Effects of Black Pepper on Oxidative Stress Markers in Acrylamide-Induced Toxicity in Male Wistar Rats

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Abstract- Background to the study: The common methods of processing carbohydrate based foods require exposure to high temperatures which often results in the formation of a toxicant called acrylamide. The aim of the present study is to determine the effect of black pepper on the oxidative stress markers of wistar rats exposed to acrylamide.

Methodology: The study involved 20 male wistar rats separated into 4 groups of 5 rats each as follows; Group 1 served as control. Animals in groups 2, 3 and 4 respectively received a daily oral dose of 30mg/kg of Acrylamide (ACR) throughout the experiment. Group 2 remained untreated (ACR only). Animals in groups 3 and 4 received in addition, 50mg/kg/day of black pepper and 150mg/kg/day of vitamin E respectively. All treatments were given orally for 14 days and thereafter animals were sacrificed and blood samples collected to determine the concentrations of oxidative stress markers in the blood using standard methods.

Results: Our results showed that daily oral administration of ACR caused significant reduction in the plasma concentrations of GSH, GPx and catalase but no significant changes in the SOD and MDA levels. However, concomitant administration of black pepper mitigated the ACR induced depletion of GPx and catalase in a similar fashion with vitamin E but caused no significant changes in the GSH, SOD and MDA.

Conclusion: Conclusively, black pepper exerts its antioxidant effects on ACR induced toxicity by improving GPx and catalase levels but not GSH, SOD and MDA. Aside from GSH levels, the antioxidant potentials of black pepper in our study showed a similar pattern as vitamin E.

Indexed Terms- Black pepper, Oxidative stress, acrylamide-induced toxicity, wistar rats

I. INTRODUCTION

The palatability of most foods consumed in most modern restaurants depends largely on the method of processing including; frying, roasting and baking. These methods mainly involve exposure of the processed food to higher thermal heating (Lingnert *et al*., 2002; Pan *et al*., 2020; Rifai & Saleh, 2020; Timmermann *et al*, 2021; Alefe, 2024) with resultant formation of a biochemical compound known as acrylamide (Zhang et al., 2021; Yan *et al*, 2023; Adimas et al., 2024). The incidence of acrylamide in foods processed by higher temperatures was first noted by the food administration agency in Sweden (SNFA) in 2002 (Ferrer-Aguirre *et al*, 2016). Several studies have linked the consumption of acrylamide contaminated food with toxicity to vital organs (Zhang *et al.*, 2021; Zheng & Xiao, 2022; Govindaraju *et al*., 2024). Acrylamide-induced toxicity is mediated by cellular oxidative stress (Sharafi-Rad *et al*, 2020; Banc *et al*, 2022). The optimal health of an organism requires maintenance of a fair balance between oxidants and antioxidants. Antioxidants play a pivotal role in disease prevention by preventing the formation of free radicals and by implication reduces cellular damage.

Most of the thermally processed foods are usually garnished with pepper such that in any popular Nigerian restaurant, foods like peppered beef, fish, roasted chicken and gizzard, roasted yam, roasted plantain and potatoes abound. This is to buttress the fact that pepper is almost always found in these thermally processed foods especially in a typical Nigerian dish. Black pepper is the commonest variety of pepper spice used in these fried, roasted or baked foods. Some of the nutritional benefits of pepper has been studied including; lipid lowering potential, organo-protective, enhanced intestinal motility and antioxidant effects (Hervert-Hernández *et al.*, 2010; Ludy *et al*., 2012; Charles *et al*., 2024; Emmanuel *et al*., 2025; Obia & Emmanuel, 2025). The present study will determine the impact of black pepper on acrylamide-induced toxicity in wistar rats.

II. MATERIALS AND METHODS

This study was carried out in the department of Human Physiology, Faculty of Basic Medical Sciences, Rivers State University with ethical approval number: RSU/FBMS/REC/24/104. The experiment involved 20 male wistar rats separated into 4 groups of 5 rats each which were acclimatized for a period of two weeks being provided with standard animal chow and water *ad libitum*. Acrylamide was procured from a chemical shop (Joechem ventures) while black pepper was purchased from the mile 3 market in Port Harcourt and processed according to the method described by Charles et al., 2024. The groups include; Group 1 which served as control and received distilled water and animal chow throughout the period of the experiment. A daily oral dose of 30mg/kg of Acrylamide was respectively used to induce toxicity (Shler *et al*., 2015) in Groups 2, 3 and 4. Group 2 remained untreated (Acrylamide only). Group 3 received in addition, 50mg/kg/day of black pepper while Group 4 received in addition, 150mg/kg of vitamin E (Ebuehi *et al*., 2012). All treatment were given orally for 14 days and thereafter animals were sacrificed and blood samples collected to determine the concentrations of oxidative stress markers in the blood. All the parameters were determined using standard methods and values recorded.

Data were analyzed using SPSS vs 23 and presented in Tables. Continuous variables were expressed as mean ± SEM. The differences between each group were analyzed using paired sample t-test and ANOVA. Values of $p < 0.05$ were considered significant with a confidence level of 95%.

III. RESULTS

Table 1: Effect of black pepper on oxidative stress markers in acrylamide-induced toxicity in male wistar rats

GROUP	GSH (mg/dl)	GP_X (U/l)	CAT (U/l)	SOD (U/l)	MDA (mg/dl)
Control	2.28 ± 0.11	0.06 ± 0.00	3.90 ± 0.17	0.33 ± 0.02	0.48 ± 0.02
ACR Only	1.78 ± 0.05[a]	0.04 ± 0.00 [a]	2.06 ± 0.31[a]	0.26 ± 0.04	0.50 ± 0.02
ACR + Black Pepper	2.12 ± 0.17	0.06 ± 0.00[b]	3.73 ± 0.46 [b]	0.46 ± 0.22	0.50 ± 0.03
ACR + Vitamin E	2.18 ± 0.08 [b]	0.06 ± 0.00[b]	3.79 ± 0.20 [b]	0.30 ± 0.04	0.49 ± 0.04

Values are expressed as Mean ± SEM (n = 5);

[a] Significantly different when compared to group 1 (control).

[b] Significantly different when compared to group 2 (acrylamide only).

IV. DISCUSSION

Antioxidants are substances which mop up free radicals or other unstable molecules. These free radicals are capable of causing cellular injury and are implicated in various human diseases (Halliwell, 2007; Sies, 2015). Antioxidant enzymes protect major molecules, such as lipids, proteins, and DNA from oxidative damage by inactivating oxidants. These antioxidant enzymes can act in a coordinated way to protect living tissues from oxidative damage (Khan *et al*, 2011). A wide range of plant-based foods; fruits, vegetables, nuts and seeds possess abundant antioxidants (Serafini & Peluso, 2016; Obia & Auquo, 2018; Maleki et al., 2019) including vitamin C, vitamin E, and polyphenols such as flavonoids, phenolic acid and carotenoids. Glutathione (GSH) is a non-enzymatic antioxidant that neutralizes reactive oxygen species (ROS) and maintains cellular redox homeostasis. The present study showed that acrylamide (ACR) significantly depleted GSH levels ($P < 0.05$). This is so because acrylamide is known to induce oxidative stress by increasing the utilization of

GSH, thus depleting its circulatory levels. ACR also inhibits the enzyme, glutathione synthetase which is essential for the production of GSH (Klaunig et al., 2010). Our findings on the effect of ACR on GSH levels agree with other studies which informed the use of ACR to induce oxidative stress in animal studies (Rifai & Saleh, 2020; Zhang et al., 2023).

Administration of black pepper did not show any significant change in the GSH levels of ACR treated rats. In a previous study, the main component of black pepper (piperine) enhanced glutathione synthetase activity (Vijayakumar *et al*., 2004) with the expected outcome of increasing GSH levels. In our study, GSH levels improved significantly upon treatment with a known antioxidant (vitamin E). In our study, administration of ACR caused significant reduction in the plasma levels of GSH, glutathione peroxidase (GPx) and catalase compared to the control group. Oral administration of black pepper caused a significant rise in the GPx and catalase levels compared to the ACR only group in a similar manner as vitamin E. The findings suggest that supplementation with black pepper and vitamin E respectively mitigated the ACR-induced GPx and catalase depletion. ACR did not affect the plasma concentrations of both supra-oxide dismutase (SOD) and malondialdehyde (MDA) in our study. Again, administration of black pepper had no significant effect on both SOD and MDA levels.

Conclusively, black pepper exerts its antioxidant effects on ACR induced toxicity by improving the GSH and catalase levels but not GPx, SOD and MDA. Asides GSH levels, the antioxidant potentials of black pepper in our study showed a similar pattern as vitamin E.

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