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Original Article

Effect of co-administration of Green Tea (*Camellia sinensis)* on Clove- (*Syzygium Aromaticum)* Induced Hepatotoxicity and Oxidative Stress in Wistar Rats

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Abstract

The study was designed to investigate the potential of oil extracts of clove (*Syzygium aromaticum)* to induce oxidative stress and hepatotoxicity in Wistar rats. The ameliorative effect due to co-administration with green tea, *Camellia sinensis* was also determined. Adult Wistar rats were exposed via oral gavage to one of the following: mineral oil (negative control), 5% green tea (GT), 12.5 mg/kg/day chlorpyrifos (CHL, positive control), 360 mg/kg/day clove oil (CO), green tea + chlorpyrifos (GT + CHL) and green tea + clove oil (GT + CO). Experimental treatment lasted three weeks, after which the animals were sacrificed and the following indices of oxidative stress and hepatotoxicity were determined in the plasma: levels of reduced glutathione (GSH), activities of catalase, glutathione peroxidase (GPx), aspartate amino transferase (AST), alanine amino transferase (ALT), and alkaline phosphatase (ALP). There was a significant decrease in plasma levels of GSH in the chlorpyrifos and *S. aromaticum* treated groups compared to the control rats. The activities of AST and ALT were higher in the chlorpyrifos and *S. aromaticum* treated groups compared to the control, however these data were only significant in the chlorpyrifos treated group. The activities of GPx, catalase

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and ALP did not differ significantly among the groups. The co-administration with *C. sinensis* resulted in less depletion of GSH as well as reduced levels of plasma AST and ALT. Overall, the results of this study show that the co-administration with *C. sinensis* has the potential to ameliorate the clove- induced oxidative stress and hepatotoxicity in rats.

Introduction

Clove (*Syzygium aromaticum*) is one of the most valuable plants widely used as food preservative and for many medicinal purposes including antimicrobial, anti-inflammatory, antioxidant and anticancer activities (1). The major constituent of clove includes phenolic compounds (eugenol acetate, gallic acid, beta-caryophyllene, vanillin and eugenol) which possess cosmetic, pharmaceutical, food and agricultural applications (2). Although, there are reports on the antioxidant property of eugenol (3, 4), however at high concentrations, eugenol could be a prooxidant, thereby leading to cytotoxicity, reactive oxygen species (ROS) production, and alteration of intracellular glutathione levels (5). Recently, studies have shown that extracts and derivatives of *S. aromaticum* were toxic to certain insect pests and microcrustaceans (6-8) while data on the toxic effect on mammals are not common. The pesticidal property of *S. aromaticum* has made it an effective and efficient alternative to conventional synthetic pesticides which are less environmentally friendly. However, since there is the possibility of residual accumulation on crops and food items, studies focusing on toxicity of *S. aromaticum* on mammalian models are imperative.

Green tea (*Camellia sinensis*) is probably the most widely drunk beverage all over the world, with estimated consumption of over 3 billion cups per day (9, 10). Several beneficial health claims have been attributed to the consumption of green tea amongst many others including the improvement of asthenia, diarrhea, bronchitis, asthma, hyperlipidemia, cellulitis, and abscesses as well as weight reduction (11, 12). Some other studies have also shown that green tea consumption is associated with a reduced risk of cardiovascular diseases, degenerative diseases, and cancer (13, 14). The potential health benefits associated with green tea consumption have been partially attributed to the antioxidative properties of polyphenols which include (-)- epigallocatechin-3-gallate (EGCG), (-)-epicatechin-3-gallate (ECG), (+)-gallocatechin (GC), (-)-epicatechin (EC), gallocatechingallate (GCG) and catechin (15, 16). Notwithstanding the aforementioned health benefits linked to consumption of green tea, there are reports of green tea resulting in liver damage when consumed at higher quantity (16, 17).

From the foregoing, studies focusing on evaluation of toxic effect of *S. aromaticum* should be encouraged especially using mammalian models. This present study is therefore designed to investigate the potential toxic effects of essential oil obtained from *S. aromaticum* using various indices like levels of reduced GSH, catalase and GPx enzymes activities (oxidative stress), and the plasma levels of enzymes such as AST, ALT, and ALP (hepatotoxicity), and also to investigate the protective role of the green tea, *C. sinensis* on *S. aromaticum* induced toxicity in Wistar rats.

Materials and Methods

Extraction of essential oils from S. aromaticum

Essential oil was extracted from clove flower buds following the procedures of Ileke and Ogungbite (18). Briefly, dried flower buds of *S. aromaticum* were obtained from a local market within Akure metropolis, and grinded using a blender. Acetone extracts of *S. aromaticum* were obtained using cold extraction method. This was done by soaking 100 g of the powder in an extraction bottle containing 300 ml of acetone. The mixture was stirred occasionally with a glass rod and extraction was terminated after 72 hours. The extract was filtered through Whatman filter paper (pore size; 0.7 microns). The extraction solvent

was evaporated using a rotary evaporator set at 56°C. The resulting extract was air dried in order to remove traces of solvent.

Experimental animals

Adult male Wistar rats weighing approximately 200 g were obtained from a commercial farm within Akure metropolis, and were placed individually in polypropylene cages, with laboratory grade pine shavings as bedding. Rats were allowed to acclimatize to experimental room conditions for two weeks prior to commencement of experiments. Rats were fed with rat chow and tap water *ad libitum*, throughout the period of experiment.

Experimental Design

The animals were randomly allocated into six groups (5 = 5 per group), and were exposed through oral gavage to one of the following treatments; oil (vehicle for extracted clove oil, thus serving as negative control), 5% green tea (GT), 12.5 mg/kg/day chlorpyrifos (CHL, positive control), 360 mg/kg/day extracted clove oil (CO), green tea + chlorpyrifos (GT + CHL) and green tea + extracted clove oil (GT + CO). Experimental treatment was done every day and lasted for three weeks. At the end of the third week of treatment, animals were sacrificed using cervical dislocation, and the blood was collected into anticoagulant bottles. The following indices of oxidative stress and hepatotoxicity were determined in the plasma: levels of reduced glutathione (GSH), activities of catalase, glutathione peroxidase (GPx), aspartate amino transferase (AST), alanine amino transferase (ALT), and alkaline phosphatase (ALP). The green tea, *Camellia sinensis* used in the study was purchased as a processed product from a commercial store in Akure, Nigeria. Experimental animals were treated and sacrificed following the approved guidelines for the use of animals by the Federal University of Technology, Akure, Nigeria.

Determination of GSH, GPx and Catalase activity in the plasma

The level of reduced GSH in the blood was determined spectrophotometrically following the methods of Ellman (19) using 5-52 -dithio-bis(2-nitrobenzoicacid(DTNB) as the substrate. GSH levels were expressed as µM GSH/ml. GPx activity was determined by the method of Paglia and Valentine (20). The principle of determination is based on the decrease in absorbance of NADPH at 340 nm, and the activity was expressed as mmol NADPH/min/ml while catalase activity was determined following the procedures of Aebi (21), the principle being the rate of H_2O_2 and its activity was expressed as rate constant of H_2O_2 decomposition (k) per ml.

Determination of plasma activity levels of AST, ALT and ALP

The plasma activity levels of AST, ALT and ALP was determined using RANDOX® diagnostics kits (Randox Laboratories Ltd, Crumlin, UK) following the manufacturer's instruction. The activities of the enzymes were expressed as Units/ml.

Statistical analyses

The plasma GSH levels, catalase, GPx, AST, ALT and ALP activities data were subjected to one-way analysis of variance test, so as to determine the difference among the different treatment groups. Tukey's multiple comparison tests was later performed in circumstances of significant difference. Statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad Software Inc., La Jolla, CA). For reporting purposes, data were expressed as mean±SE, and statistical significance was assumed at $p \leq 0.05$.

Results

Plasma levels of GSH, GPx and catalase activities

The results of levels of GSH, GPx and catalase activities are presented in Figs. 1, 2, and 3 respectively. There was a significant difference in the levels of GSH among the groups (p=0.0031). The rats that were treated with the extracted clove oil and chlorpyrifos had significant lower levels of GSH in comparison to the oil and green tea treated groups. There was no significant difference between the mixture groups (GT + CO & GT + CHL) and the oil-treated control group and the green tea treated group. The plasma GPx and catalase activities did not differ significantly among the groups (p=0.2671 and 0.5112

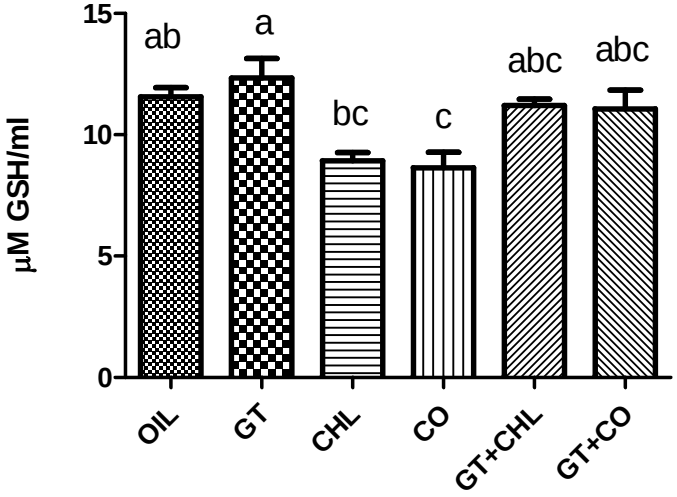

Fig. 1 : GSH levels (µM GSH/ ml) in the plasma of Wistar rats. Each point is the mean±standard error (n=5). Bars with different letters are significantly different in pairwise comparison.

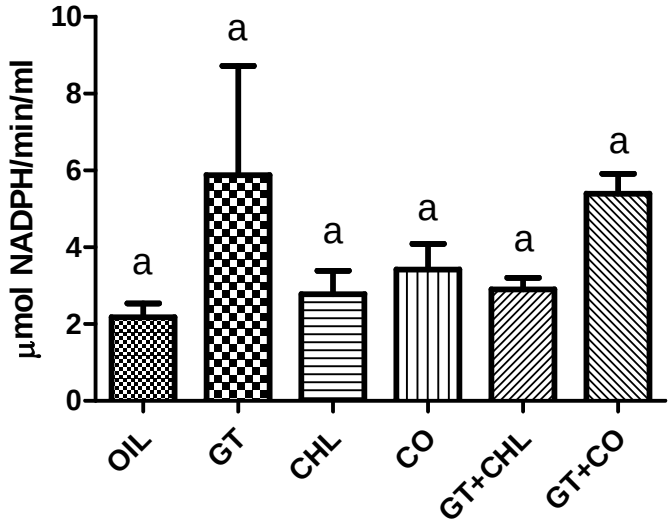

Fig. 2 : GPx activity (mmol NADPH/min/ml) in the plasma of Wistar rats. Each point is the mean±standard error (n=5). Bars with different letters are significantly different in pairwise comparison.

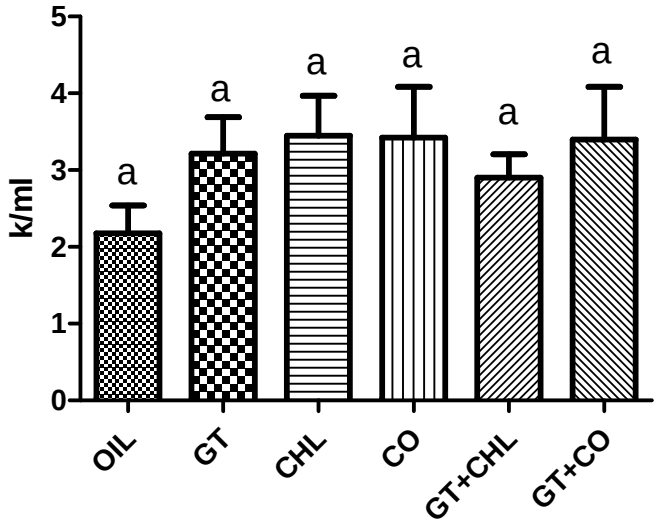

Fig. 3 : Catalase activity (rate of H_2O_2 decomposition (k)/ml) in the plasma of Wistar rats. Each point is the mean±standard error (n=5). Bars with different letters are significantly different in pairwise comparison.

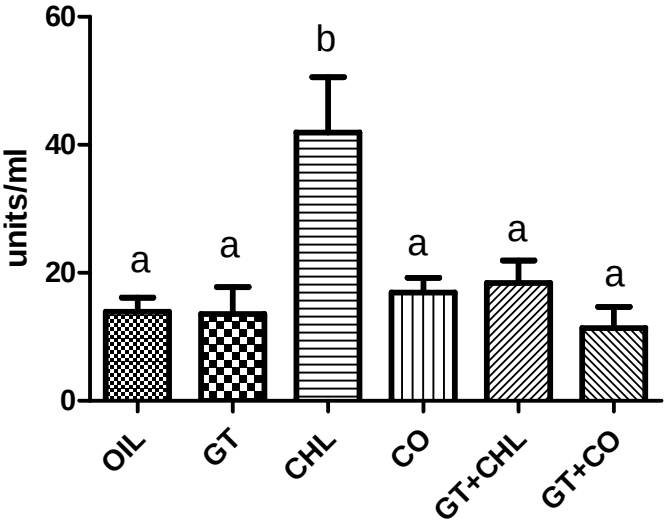

Fig. 4 : AST activity (units/ml) in the plasma of Wistar rats. Each point is the mean±standard error (n=5). Bars with different letters are significantly different in pairwise comparison.

for GPx and catalase respectively). There was preponderance for decreased GPx activity in the groups that were treated with chlorpyrifos, clove oil and the mixture of green tea and chlorpyrifos but this trend was not statistically significant.

Plasma AST, ALT and ALP activities

The plasma activity levels of AST, ALT and ALP are shown in Figs. 4, 5 and 6 respectively. There were significant differences in the activities of AST and ALT among the groups (p=0.0012 and 0.0358; AST and ALT respectively). Wistar rats administered with chlorpyrifos had significantly higher activities of AST and ALT compared to other groups in which the activities of AST and ALT were statistically the same. Also, pairwise comparison between the groups treated with chlorpyrifos and the mixture of chlorpyrifos and green tea indicated a significant reduction in the activities of AST and ALT. The activity of ALP did not differ significantly among the

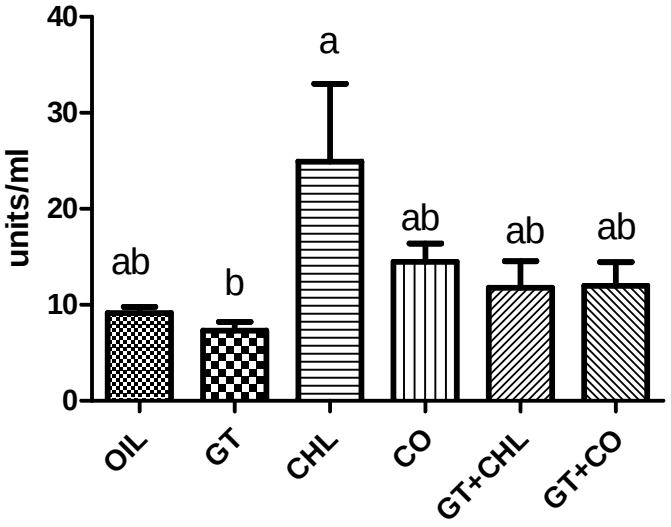

Fig. 5 : ALT activity (units/ml) in the plasma of Wistar rats. Each point is the mean±standard error (n=5). Bars with different letters are significantly different in pairwise comparison.

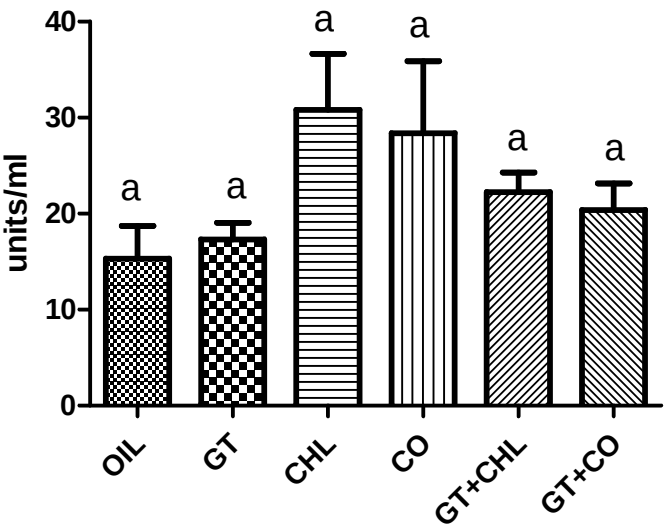

Fig. 6 : ALP activity (units/ml) in the plasma of Wistar rats. Each point is the mean±standard error (n=5). Bars with different letters are significantly different in pairwise comparison.

groups (p=0.1496). The groups that were treated with chlorpyrifos and extracted clove oil appeared to have higher ALP activities compared to the other groups although this difference was not statistically significant.

Discussion

The cellular levels of reduced glutathione have been widely used as a biomarker of oxidative stress in animals. As a defense mechanism against oxidative stress, glutathione in its reduced state donates its thiol group to the reactive oxygen species, thus neutralizing them, and consequently leading to depletion of the cellular level of reduced glutathione (22). The significant reduction in the levels of reduced glutathione in the rats that were treated with clove oil and chlorpyrifos was an indication of GSH depletion in response to oxidative stress. Previous studies have shown that the exposure of animals to certain organophosphate pesticides resulted in depleted level of reduced glutathione (23, 24).

The activity levels of antioxidant enzymes such as catalase and glutathione peroxidase has often being employed as biomarkers of oxidative stress in animals. Catalase acts to detoxify hydrogen peroxide by converting it to water and molecular oxygen while glutathione peroxidase in addition to its role in detoxifying hydrogen peroxide also converts lipids hydroperoxides to their corresponding alcohols. In the present study there was no significant difference in the activity levels of both catalase and glutathione peroxidase among the treatment groups. The lack of difference in activity of the two enzymes measured in this study, notwithstanding the significant difference in the levels of reduced glutathione could be wrongly interpreted to mean lack of oxidative stress due to treatment with clove oil or the known toxic chlorpyrifos. This suggests the need to employ multiple biomarkers when studying the toxic effects of certain substances on animals. The results of this study therefore demonstrated that the treatment of Wistar rats with clove oil has the tendency to cause oxidative stress. The result is in tandem with the findings of Cortes-Rojas et al (1). which reported that eugenol, the major constituent of clove, acts as an antioxidant at low concentrations (5-10 µmol/ L) but could serve as a prooxidant at a high concentration (500 µmol/L) resulting in increased production of reactive oxygen species.

The liver has been shown to be the centre of assault to numerous toxic substances in the body showing various abnormalities like degeneration of hepatocytes, infiltration with inflammatory cells, vacoulation of cells, hypertrophy etc. (24, 25). Also,

the biochemical quantification of activities of liver enzymes such as AST and ALT has been used as biomarkers of liver damage (26, 27). A high level of these enzymes in the blood is an indication of injury to the liver (28). The present data showed that the treatment of Wistar rats with chlorpyrifos caused significant damage to the liver cells with little support for *S. aromaticum* induced hepatotoxicity. Previous studies have actually shown that chlorpyrifos is hepatotoxic (29, 30). Although, the data from the present study do not find significant hepatotoxic effect due to clove oil administration, however few studies have shown that clove oil was hepatotoxic to rodents and man (31, 32).

The green tea, *Camellia sinensis* has been reported to be of significant health importance to man and other animals (13, 14). In this study, rats that were co-administered with clove oil and green tea, there was approximately 28% less GSH depletion while the AST and ALT levels were reduced by approximately 33 and 17% respectively. Similarly, in rats that were co-administered with chlorpyrifos and green tea, the depletion of GSH was less by 25% while the inhibition of glutathione peroxidase was reduced by almost 97%. The plasma levels of AST and ALT were reduced approximately 56 and 57% respectively. The findings were in agreement with the data from other studies that have reported the potential for *C. sinensis* to have anti oxidative and anti hepatotoxic effects in animals (33, 34). The antioxidant and hepatoprotective property of green tea may be due to the presence of catechin and (-)- epigallocatechin-3-gallate (EGCG) (15, 35).

In conclusion, we show in this study that the administration of *S. aromaticum* and chlorpyrifos to Wistar rats resulted in oxidative stress evidenced by decreased level of plasma GSH and decreased activity of glutathione peroxidase. The relative high level of liver enzymes in the plasma is also an indication that extracted oil of *S. aromaticum* and chlorpyrifos could cause liver damage in rats. Overall, the results of this study provide minimal support for protective ability of *C. sinensis* against *S. aromaticum* induced oxidative stress and hepatotoxicity in rat.

References

1. Cortes-Rojas DF, Souza CR, Oliveira WP. Clove (*Syzygium aromaticum*): a precious spice. *Asian Pac J Trop Biomed* 2014; 2: 90–96.
2. Sheweita SA, El-Hosseiny LS, Nashashibi MA. Protective effects of essential oils as natural antioxidants against hepatotoxicity induced by cyclophosphamide in mice. *Plos One* 2016; 11: e0165667.
3. Gulcin I. Antioxidant activity of eugenol: a structure-activity relationship study. *J Med Food* 2011; 14: 975–985.
4. Nagababu E, Rifkind JM, Boindala S, Nakka L. Assessment of antioxidant activity of eugenol in vitro and *in vivo*. *Methods Mol Biol* 2010; 610: 165–180.
5. Atsumi T, Fujisawa S, Tonosaki K. A comparative study of the antioxidant/prooxidant activities of eugenol and isoeugenol with various concentrations and oxidation conditions. *Toxicol in Vitro* 2005; 19: 1025–1033.
6. Cansian RL, Vanin AB, Orlando T, Piazza SP, Puton BMS, Cardoso RI et al. Toxicity of clove essential oil and its ester eugenyl acetate against *Artemia salina. Braz J Biol* 2017; 77: 155–161.
7. Kafle L, Shih CJ. Toxicity and repellency of compounds from clove (*Syzygium aromaticum*) to red imported fire ants *Solenopsis invicta* (Hymenoptera: Formicidae). *J Econ Entomol* 2013; 106: 131–135.
8. Mahfuz I, Khalequzzaman M. Contact and fumigant toxicity of essential oils against *Callosobruchus maculatus*. *Univ J Zool Rajshahi Uni* 2007; 26: 63–66.
9. Labbé D, Têtu B, Trudel D, Bazinet L. Catechin stability of EGC- and EGCG-enriched tea drinks produced by a two-step extraction procedure. *Food Chem* 2008; 111: 139–143.
10. Chen L, Zhou ZX. Variations of main quality components of tea genetic resources (*Camellia sinensis* (L) O Kuntze) preserved in the China National Germplasm Tea Repository. *Plant Foods Hum Nutr* 2005; 60: 31–35.
11. Schönthal AH. Adverse effects of concentrated green tea extracts. *Mol Nutr Food Res* 2011; 55: 874–885.
12. Kao YH, Chang HH, Lee MJ, Chen CL. Tea, obesity, and diabetes. *Mol Nutr Food Res* 2006; 50: 188–210.
13. Wolfram S. Effects of green tea and EGCG on cardiovascular and metabolic health. *J Am Coll Nutr* 2007; 26: 373S–388S.
14. Shula Y. Tea and cancer chemoprevention: a comprehensive review. *Asian Pac J Cancer Prev* 2007; 8: 155–166.
15. Castro J, Pregibon T, Chumanov K, Marcus RK. Determination of catechins and caffeine in proposed green tea standard reference materials by liquid chromatography-particle beam/electron ionization mass spectrometry (LC-PB/EIMS). *Talanta* 2010; 82: 1687–1695.
16. Mazzanti G, Sotto AD, Vitalone A. (2015). Hepatoxicity of green tea an update. *Arch Toxicol* 2015; 89; 1175–1191.

17. James KD, Forester SC, Lambert JD. Dietary pretreatment with green tea polyphenol, (-)-epigallocatechin-3-gallate reduces the bioavailability and hepatotoxicity of subsequent oral bolus doses of (-)-epigallocatechin-3-gallate. *Food Chem Toxicol* 2015; 76: 103–108.
18. Ileke KD, Ogungbite OC. *Alstonia boonei* De Wild oil extract in the management of mosquito (*Anopheles gambiae*), a vector of malaria disease. *J Coastal Life Med* 2015; 3: 557–563.
19. Ellman GL. Determination of sulfhydryl group. *Arch Biochem Biophys* 1959; 82: 70–74.
20. Paglia DE, ValentineWN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; 70: 158–169.
21. Aebi H. Catalase in vitro. *Methods Enzymol* 1984; 105: 121–126.
22. Bannai S, Tateishi N. Role of membrane transport in metabolism and function of glutathione in mammals. *J Membr Biol* 1986; 89: 1–8.
23. Olsvik PA, Berntssen MHG, Søfteland L (2015) Modifying effects of vitamin E on chlorpyrifos toxicity in Atlantic salmon. PLoS ONE 10: e0119250. doi:10.1371/journal.pone.0119250.
24. Mossa AH, Swelam ES, Mohafrash SMM. Sub-chronic exposure to fipronil induced oxidative stress, biochemical and histopathological changes in the liver and kidney of male albino rats. *Toxicol Rep* 2015; 2: 775–784.
25. Maharajan A, Kitto MR, Paruruckumani PS, Ganapiriya V. Histopathology biomarker responses in Asian sea bass, *Lates calcarifer* (Bloch) exposed to copper. *J Basic Appl Sci* 2016; 77: 21–30.
26. Adeyemi JA, Adewale OO, Oguma AY. Mortality, oxidative stress and hepatotoxicity in juvenile African catfish*, Clarias gariepinus*, Burchell exposed to lead and cypermethrin. *Bull Environ Contam Toxicol* 2014; 92: 529–533.
27. Oduola T, Bello I, Adeosun G, Ademosun A, Raheem G, Avwioro G. Hepatotoxicity and nephrotoxicity evaluation in Wistar albino rats exposed to *Morinda lucida* leaf extract. *North Am J Med Sci* 2010; 2: 230–233.
28. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. *Can Med Assoc J* 2005; 172: 367–379.
29. Deng Y, Zhang Y, Lu Y, Zhao Y, Ren H. Hepatotoxicity and nephrotoxicity induced by the chlorpyrifos and chlorpyrifos-methyl metabolite, 3,5,6-trichloro-2-pyridinol, in orally exposed mice. *Sci Total Environ* 2016; 544: 507–514.
30. Uzun FG, Kalender Y. Chlorpyrifos induced hepatotoxic and hematologic changes in rats: the role of quercetin and catechin. *Food Chem Toxicol* 2013; 55: 549–556.
31. Agbaje E, Adeneye AA, Daramola AO. Biochemical and toxicological studies of aqueous extract of *Syzigium aromaticum* (L.) Merr. & Perry (Myrtaceae) in rodents. *Afr J Tradit Complement Altern Med* 2009; 6: 241–254.
32. Hartnoll G, Moore D, Douek D. Near fatal ingestion of oil of cloves. *Arch Dis Child* 1993; 69: 392–393.
33. Elsayed AI, Mohamed AH, Hala AM, Mona MH. The protective and ameliorative effect of green tea extract on antioxidant status of brain tissue exposed to oxidative stress. *Pyrex J Biomed Res* 2015; 1: 59–67.
34. Ibrahim MA, Khalaf AA, Galal MK, Ogaly HA, Hassan AHM. Ameliorative influence of green tea extract on copper nanoparticle-induced hepatotoxicity in rats. *Nanoscale Res Lett* 2015; 10: 363. doi.org/10.1186/s11671-015-1068-z.
35. Yu DK, Zhang CX, Zhao SS, Zhang SH, Zhang H, Cai SY et al. The anti-fibrotic effects of epigallocatechin-3-gallate in bile ductligated cholestatic rats and human hepatic stellate LX-2 cells are mediated by the PI3K/Akt/Smad pathway. *Acta Pharmacol Sin* 2015; 36: 473–482.