and Bhatnagar, D., 2005. Protective role of vitamin E pre-treatment on N-nitrosodiethylamine induced oxidative stress in rat liver. *Chem. Biol. Interact.,* **156**:101-111.

Bhattacharjee, R., 2006. Effect of vitamin E aerosol on antioxidant imbalance in idiopathic pulmonary fibrosis. *Lancet*, **338**: 215-216.

Boca, R., 1986. Antioxidants in higher plant activated oxygen mediated metabolic functions of leaf peroxisomes. *Physiol. Pl.*, **104**: 673-680.

Cooney, J., Packard, C.J., O'Reilly, D.S.J., Muriel, J. and Caslake, B., 1997. Lipoprotein metabolism. *J. clin. Endocrinol. Metab*., **829**: 3952-3954.

Halliwell, B. and Gutteridge, J., 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J*., **219**: 1-14. https://doi.org/10.1042/bj2190001

Hamid, A., 2006. Diphenyl dimethyl bicarboxylate as an effective treatment for chemical induced fatty liver in rats. *Afr. J. biomed. Res*., **9**: 77-81.

Hamid, A., Ilyas, M. and Kalsoom, S., 2017. Effect of wheat and corn bran and barley and sorghum ß-glucan extracts on the plasma cholesterol level of dietary-induced hypercholesterolemic rats. *Pakistan J. Zool.,* 49(5). 1631-1637. http://dx.doi.org/10.17582/journal.pjz/2017.49.5.1631.1637

Harper, M.E., Bevilacqua, L., Hagopian, K., Weindruch, R. and Ramsey, J.J., 2004. Ageing, oxidative stress and mitochondrial uncoupling. *Acta Physiol. Scand*., **184**: 321-331. https://doi.org/10.1111/j.1365-201X.2004.01370.x

Hartel, A., Helger, R. and Lang, H., 1969. Determination of blood sugar by the o-toluidine method without glacial acetic acid. *Z. Klin. Chem. Klin. Biochem*., **7**: 14-17.

Hickenbottom, R.S. and Hornbrook, K.R., 1971. Effect of carbon tetrachloride on mechanism of liver glycogen in rat. *J. Pharmacol. exp. Ther*., **178**: 383-394.

Ilavarasan, R., Vasudevan, M., Anbazhagan, S. and Venkataraman, S., 2003. Antioxidant activity of Thespesiapopulnea bark extracts against carbon tetrachloride induced liver injury in rats. *J. Ethnopharmacol*., **87**: 227-230. https://doi.org/10.1016/S0378-8741(03)00147-8

Jiang, Q. and Elson, S., 2000. Gamma-tocopherol and its major metabolite, in contrast to alpha-tocopherol, inhibit cyclooxygenase activity in macrophages and epithelial cells. *Proc. natl. Acad. Sci*., **97**: 11494-11499. https://doi.org/10.1073/pnas.200357097

Lin, J.M., Lin, C.C., Chiu, H.F., Yang, J.J. and Lee, S.G., 1993. Evaluation of anti-inflammatory and liver protective effects of *Anoectochilus formosanus*, *Ganoderma lucidum* and *Gynostemma pentaphyllum* in rats. *Am. J. Chin. Med*., **21**: 59-69. https://doi.org/10.1142/S0192415X9300008X

Lin, M.T. and Beal, M.F., 2006. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature*, **443**: 787-795. https://doi.org/10.1038/nature05292

Lowry, O.H., Rosebrogh, N.J., Farr, A.L. and Randal, R.J., 1951. Protein measurement with the folin phenol reagent. *J. biol. Chem*., **193**: 265-275.

Messarah, M., Amamra, W., Boumendjel, A., Barkat, L., Bouasla, I., Abdennour, C., Boulakoud, M.S. and Feki, A., 2013. Ameliorating effects of curcumin and vitamin E on diazinon-induced oxidative damage in rat liver and erythrocytes. *Toxicol. Ind. Hlth.,* 29:77–88.

Natelsen, S.M., Scott, L. and Beffa, G., 1951. A rapid method for determination of urea in biological fluids by men's of reaction between diacetyl and urea. *Am. J. Chem. Pathol*., **21**: 275.

Nathan, C. and Cunningham-Bussel, A., 2013. Beyond oxidative stress: An immunologists guide to reactive oxygen species. *Nat. Rev. Immunol.,* **13**: 349-361. https://doi.org/10.1038/nri3423

Noreen, A., Bukhari, D.A. and Rehman, A., 2018. Reactive oxygen species: Synthesis and their relationship with cancer-a review. *Pakistan J. Zool.,* **50**: 1951-1963. http://dx.doi.org/10.17582/journal.pjz/2018.50.5.1951.1963

Obi, F.O., Omogbai, L.A., Oriafo, O.S.J. and Ovat, O.D., 2001. Effect of a short time post carbon tetrachloride treatment interval on rat plasma enzyme levels and percentage mortality. *J. appl. Sci. environ. Manage.,* **5**: 5-8.

Pietrangelo, A., 1990. Metals, oxidative stress and hepatic fibrogenesis. *Semin. Liver Dis*., **16**: 13-30. https://doi.org/10.1055/s-2007-1007215

Ravagnan, L., Roumier, T. and Kroemer, G., 2002. Mitochondria, the killer organelles and their weapons. *J. Cell. Physiol*., **192**: 131-137. https://doi.org/10.1002/jcp.10111

Reitman, S. and Frankel, S., 1957. A colometric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am. J. clin. Pathol*., **28**: 56-63. https://doi.org/10.1093/ajcp/28.1.56

Ritesh, K.R., Suganya, A., Dileepkumar, H.V., Rajashekar, Y. and Shivanandappa, T., 2015. A single acute hepatotoxic dose of $CCl_4$ causes oxidative stress in the rat brain. *Toxicol. Rep*., **2**: 891-895. https://doi.org/10.1016/j.toxrep.2015.05.012

Rood, A.S., McGavran, P.D., Aanenson, J.W. and Till, J.E., 2001. Stochastic estimates of exposure and cancer risk from carbon tetrachloride released to the air from the rocky flats plants. *Risk Anal*., **21**: 675-695. https://doi.org/10.1111/0272-4332.214143

Schneider, W.C., 1957. Determination of nucleic acidby pentose analysis. *Methods Enzymol*., **3**: 680-684. https://doi.org/10.1016/S0076-6879(57)03442-4

Shakoori, A.R. and Ahmed, M.S., 1973. Studies on liver of chicken, *Gallus domesticus*. Liver growth and nucleic acid content. *Pakistan J. Zool*., **5**: 111-117.

Sheweita, S.A., Gabar, A. and Bastawy, M., 2001. Carbon tetrachloride induced changes in activity of phase II drug metabolizing enzymes in the liver of male rats: Role of antioxidants. *Toxicol*ogy, **65**: 217-224. https://doi.org/10.1016/S0300-483X(01)00429-2

Sotelo, F., Martinez, D., Muriel, P., Santillan, R.L., Castillo, D. and Yahuaca, P., 2002. Evaluation of the effectiveness of *Rosmarinus officinalis* (Lamiaceae) in the alleviation of $CCl_4$ induced acute hepatotoxicity in the rat. *J. Ethnopharmacol*., **81**: 145-154. https://doi.org/10.1016/S0378-8741(02)00090-9

Wu, C.H., Xu, X.Y., Tian, G.S. and Yu, Y.Y., 2006. Serum autoantibodies of patients with chronic hepatitis C and the significance thereof in infection of hepatitis C virus. *Chin. J. Cardiol*., **86**: 390-393.

Zak, B., 1957. Estimation of cholesterol by Zak's method. *Am. J. clin. Pathol*., **27**: 570-583.

Zollner, N. and Kirsch, K., 1962. Micro determination of lipids by sulpho-phospho-vanillin reaction. *Z. Ges. Exp. Med*., **135**: 545-561.