Toxicology International, *29(3) : 353-361; 2022. DOI: 10.18311/ti/2022/v29i3/29822*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Contents available at: www.informaticsjournals.com/index.php/toxi



Protective Effects of Black Tea (*Camellia sinensis*) Extract on Endosulfan Induced Oxidative Stress, Inflammation and Hepatic Damage in Rats

Maitrayee Banerjee[1]*, Oly Banerjee[2], Siddhartha Singh[2] and Sandip Mukherjee[2]

[1]Department of Physiology, Krishnagar Government College, Krishnagar, Nadia – 741101, West Bengal, India; maitrayee_77@yahoo.co.in

[2]Department of Physiology, Serampore College, 9 William Carey Road, Hooghly – 712201, West Bengal, India

ARTICLE INFO

KEYWORDS: Endosulfan, Hepatotoxicity, Liver, Oxidative Stress, Proinflammatory Cytokines

ARTICLE HISTORY
Received: 18-03-2022
Revised: 15-04-2022
Accepted: 30-04-2022

Abstract

With the agricultural expansion the use of pesticides is increasing rapidly in developing countries. Endosulfan, an organochlorine insecticide, is a broad-spectrum effective compound used in wide variety of agricultural crops but known to generate free radicals in the liver and caused hepatotoxicity. Thus, we considered it necessary to explore the protective effect of black tea extract against endusulfan mediated hepatotoxicity. 18 male albino Wistar rats were divided into 3 groups: Control, endosulfan treated (5mg/kg body weight/day) and endosulfan+black tea extract treated (1ml of 2.5gm%/100gm of body weight/day). After 30 days of treatment period, all the animals were sacrificed, and blood and liver tissue were collected. Serum and tissue cholesterol, serum liver function parameters, liver oxidative stress parameters and serum proinflammatory cytokines were measured. Liver sections were stained with haematoxylene and eosine and histological evaluation was done. Results revealed that endusulfan induces oxidative stress in liver by altering oxidant/antioxidant balance, and causes inflammation resulting into hepatic damage. Black tea extract supplementation shows considerable protection against endosulfan mediated changes in liver. Thus, black tea extract exerts ameliorative effect against endosulfan mediated liver toxicity.

**Author for correspondence*

1. Introduction

The industrialization of the agricultural sector has increased the chemical burden on natural ecosystems. For many years, food chain and the environment are contaminated with insecticides because of their widespread use in animal husbandry and agriculture[1]. However, their side effects seem to have huge environmental health risk due to their known ability to cause a large number of negative health and environmental effects.

Endosulfan, a polycyclic chlorinated hydrocarbon insecticide is a broad-spectrum compound used widely to control a variety of pests in agriculture and is commonly used to control the insects on cocoa, coffee, cashew, tobacco, and cotton plants, and also in grains, tea, fruits, and vegetables among others. It is of environmental concern because it is highly toxic to aquatic organisms, plants, animals and human being[2] and is well known on non-target organisms. It has also been responsible for many severe poisonings, including several fatal cases[3]. The major degradation product of endosulfan, endosulfan sulfate has been shown to be more toxic than the parent compounds[4-5]. In addition, endosulfan is a suspected endocrine-disruptor compound. Reproductive toxicty of endosulfan has been shown in some studies. It reduces circulating Follicle Stimulating Horinone (FSH) and Luteinisig Hormone (LH)[5]. It has also been associated with decrease in Daily Sperm Production (DSP), sperm count and increase in the sperm abnormalities in males[6].

Endosulfan is a hydrophobic molecule that binds to biological membranes and enhances lipid peroxidation. The role of oxidative stress and lipid peroxidation in endosulfan toxicity has been shown in many organs including the brain[7], erythrocyte[8,9], peripheral blood mononuclear cells[10], liver & kidney[11] and testis[12]. Oxidative stress occurs as a consequence of unbalance between cellular antioxidant system and production of free radicals.

Black tea has an antioxidant property. Black tea consumption has been shown to be useful for prevention of many debilitating human diseases that include maintenance of cardiovascular and metabolic health[13]. Black tea extract protects against the free-radical mediated oxidative stress in hepatocytes of animals with pesticide-induced liver injury[14]. It has also been reported to increase serum basal estradiol level in oophorectomized rat[15].

The present study was designed to investigate the potential of black tea extract to neutralize the toxic effects of endosulfan on hepatic system in rat.

2. Material and Methods

2.1 Animals

Male albino Wistar rats of 18-20 weeks, weighing between 120–140g were obtained from Saha Enterprise, Birati, Kolkata-700051 (Registration Number:1828/PO/Bt/S/15/CPCSEA) authorized by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), (Animal Welfare Division, Government of India). These animals were acclimatized in an environmentally controlled animal house with 12h light/dark schedule and fed with a standard laboratory diet and water was supplied *ad libitum* throughout the experimental period. Good hygiene was maintained by constant cleaning and removal of feces and spilled feed from the cages daily. All the experiments were done according to the guidelines of Institutional Animal Ethics Committee, Serampore College.

2.2 Preparation of Black Tea Extract

The black tea extract was prepared from Curl, Tear and Crush (CTC) Broken Orange Pickoe (BOP) grade black clonal tea. Preparation of aqueous extract of black tea was done by following the method as described elsewhere[16]. Briefly, 1.25g of tea leaves was added to 25ml of boiling water and was steeped for 15min. The infusion was cooled to room temperature and then filtered. The tea leaves were extracted a second time with 25ml of boiling water and filtered, and the two filtrates were combined to obtain a 2.5% aqueous-tea extract. The resulting clear solution was similar to tea brews consumed by human. This Black Tea Extract (BTE) was fed to animals by gavage technique at a dose of 1 ml/100 g body weight/day at a temperature of 37° C[16].

2.3 Experimental Design

After 7 days of acclimatization, animals were divided into three groups (6 rats in each group): (A) Control (CON); (B) Endosulfan treated (EDS); (C) endosulfan and black tea extract treated (EDS+ BTE). Corn oil

was used to prepare endosulfan. Rats in group B and C were treated with endosulfan (5mg/kg body weight/day)[16] orally for 30 days, while the rats in group C were additionally supplemented with black tea extract (2.5 gm%) (1ml/100gm of body weight/day) orally for 30 days[17-18]. The control animals were treated with corn oil (as vehicle: 2mg.kg body weight/day) in a similar manner like Group B and C.

2.4 Collection of Blood and Preparation of Tissue Extract

At the end of experimental period, animals of all the groups were anaesthetized with pentobarbitone sodium (60mg/kg body weight, intraperitonially) and sacrificed by cervical dislocation which is one of the recommended physical methods of euthanasia for collection of liver tissue and blood specimens. The liver tissues were quickly removed and cleansed for histopathological and biochemical analyses. The blood samples of different groups were collected by syringe directly through cardiac puncture. Serum was separated for the assay of different biochemical parameters. One portion of liver tissue was weighed and placed in ice-cold Phosphate Buffered Saline (PBS), pH 7.4. The homogenate was processed according to Das *et al.*[19]. For the estimation of tissue cholesterol, a part of tissue was homogenized in ether alcohol mixture. All sampling were performed between 1 pm to 3 pm in order to avoid diurnal variation on the parameters to be studied.

2.5 Biochemical Estimations

Tissue and serum cholesterol level were assayed using kit (cholesterol oxidase/peroxidase). Protein content was measured following Lowry *et al.*[20]. Activity of serum alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase were estimated using biochemical kit. Malondialdehyde (MDA) production was estimated according to the method of Wills *et al.*[21].

2.6 Estimation of Nitric Oxide Production

Nitric oxide decomposes very fast in aerated solutions to produce stable nitrite/nitrate products. Therefore, nitrite accumulation was measured by Griess reaction and was used as a marker of NO production in the current study[22]. Sodium nitrite standard curve was used to estimate the amount of nitrite (micromolar unit) present in the hepatic tissue.

2.7 Estimation of Lipid Peroxidation

The role of lipid peroxidase was assessed by measuring the formation of malondialdehyde (MDA) which acts as an index of lipid peroxidation level. Quantitative measurement of lipid peroxidation was done following the Thio-Barbituric Acid (TBA) test[21]. The amount of MDA formed was quantitated with TBA and used as a marker of lipid peroxidation. The results were expressed as nanomoles of MDA per milligram of protein using the molar extinction coefficient (ε =1.56 105cm2/mM).

2.8 Estimation of Superoxide Dismutase (SOD) Activity and Catalase (CAT) Activity

The SOD activity was measured following the Nitro-Blue Tetrazolium (NBT) method, which is based on the inhibition of NBT reduction by SOD[23]. The relative absorbance was then converted into unit of SOD activity/mg protein, where one unit of SOD activity was equivalent to the quantity of SOD that resulted in a 50% reduction in the background rate of NBT reduction. Catalase activity was measured according to the method of Aebi by following the decomposition of H_2O_2 at 240 nm[24]. The difference in absorbance per unit time was used as a measurement of CAT activity. The values were expressed as U/mg protein.

2.9 Estimation of Reduced Glutathione (GSH) Level

GSH level was measured using 5,5'-Di-Thiobis-2-Nitro-Benzoic acid (DTNB). The absorbance of reduced chromogen was followed spectrophotometrically at 412nm. GSH level was estimated from a standard curve and expressed as mM/mg protein[25].

2.10 Determination of Cytokine Level

Commercially available rat ELISA kit obtained from Raybiotech (USA) was used for estimation of Serum TNF-α and IL-6 level. All samples were assayed in duplicate. The intra-assay variation was 6.3% and 5.7%, respectively for TNF-α and IL-6.

2.11 Preparation of Tissues for Microscopic Examination

The remaining part of the liver tissue was fixed in Carnoy's solution, dehydrated in graded alcohol, embedded in paraffin and 5-mm sections were prepared. Paraffin tissue sections were stained with haematoxylene – eosin, examined under light microscope and were photographed using microscope (Karl Zeiss).

2.12 Statistical Analysis

Results were presented as mean ± SEM (standard error of mean). The data were statistically analyzed using the Kruskal-Wallis non-parametric ANOVA, followed by Mann-Whitney U multiple comparison test for pairwise comparison of the means. Results were considered to be statistically significant when p values are less than 0.05 ($p \leq 0.05$).

3. Result

Table 1 shows the extent of injury to the liver post endosulfan treatment and they returned towards near control values following supplementation with black tea extract. In the present study, the effect of oral exposure to endosulfan on the activities of some serum liver enzymes; AST, ALT, ALP was studied along with serum and liver cholesterol levels in rats. Results revealed that endosulfan significantly increased the activities of serum AST ($p<0.01$), ALT ($p<0.01$), ALP ($p<0.01$) along with serum and tissue cholesterol levels ($p<0.01$) as compared, respectively, to the activities obtained for the control, suggesting the incidence of hepatic damage. Supplementation with aqueous extract of black tea significantly reduced the activities of these enzymes ($p<0.01$) as well as serum and tissue cholesterol levels significantly ($p < 0.01$) towards the control level except

Table 1. Effect of aqueous extract of black tea on endosulfan-induced changes in serum cholesterol, liver function parameters and proinflammatory cytokine level in rats

Parameters	Group A	Group B	Group C	Kruskal Wallis test*	Mann Whitney test	
					Gr A *vs* Gr B	Gr B *vs* Gr C
Serum cholesterol (mg/dL)	66.75±0.85	42.5±1.6	57.25±0.48	P<0.01	P<0.01	P<0.01
Serum alkaline phosphatase (U/L)	38.0±1.95	42.5±1.7	56.5±1.04	P<0.01	P<0.05	P<0.01
Serum aspartate amino-transferase (U/L)	16.25±1.78	96.0±3.22	75.76±3.45	P<0.001	P<0.01	P<0.01
Serum alanine amino-transferase (U/L)	10.22±2.34	89.76±3.79	61.34±3.85	P<0.001	P<0.01	P<0.01
Serum IL-6 (pg/ml)	31.13±1.87	42.03±2.33	38.76±3.70	P<0.05	P<0.05	NS
TNF-α (pg/ml)	13.79±1.31	37.66±2.03	21.39±1.51	P<0.001	P<0.01	P<0.01

Data are presented as mean ±SEM, where n=6. *Significance level based on Kruskal Wallis non-parametric ANOVA (P<0.01). Intergroup significant difference was based on Mann Whitney U multiple comparison tests. NS: Not significant.

serum alkaline phosphatase level which was further increased on supplementation of aqueous extract of black tea.

To investigate the involvement of oxidative stress in endosulfan induced hepatotoxicity, different indices of oxidative and antioxidative stress parameters were studied. Results confirmed that MDA and NO levels; hallmarks of lipid peroxidation and inflammatory response were significantly increased in the animals treated with endosulfan (Table 2). Results also revealed that exposure to endosulfan caused a significant decrease in the GSH level of the hepatocytes as compared to the control group. In addition, notable decrease in the activities of CAT and SOD were observed in the liver tissue samples of endosulfan treated animals as compared to control animals (Table 2).

Pro-inflammatory cytokine levels in the serum including TNF-α and IL-6 were measured in this study. Endosulfan was shown to elevate serum TNF-α and IL-6 levels significantly as compared to control animals. Endosulfan induced changes in the oxidative stress markers were markedly attenuated with the supplementation of black tea extract. Rise in the levels of TNF-α were also seen to be reverted towards control values following supplementation with black tea extract. Microscopic examinations of liver tissues revealed that oral exposure to endoslfan resulted in histopathological disintegration and liver cell necrosis, compared to the liver tissues of control group animals. The liver section of rats in the control group showed normal hepatocytes with prominent nucleus, well-preserved cytoplasm and central vein (Figure 1A). Whereas, endosulfan treated liver sections showed alterations in the liver architecture along with disarrangement of the hepatic strands (Figure 1 B and C). Endosulfan causes marked histopathological changes including Kupffer cell activation and portal infiltration with mononuclear inflammatory cells. Taken together, the results provided a strong indication that oral

Table 2. Effect of aqueous extract of black tea on endosulfan-induced changes in hepatic cholesterol, protein and oxidative stress parameters in rats

Parameters	Group A	Group B	Group C	Kruskal Wallis test*	Mann Whitney test	
					Gr A *vs* Gr B	Gr B *vs* Gr C
Liver cholesterol (mg/gm of tissue)	65.06±1.52	39.2±4.03	43.26±4.02	P<0.001	P<0.01	NS
Protein (gm/gm of tissue)	0.488±0.08	0.424±0.09	0.366±0.03	P<0.05	P<0.05	P<0.01
MDA level (nanomole/mg of protein)	323.5±22.66	566.75±38.64	220.75±19.14	P<0.001	P<0.01	P<0.01
NO level (μM/mg protein)	53.11±1.89	140.21±4.12	125.11±3.97	P<0.001	P<0.01	P<0.01
SOD activity (U/mg protein)	178.19±6.78	143.01±4.27	154.62±6.27	P<0.01	P<0.01	NS
CAT activity (U/mg protein)	8.28±0.98	5.91±0.92	6.87±0.63	P<0.01	P<0.01	P<0.01
GSH (mM/mg protein)	100.13±2.21	71.56±2.06	87.91±3.01	P<0.01	P<0.01	P<0.01

Data are presented as mean ±SEM, where n=6. Data are presented as mean ±SEM, where n=6. *Significance level based on Kruskal Wallis non-parametric ANOVA (P<0.01). Intergroup significant difference was based on Mann Whitney U multiple comparison tests. NS: Not Significant.

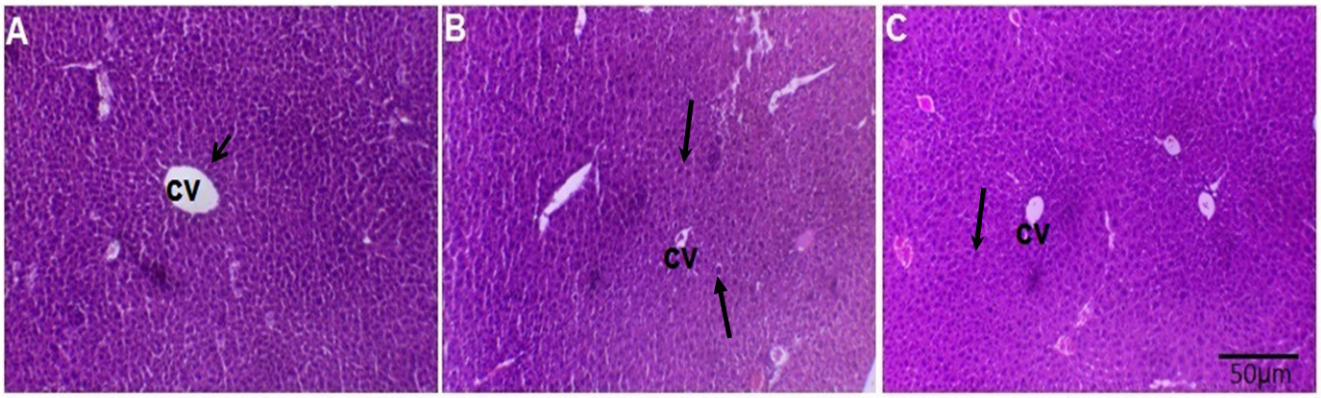


Figure 1. Representative photomicrograph of haematoxylene and eosine stained section (×10) showing morphology of liver in control **(A)**, endosulfan treated **(B)** and endosulfan + black tea extract treated **(C)**. In (A) no pathological changes were seen. Normal architecture of liver tissue with central vein and surrounding hepatocytes (→) were maintained. In (B) endusulfan treated: Kupffer cells activation (→) and portal infiltration with mononuclear inflammatory cells (→). (C) endosulfan + black tea extract treated: Kupffer cells activation (→) and but no sign of mononuclear inflammatory cells.

exposure to endosulfan might result in hepatotoxicity that could be ameliorated with oral supplementation of black tea extract.

4. Discussion

Pesticides have a considerable role in modern agriculture system by contributing persistent and nearly complete control against toxic pests with limited expense and effort. Pesticides have undoubtedly been able to increase crop yields by killing different types of pests that usually cause substantial amount of damage. Coincidently, these chemicals are acknowledged as potent environmental toxicant with unwanted effects on non-target organisms. Among various other pesticides, the chlorinated hydrocarbon insecticides are usually much more persistent and have long residual functions that make them ubiquitous in agriculture and forestry around the world. Endosulfan is one such environmental toxicant of organochloride pesticide origin that is broadly used in insect management and is absorbed by both animals and humans through the skin gastrointestinal tract and lungs. Adipose tissue is known to be the main site of organochloride storage in the body. Cytochrome P450 oxygenase system metabolizes endosulfan from lipophilic xenobiotic to hepatotoxic intermediates in the liver, forming highly reactive free radicals that results in oxidative damage. Free radicals generated during oxidative stress cause lipid peroxidation of cell membranes which is in turn prevented by antioxidant enzymes[25]. It has earlier been reported that organophosphorus pesticides upon acute exposure may lead to oxidative stress in animals and humans[27,28]. Oxidative stress, generated by xenobiotics, induces disturbances in antioxidant enzyme systems[29]. Free radicals play key role in pesticide and other environmental chemical mediated toxicity by generating free radicals and altering enzymatic and non enzymatic antioxidant system[30].

In the current study, we observed that endosulfan negatively impacted antioxidative enzymes in hepatic tissue and, thereby causing liver damage as a result of oxidative stress. Results of the present study showed that oral administration of endosulfan lead to oxidative damage to liver as evidenced by elevated levels of MDA and NO which are known as important oxidative stress markers leading to generation of free radicals. Results also documented that this increase in lipid peroxidation and NO level was associated with a simultaneous reduction in the GSH level. The involvement of the two main cellular antioxidative enzymes, CAT and SOD to protect cells against endosulfan induced oxidative stress was also estimated in the current study. The reduction in the activities of SOD and CAT in liver tissue indicates an impaired antioxidant status. Oxidative stress mainly takes

place by producing reactive oxygen species that ultimately reacts with biological molecules, leading to damage to membranes and other tissues[31]. Therefore, reduction in GSH content as well as in the activities of SOD and CAT suggest net suppression of the total antioxidant capacity in liver tissue. Endosulfan mediated increase in lipid peroxidation level in the liver homogenate indicates underlying membrane damage arising from a considerable loss of membrane integrity in the liver parenchymal cells. The increase in the activity of serum AST, ALT, alkaline phosphatase and tissue cholesterol levels are outcome of this phenomenon. It was earlier established that excessive ROS production and oxidative stress initiate an intracellular cascade signaling that increases the expression of the proinflammatory gene as well as the generation of pro inflammatory cytokines[32]. Results of the present investigation revealed increases in serum TNF-α and IL-6 levels following endosulfan administration which might have occurred in response to endosulfan-induced oxidative damage in the liver. Endosulfan induced alterations in the activity of the enzymes in rats that were reflected in the histology of the liver which showed some distortions in the hepatic plates and distribution as the stress on the sinusoid changes due to impairment in the activity of the liver enzyme, a manifestation of liver damage. This finding is in line with previous reports that associated extremely high serum liver enzyme with ingestion of extremely high amount endosulfan and the congestion of liver with perivenular steatosis presumably was linked to endosulfan intoxication at forensic autopsy[31,32].

Supplementation with aqueous extract of black tea considerably lowered NO and MDA levels and enhanced the levels of GSH towards control values indicating its protective role against endosulfan mediated hepatic toxicity. Activities of the phase II detoxifying enzymes in liver i.e., SOD and CAT were also significantly increased in endosulfan-treated rats following supplementation with black tea extract further reinforcing the cellular antioxidant machinery. Aqueous extract of black tea significantly reduced serum levels of both the pro inflammatory cytokines (TNF-α and IL-6). The present data also indicated that the levels of AST, ALT and ALP were significantly decreased in rats that were treated with endosulfan along with black tea extract. This finding suggests an active protective role by the components present in black tea in ameliorating free radical-induced damage. Microscopic examination of endosulfan treated liver that was supplemented with black tea extract revealed partially ameliorated hepatocytes.

Thereby, the current data suggest that supplementation of black tea extract play a significant role in repairing the distortion of the histological and biochemical modifications induced by endosulfan in liver. The possible explanation for the cholesterol lowering effect of tea may be due to limited cholesterol absorption in the intestine by tea as previous reports documented tea catechins, specifically gallate esters to be hypocholesterolemic which decreases cholesterol absorption in experimental animals[33].

5. Conclusion

In conclusion, our results elaborated that the active components present in aqueous black tea extract exhibit a significant protective role against free radical-induced oxidative damage in rat liver following oral endosulfan exposure. We speculate that high flavonoids levels in black tea extract might be responsible for protecting liver tissue from oxidative damage by scavenging oxygen-free radicals. Further, tea consumption is known to increase the antioxidants capacity of the blood and thereby black tea extract mediated increase in antioxidant capacity might lead to neutralization of endosulfan-induced oxidative stress and amelioration of liver toxicity.

6. Conflict of Interest

We declare that we have no conflict of interest.

7. References

1. Raj J, Mohineesh, Ray R, Dogra TD, Raina A. Acute oral toxicity and histopathological study of combination of endosulfan and cypermethrin in Wistar rats. Toxicol Int. 2013; 20(1):61-67. https://doi.org/10.4103/0971-6580.111575. PMid:23833440 PMCid: PMC3702130.
2. Carvalho FP, Villeneuve JP, Cattini C, Tolosa I, Montenegro-Guillén S, Lacayo M, Cruz A. Ecological risk assessment of pesticide residues in coastal lagoons of Nicaragua. J Environ Monit. 2002; 4(5):778-787. https://doi.org/10.1039/B203728A. PMid:12400931.

3. Sutherland TD, Horne I, Weir KM, Russell RJ, Oakeshott JG. Toxicity and Residues of Endosulfan Isomers. In: Ware GW, New York, NY, Editor. Reviews of Environmental Contamination and Toxicology. Vol 183, Springer; 2004. https://doi.org/10.1007/978-1-4419-9100-3_4. PMid:15369323.
4. Bajpayee M, Pandey AK, Zaidi S, Mussarat J, Parmar D, Mathur N. *et al*. DNA damage and mutagenicity induced by endosulfan and its metabolites. Environ Mol Mutagen. 2006; 47(9):682-692. https://doi.org/10.1002/em.20255. PMid:16933317.
5. Singh SK, Pandey RS. Effect of subchronic endosulfan exposure on plasma gonadotropins, testosterone and enzymes of androgen biosynthesis in rat. Indian J Exp Biol. 1990; 28(10):953-956. PMID:2279768.
6. Sinha N, Narayan R, Shanker R, Saxena DK. Endosulfan-induced biochemical changes in the testis of rats. Vet Hum Toxicol. 1995; 37(6):547-549. PMID:8588293.
7. Zervos IA, Nikolaidis E, Lavrentiadou SN, Tsantarliotou MP, Eleftheriadou EK, Papapanagiotou EP, Fletouris DJ, Georgiadis M, Taitzoglou IA. Endosulfan-induced lipid peroxidation in rat brain and its effect on t-PA and PAI-1: Ameliorating effect of vitamins C and E. J Toxicol Sci. 2011; 36(4):423-433. https://doi.org/10.2131/jts.36.423. PMid:21804306.
8. Saxena R, Garg P, Jain DK. In vitro anti-oxidant effect of vitamin E on oxidative stress induced due to pesticides in rat erythrocytes. Toxicol Int. 2011; 18(1):73-76. https://doi.org/10.4103/0971-6580.75871. PMid:21430928 PMCid:PMC3052592.
9. Aggarwal M, Naraharisetti SB, Sarkar SN, Rao GS, Degen GH, Malik JK. Effects of subchronic coexposure to arsenic and endosulfan on the erythrocytes of broiler chickens: a biochemical study. Arch Environ Contam Toxicol. 2009; 56(1):139-148. https://doi.org/10.1007/s00244-008-9171-0. PMid:18443843.
10. Ahmed T, Tripathi AK, Ahmed RS, Das S, Suke SG, Pathak R. Endosulfan-induced apoptosis and glutathione depletion in human peripheral blood mononuclear cells: Attenuation by N-acetylcysteine. J Biochem Mol Toxicol. 2008; 22(5):299-304. https://doi.org/10.1002/jbt.20240. PMid:18972393.
11. Choudhary N, Joshi SC. Reproductive toxicity of endosulfan in male albino rats. Bull Environ Contam Toxicol. 2003; 70(2): 0285-0289. https://doi.org/10.1007/s00128-002-0189-0. PMid:12545360.
12. Bouabdallaha N, Mallemab L, Abdennoura C, Chouabbiaa A, Tektak M. Toxic impacts of a mixture of three pesticides on the reproduction and oxidative stress in male rats. J Anim Behav Biometeriol. 2022; 10:2204. https://doi.org/10.31893/jabb.22004.
13. Graham HN. Green tea composition, consumption, and polyphenol chemistry. Prev Med. 1992; 21(3):334-350. https://doi.org/10.1016/0091-7435(92)90041-F.
14. Khan SM, Sobti RC, Kataria L. Pesticide-induced alteration in mice hepato-oxidative status and protective effects of black tea extract. Clin Chim Acta. 2005; 358(1-2):131-138. https://doi.org/10.1016/j.cccn.2005.02.015. PMid:15885683.
15. Das AS, Das D, Mukherjee M, Mukherjee S, Mitra C. Phytoestrogenic effects of black tea extract (*Camellia sinensis*) in an oophorectomized rat (*Rattus norvegicus*) model of osteoporosis. Life Sci. 2005; 77(24):3049-3057. https://doi.org/10.1016/j.lfs.2005.02.035. PMid:15996685.
16. Uboh FE, Asuquo EN, Eteng MU. Endosulfan-induced hepatotoxicity is route of exposure independent in rats. Toxicol Ind Health. 2011; 27(6):483-488. https://doi.org/10.1177/0748233710387011. PMid:21543461.
17. Silva MH, Gammon D. An assessment of the developmental, reproductive, and neurotoxicity of endosulfan. Birth Defects Res B Dev Reprod Toxicol. 2009; 86(1):1-28. https://doi.org/10.1002/bdrb.20183. PMid:19243027.
18. Wei H, Zhang X, Zhao JF, Wang ZY, Bickers D, Lebwohl M. Scavenging of hydrogen peroxide and inhibition of ultraviolet light-induced oxidative DNA damage by aqueous extracts from green and black teas. Free Radic Biol Med. 1999; 26(11-12):1427-1435. https://doi.org/10.1016/S0891-5849(99)00005-2.
19. Das AS, Banerjee M, Das D, Mukherjee S, Mitra C. Black tea may be a prospective adjunct for calcium supplementation to prevent early menopausal bone loss in a rat model of osteoporosis. J Osteoporos. 2013; 2013:760586. https://doi.org/10.1155/2013/760586. PMid:23984184 PMCid:PMC3741960.
20. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reaction. J Biol Chem. 1951; 193(1):265-271. https://doi.org/10.1016/S0021-9258(19)52451-6. PMID: 14907713.
21. Wills ED. Evaluation of Lipid Peroxidation in Lipids and Biological Membranes. In: Snell K, Mullock B, Oxford (England), Editor. Biochemical Toxicology: A Practical Approach; 1987.
22. Raso GM, Meli R, Gualillo O, Pacilio M, Carlo RD. Prolactin induction of nitric oxide synthase in rat C6

glioma cells. J Neurochem. 1999; 73(6):2272-2277. https://doi.org/10.1046/j.1471-4159.1999.0732272.x. PMid:10582584.

23. Sun YI, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34(3):497-500. https://doi.org/10.1093/clinchem/34.3.497. PMid:3349599.
24. Aebi H. Catalase in vitro. Methods Enzymol. 1984; 105:121-126. https://doi.org/10.1016/S0076-6879(84)05016-3.
25. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959; 82(1):70-77. https://doi.org/10.1016/0003-9861(59)90090-6.
26. Omurtag GZ, Tozan A, Şehirli AÖ, Şener G. Melatonin protects against endosulfan-induced oxidative tissue damage in rats. J Pineal Res. 2008; 44(4):432-438. https://doi.org/10.1111/j.1600-079X.2007.00546.x. PMid:18205731.
27. Halliwell B, Gutteridge JM. Free Radicals in Biology and Medicine. New York:Oxford University Press; 2015. https://doi.org/10.1093/acprof:oso/9780198717478.001.0001.
28. Banerjee BD, Seth V, Ahmed RS. Pesticide-induced oxidative stress: perspective and trends. Rev Environ Health. 2001; 16(1):1-40. https://doi.org/10.1515/REVEH.2001.16.1.1. PMid:11354540.
29. Gabbianelli R, Falcioni G, Nasuti C, Cantalamessa F. Cypermethrin-induced plasma membrane perturbation on erythrocytes from rats: reduction of fluidity in the hydrophobic core and in glutathione peroxidase activity. Toxicol. 2002; 175(1-3):91-101. https://doi.org/10.1016/S0300-483X(02)00058-6.
30. Banerjee BD, Seth V, Bhattacharya A, Pasha ST, Chakraborty AK. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicol Lett. 1999; 107(1-3):33-47. https://doi.org/10.1016/S0378-4274(99)00029-6.
31. Boereboom TJ, Van Dijk A, Van Zoonen P, Meulenbelt J. Nonaccidental endosulfan intoxication: a case report with toxicokinetic calculations and tissue concentrations. Clin Toxicol. 1998; 36(4):345-352. https://doi.org/10.3109/15563659809028031. PMid:9711201.
32. Sebastian R, Raghavan SC. Exposure to Endosulfan can result in male infertility due to testicular atrophy and reduced sperm count. Cell Death Discov. 2015; 1:15020. https://doi.org/10.1038/cddiscovery.2015.20. PMid:27551453 PMCid:PMC4979443.
33. Ikeda I, Imasato Y, Sasaki E, Nakayama M, Nagao H, Takeo T, Yayabe F, Sugano M. Tea catechins decrease micellar solubility and intestinal absorption of cholesterol in rats. Biochim Biophys Acta. 1992; 1127(2):141-146. https://doi.org/10.1016/0005-2760(92)90269-2.