Nigerian Journal of Physics (NJP)

ISSN: 1595-0611

Volume 32(2), June 2023

Effects of Supplementing Feed with Irradiated Tomatoes on Liver Biochemicals and Antioxidant Activities of Male Wistar Rats

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ABSTRACT

Irradiation of tomatoes has been found to delay ripening, extend shelf-life and inhibit microbial growth. However, little is heard about the consequence of consuming irradiated tomatoes. Thus, this study supplements low-dose irradiated tomatoes into animal feed and then evaluates its effects on liver biochemicals and antioxidants of male Wistar rats. Tomatoes of RF variety were irradiated with x-ray doses of 1.0, 1.5, 2.0, and 2.5 Gy and thereafter mixed with the animal feed at 30% of tomatoes to 70% of the feed and grouped accordingly. Control and non-irradiated tomato groups were also made for the study. The rats were fed with the prepared feed for six weeks and later sacrificed; samples of blood and liver were collected, and thawed to obtain serum and tissue homogenate which were later used for laboratory analysis of the biochemicals and antioxidants. There was a significant increase in the activities of the hepatic enzymes, with a significant decrease in the activities of serum proteins of the animals fed with the irradiated tomatoes mixed feed compared to those of the control at $p < 0.05$. A significant decrease was also obtained in the activity of the antioxidants with a concomitant increase in the level of MDA activity of the rats fed with the irradiated tomato mixed-feed at $p < 0.05$. The study observed hepatotoxicity following the consumption of the irradiated tomatoes mixed feed. Hence, the use of irradiation in preserving and extending tomato shelf-life and quality needs thorough reconsideration.

Keywords:
Antioxidants,
Biochemicals,
Food supplement,
Liver,
Tomato irradiation.

INTRODUCTION

Tomato is an edible berry of the plant *Solanum lycopersicum* but a perishable vegetable that can be consumed raw or processed. Thus, it is an integral part of human diets that is produced worldwide. Tomato production accounts for about 187 million tonnes, with China leading the world's tomato production with about 65 million tonnes followed by India with 20.6 million tonnes. Nigeria ranked 12th on the world list and 2nd in Africa with about 4.1 million tonnes as provided by the Food and Agricultural Organization Statistics of the year 2022. Tomatoes are rich sources of several nutrients and secondary metabolites, minerals, vitamins C and E, β-carotene, lycopene, organic acids, and many more (Agarwal et al., 2001; Giovanelli and Paradiso, 2002; Bugianesi et al., 2004), all of which helped in preventing the formation and neutralizing various forms of free radicals (Jacob et al., 2010). The consumption of tomatoes is strongly linked to a significant proportion of antioxidant associated with anti-inflammatory activity, and reduce the risk of cancers and many other cardiovascular disorders (Seren et al., 2008; Freeman and Reimers, 2010).

Irrespective of these benefits, tomato fruits are perishable and face several post-harvest challenges that affect their acceptability. Thus, irradiation of tomatoes has been reported as an alternative method of preserving, reducing losses, and, improving post-harvest handling. In the last two decades, several studies have reported the irradiation of tomatoes as a means of extending their shelf-life and quality (Akter and Khan, 2012; Adam et al, 2014; Kirthy, 2014; Kramer et al., 2014, Singh et al., 2016, Loro et al., 2018; Dyshlyuk et al, 2020; Gyimah et al., 2020, Boonsua et al., 2021). However, to the best of our findings and knowledge, no one has been reported about the likely effects either positive or negative that may be associated with or accompany the supplementation of irradiated tomatoes

into human or animal food. The liver is the main organ that metabolizes xenobiotics and endogenous molecules to maintain metabolic homeostasis in the organism. Thus, it is a target of many injuries that results in dysregulated hepatic homeostasis and leads to hepatic diseases (Muriel, 2007a, 2007b; Li et al., 2015; Abou-Seif, 2016, Appak-Baskoy et al., 2019). Therefore, liver diseases pose a serious challenge to international public health, as any injury to the organ or impairment of its functions may lead to severe implications on an individual's health. Hence, this study incorporates irradiated tomatoes into the animal's feed and then evaluates the effects on the liver's biochemicals and antioxidant activities.

MATERIALS AND METHODS

Tomatoes irradiation

Freshly harvested tomato fruits of the Roma VF variety were divided into five (5) groups. Group I was not irradiated. Groups II – V were subjected to x-ray irradiation using MARS Fixed X-ray, (Allengers Medical Systems Ltd.) operated at a varying dose of 1.0, 1.5, 2.0, and 2.5 Gy.

Feed preparation

The tomato fruits of groups I – V were blended into paste separately and thoroughly mixed with already pulverized animal feed at 30% of the paste to 70% of the feed, tagged accordingly, and then allowed to dry at room temperature for two weeks. Another group tagged VI containing 100% animal feed was also prepared. Each feed sample was retracted, pelletized, and thereafter regrouped into groups F_1, F_2, F_3, F_4, F_5, F_6. Group F_1 contains 100% of animal feed. Group F_2 contains 30% of non-irradiated tomatoes and 70% of animal feed. Groups F_3 – F_6 contain 30% of irradiated tomatoes and 70% of animal feed at the dose of 1.0, 1.5, 2.0 and 2.5 Gy respectively.

Experimental animals

A total number of fifty-four (54) male Wistar rats (*Rattus norvegicus*) between 120 ± 10 g body weights were used in this study. The animals were housed inside laboratory cages under laboratory conditions with twelve (12) hours of the light-dark cycle at 25 ± 2°C. The animals were allowed to acclimatize for two (2) weeks during which they were fed with normal animal feed and water ad libitum pending the experimental period.

Experimental design

At the expiration of the acclimatization period, the animals were randomized into six (6) groups (A, B, C, D, E, F) of nine (9) rats per group and fed with the prepared feeds F_1 - F_6 for six (6) weeks. Rats in group A served as the baseline control and were fed with F1 feed, Rats in group B serve as the experimental control and were with fed F_2 feed. Rats in groups C – F were fed with F_3 – F_6 feed respectively.

Collection of blood and organ samples

At the expiration of the feeding period, the rats were fasted for 18 h and thereafter anesthetized and blood samples were collected via cardiac puncture. The blood collected was dispersed into lithium heparin tubes and allowed to clot at room temperature for 45 min, after which the tubes were retracted and then centrifuged at 3000 rpm for 15 minutes at 4°C to obtain serum. The obtained serum was dispersed into plain sample bottles. In addition, liver tissue was excised from the rats and 1 g of the organ was cut out, washed in normal saline, homogenized on ice with 10 mM phosphate buffer (pH 7.4) at 5 ml of tissue weight, and thereafter centrifuged at 10,000 rpm to obtain supernatant. The serum, and supernatant of the tissue homogenate were stored at -80°C pending analysis.

Biochemical analysis

Each serum sample was collected in aliquots and assay for alkaline phosphate (ALP), aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), total protein (TP), total bilirubin (TBLI) using reagent kits procured from a reputable supplier and following the manufacturer's instruction. Each tissue homogenate was also assayed for the level of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and malondialdehyde (MDA) following the protocol described in the appropriate reagent kits. All standards, samples and controls were run in duplicate.

Data analysis

GraphPad Prism software package was employed for the analysis of the results and then presented as mean ± standard error of the mean (SEM). The data were also subjected to statistical analysis through analysis of variance (ANOVA) with Dunnett's multiple comparison posthoc test. $P < 0.05$ was set as the level of significance.

RESULTS AND DISCUSSION

Level of biochemicals activities

The results obtained for the biochemical activities comprising the hepatic enzymes and protein of the animals are presented in Table 1. The results showed a significant increase in both the ALT and AST levels of the animals fed with the tomato mixed feed (groups B – F) irrespective of the irradiation doses compared to the control animals (group A). There was also a significant increase in the ALT and AST activities of the groups fed with high doses of the irradiated tomato mixed feed (groups E and F) compared to the animals in group B

which were fed with the non-irradiated tomato mixed feed. A similar trend was observed in the ALP activities of the animals in groups B – F when compared to group A. However, in this case, all the animals fed with the irradiated tomato mixed feed (groups C – F) experienced a significant increase in the ALP activities when compared to those in group B. There was a significant decrease in the TP level of the animals fed with the tomato mixed feed (groups B – F) compared to the control animals (group A). Likewise, the TP levels of the animals in groups C – F were statistically significant when compared to those of group B. The results follow the same pattern for the ALB and GLB levels of the animals fed with the tomato mixed feed (groups B – F) compared to the control animals (group A). However, only the animals in groups D – F for the ALB and groups E – F for the GLB experienced a significant decrease when compared with the animals in group B.

Table 1: Levels of hepatic enzymes and protein in the rats (n = 9)

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/dl)	ALB (g/dl)	GLB (g/dl)
A	44.07 ± 1.08	27.05 ± 1.43	13.49 ± 0.33	5.10 ± 0.13	2.18 ± 0.09	3.51 ± 0.06
B	49.16 ± 1.78	34.82 ± 1.63	25.13 ± 0.31[a]	4.45 ± 0.03	1.89 ± 0.01[a]	2.61 ± 0.06[a]
C	55.12 ± 1.19[a]	35.90 ± 0.11[a]	32.06 ± 1.15[a,b]	3.61 ± 0.17[a,b]	1.72 ± 0.01[a]	2.51 ± 0.08[a]
D	56.62 ± 1.21[a]	37.89 ± 0.92[a]	32.67 ± 1.71[a,b]	3.01 ± 0.19[a,b]	1.58 ± 0.05[a,b]	2.30 ± 0.08[a]
E	62.63 ± 1.07[a,b]	44.30 ± 2.64[a,b]	37.87 ± 1.67[a,b]	2.97 ± 0.18[a,b]	1.49 ± 0.07[a,b]	1.54 ± 0.13[a,b]
F	69.37 ± 4.7[a,b]	54.04 ± 3.34[a,b]	46.91 ± 0.73[a,b]	2.80 ± 0.22[a,b]	1.26 ± 0.07[a,b]	1.42 ± 0.12[a,b]

ALT – alanine aminotransferase; AST – aspartate aminotransferase, ALP – alkaline phosphatase; TP – total protein, ALB – albumin; GLB – globin ([a]significant when compared to group A at $P < 0.05$. [b]significant when compared to group B at $P < 0.05$)

Level of antioxidants activities

The results obtained for the indicators of antioxidants are presented in Table 2. The results showed a significant decrease in both the SOD and CAT activities of the animals fed with the tomato mixed feed (groups B – F) irrespective of the irradiation doses compared to the control animals (group A). There was also a significant decrease in the SOD and CAT levels of the groups fed with the irradiated tomato mixed feed (groups C and F) compared to the animals in group B which were fed with the non-irradiated tomato mixed feed. There was a significant decrease in the GSH concentration of the animals in groups B – F when compared to those in groups A and B respectively. The results showed a significant increase in the MDA concentration of the animals in groups B – F when compared to those in group A. Only the animals in group F revealed a significant increase in MDA concentration when compared to group B.

Table 2: Level of liver antioxidant activities in the rats (n = 9)

Groups	SOD (µmol/g tissue)	CAT (µmol/g tissue)	GSH (µmol/g tissue)	MDA (µmol/g tissue)
A	4.35 ± 0.06	1.27 ± 0.05	2.74 ± 0.19	10.66 ± 0.73
B	3.55 ± 0.13[a]	1.15 ± 0.05[a]	2.66 ± 0.13	15.28 ± 0.22[a]
C	2.61 ± 0.14[a,b]	0.67 ± 0.06[a,b]	1.67± 0.21[a,b]	15.79 ± 0.20[a]
D	2.39 ± 0.06[a,b]	0.64 ± 0.05[a,b]	1.54 ± 0.14[a,b]	16.49 ± 0.48[a]
E	1.79 ± 0.17[a,b]	0.63 ± 0.05[a,b]	1.47 ± 0.16[a,b]	16.74 ± 0.63[a]
F	1.33 ± 0.13[a,b]	0.57 ± 0.04[a,b]	1.34 ± 0.07[a,b]	18.86 ± 0.80[a,b]

SOD – superoxide dismutase; CAT – catalase; GSH - glutathione; MDA – malondialdehyde ([a]significant when compared to group A at $P < 0.05$. [b]significant when compared to group B at $P < 0.05$)

Discussion

The liver is a major metabolic organ and plays a key role in lipid metabolism. It is the hub of fatty acid synthesis, which are broken down to generate aminotransferases such as ALT and AST, synthesize various lipoproteins which transport the fatty acids, triglycerides, and cholesterol to and from body cells, and use cholesterol to make bile salts. The ALT helps convert proteins into energy for the liver cells, AST helps to metabolize amino acids and ALP is important for breaking down of proteins. Elevated levels of hepatic enzymes are indicative of cellular leakage and loss of functional integrity of cell membranes in the liver (Choudhary and Devi, 2014; Dasgupta and Wahed, 2021). The present study demonstrated a significant increase in ALT, AST and ALP activities of the rats fed with the irradiated tomato mixed feed, thus suggestive of liver damage.

Proteins form the major portion of dissolved substances in the plasma. Albumin which is synthesized in the liver

constitutes a major part of the total proteins in the body, the other part being globulin. The body needs albumin to fight infections, distribution of extracellular fluid, regulation of osmotic pressure, and acts as a transport agent for a wide variety of substance such as hormones, lipids, vitamins, and many more. Lower than normal levels of albumin and total protein may indicate liver damage or disease. Serum albumin is likely to be low in chronic liver disease and serves as a guide to the severity of the liver disease (Dasgupta and Wahed, 2021). The findings in this study showed a significant decrease in the TP, ALB and GLB activities of the animals fed with the irradiated tomato mixed feed, thus suggestive of liver damage.

Exposure of the liver to a foreign substance, whether toxic or not may produce oxygen-derived free radicals termed reactive oxygen species (ROS). These species namely hydroxyl radical (OH^{-}), superoxide radical anion (O^{2-}), and hydrogen peroxide (H_2O_2) cause severe damage to macromolecules, tissues and organs through the process of lipid peroxidation, protein modification, and DNA strand breaks (Shun and Chen, 1998; Zaidi and Banu, 2004). Reflecting the imbalance between the systemic manifestation of ROS and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage result in oxidative stress (Katerji et al., 2019). The importance of oxidative stress is commonly emphasized in the pathogenesis of various degenerative diseases, such as diabetes, cancer, cardiovascular disorders or neurodegenerative diseases (Halliwell and Gutteridge, 1999; Abuja and Albertini, 2001; Apostolova et al., 2011). Protective actions against ROS are performed by enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) as well as nonenzymatic compounds such as tocopherol, vitamin E, beta-carotene, ascorbate (Mao et al., 2011; Kateri et al., 2019), with the SOD and CAT forming the first line of cellular defense against oxidative damage (Mentese et al., 2022; Demir et al., 2022). When the capacity of these antioxidants system decreases, the level of inactivated ROS rises.

Lipid peroxidation is also a significant determinant of the degree of free radical generation, with MDA being one of the products and an important marker of the process of oxidative stress (Halliwell and Gutteridge, 1994; Chaudiere and Ferrari-Iliou, 1999; Katerji et al., 2019). Lipid peroxidation leads to the generation of free radicals (such as peroxyl, alkoxyl, and aldehyde), which cause cell damage and lead to the release of marker enzymes. When the liver and kidney cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the bloodstream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatic and renal cellular damage (Pagana and Pagana, 2002; Abou-Seif et al, 2016; Dasgupta and Wahed, 2021). The present findings revealed a significant decrease in SOD, CAT, and GSH in the rats fed with tomato mixed feed with a prominent decrease in the irradiated groups and a concomitant increase in MDA's lipid peroxidation suggestive of decreased oxidative balance.

CONCLUSION

The results obtained in this study for the liver biochemicals and antioxidant activities inferred that supplementing low-dose x-rays irradiated tomatoes into vertebrate food may result in acute liver damage. To the best of our findings and knowledge, no study has been reported within the scope of this study and this, therefore, limits its wide comparison. Thus, while several efforts are ongoing towards promoting tomato irradiation, more *in-vivo* study should be conducted so as to enlighten the general public on the likely biological effect of consuming irradiated products.

ACKNOWLEDGMENTS

The authors appreciate the technical support of the laboratory staff of Davejosh Global Research Laboratory, Ogbomoso.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AVAILABILITY OF DATA AND MATERIAL

All data generated or analysed during this study are included in the article.

ETHICAL APPROVAL

Animal housing and handling were performed according to the recommendations of the Ethics Committee, Ladoke Akintola University of Technology, Ogbomoso. All animals received humane care in compliance with laid down guidelines and criteria as outlined in the National Research Council (2011) of the National Academy of Sciences on Guide for the Care and Use of Laboratory Animals, Eight Edition

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