EFFECT OF CHITOSAN SUPPLEMENTATION ON 1,2 - DIMETHYLHYDRAZINE-INDUCED INTESTINAL DYSBIOSIS AND HEPATOXICITY IN MALE WISTAR RATS

BY

ADEBIYI, VICTORIA AYOMIDE

(PG 21/0246)

B.Sc. Biochemistry (Abeokuta)

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ABSTRACT

Intestinal dysbiosis, a condition that depicts an imbalance in the gut microbiota has been posited to disrupt the gut-liver axis. This disruption allows inflammatory molecules and toxins to reach the liver, causing inflammation. 1, 2-dimethylhydrazine (DMH) is a toxicant that alters intestinal homeostasis by altering microbial composition, increasing inflammation and damaging epithelial cells. Chitosan (CTS), derived from the deacetylation of chitin, has been noted for its prebiotic potential in promoting beneficial gut microbiota, reducing intestinal permeability and systemic inflammation. This study evaluated the effect of chitosan (CTS) supplementation on DMH - induced intestinal dysbiosis and hepatotoxicity in rats. Forty-two adult male Wistar rats were randomly assigned to six groups (n = 7): Group 1 (Normal control), Group 2 (DMH only), Group 3 (7.5% CTS), Group 4 (DMH + 2.5% CTS), Group 5 (DMH + 5% CTS) and Group 6 (DMH + 7.5% CTS). The rats were treated for 16 weeks; the first eight weeks, all animals were fed standard diet. Groups 1 and 3 were administered buffered saline while Groups 2,4, 5 and 6 received DMH (65mg/kg body weight) subcutaneously once weekly. For the subsequent eight weeks, Groups 1 and 2 continued with standard diet while groups 3-6 received 7.5%, 2.5%, 5.0%, and 7.5% chitosan supplemented diets, respectively. Samples of blood, liver, ileum, cecum and colon were collected for biochemical, microbial, histological, and gene expression analyses. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test, with $p < 0.05$ considered significant. The DMH exposure increased microbial diversity, enriching fungal species like *Aspergillus niger, Rhizopus, Rhodotorula* and *Fusarium*. Chitosan at 7.5% notably reduced microbial diversity compared to lower CTS concentrations of 2.5 and 5%. Liver damage induced by DMH was evident by significantly ($p < 0.05$) increasing alanine aminotransferase and decreasing aspartate amino transferase activities by 7.9% and 40.8%, respectively, compared to

control. Oxidative stress was induced in DMH control group by increased MDA in serum (14.7%), cecum (7.4%) and colon (12.4%) with decreased MDA in the ileum (17.8%). Activities of CAT and SOD decreased in the serum (66.7%), ileum (5.4% and 25%), cecum (7.4 and 267%) and colon (21.5 and 200%) of DMH control group when compared to control. However, post-treatment with chitosan alleviated these imbalances. Also, the expression of pro-inflammatory markers, interleukin-6 and tumor necrosis factor-α, was notably upregulated in the liver, cecum and colon of the DMH control group. Conversely, treatment with chitosan significantly ($p < 0.05$) mitigated observed effects. Histopathology showed mild regenerative nodules, colonic polyps and crypt hyperlasia in the liver, colon and rectum of the DMH only group, respectively, while the chitosan-treated groups showed normal histologic structure. Comparing 2.5%, 5.0% and 7.5% dosing, chitosan showed dose-dependent prebiotic effects, and 7.5% was most effective in improving gut microbiota balance and reducing systemic inflammation through the modulation of the gut-liver axis.