

## RESEARCH ARTICLE

**Effects of Ethanol on Hematological, Histopathological, and Antioxidant on Indomethacin-induced Heat-stressed Rats**

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**ABSTRACT**

The non-steroidal anti-inflammatory medicine indomethacin causes pathogenesis that involves reactive oxygen species and lipid peroxidation. This study examined how ethanol and indomethacin affected heat-stressed rats' hematological, histopathological, and antioxidant functions. Three groups of 18 male albino rats were created. Group A acted as the standard control group and received saline as usual for 15 days. Group B was given 0.2 mL of ethanol orally each day. In group C, indomethacin 10 mg/kg/day was administered orally once daily. Rats given ethanol and indomethacin had significantly lower levels of red blood cells, packed cell volume, and hemoglobin compared to the control group. White blood cell (WBC), lymphocyte (LYM), and neutrophil (NEUT) levels were significantly altered by the addition of ethanol and indomethacin, with indomethacin administration significantly increasing WBC and NEUT in comparison to ethanol administration. Compared to the stress control group, the levels of malondialdehyde and superoxide dismutase were significantly elevated and decreased, respectively, in the rats supplemented with ethanol and indomethacin. In addition, rats given ethanol and indomethacin showed significantly reduced catalase, glutathione, and glutathione peroxidase activities. The stomachs of rats given indomethacin and ethanol treatment underwent histological analysis, which revealed varying degrees of architectural deformities. These results imply that supplementation with ethanol and indomethacin may harm rats under heat stress.

**Keywords:** Biochemistry, hematology, histopathology, oxidative stress

**INTRODUCTION**

When the body is exposed to high temperatures for an extended length of time, a condition called heat stress develops that causes physiological and metabolic changes. Researchers have looked into the usage of several supplements, such as ethanol and indomethacin, to lessen the harmful consequences of heat stress. The previous studies looked into using several supplements to lessen the

harmful effects of heat stress. Antioxidants, such as Vitamins C and E, have been demonstrated to lessen oxidative damage in rats under heat stress.<sup>[1]</sup> The potential for other supplements, such as beta-glucans and plant extracts, to enhance the immune response and lessen inflammation in heat-stressed animals has also been researched.<sup>[2]</sup> According to the study's conclusions, adding ethanol and indomethacin to the rats' diets significantly affected how they responded to oxidative and hematological stress. Compared to the other groups, the group that received both supplements showed appreciable improvements in their hematological parameters

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and decreased oxidative stress markers. These findings point to ethanol plus indomethacin as a potentially effective treatment for heat exhaustion. The research by Adeniyi *et al.* (2021)<sup>[3]</sup> expands on the body of knowledge regarding the potential application of supplements in reducing the harmful effects of heat stress. Further research is required to determine the effectiveness of these supplements in human populations and their long-term effects. However, these findings offer new information about effects of ethanol and Indomethacin on hematological, histopathological, and antioxidants on heat-stressed Rats.

## MATERIALS AND METHODS

### Ethical Approval

The experimental protocols were carried out per Ekiti State University's policies for handling and using experimental animals. Following the National Society of Medical Research guidelines, all subjects got adequate care.

### Protocol

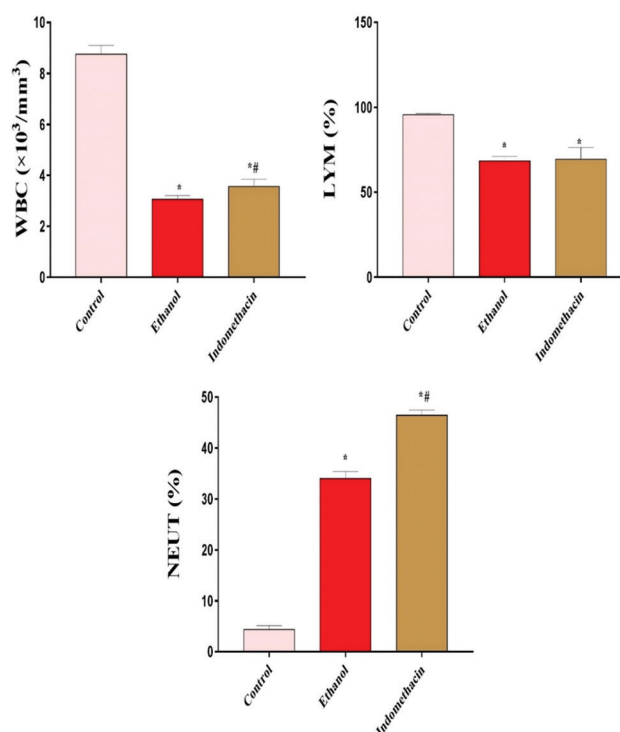
Male albino Wistar rats (150–250 g) were acquired from the Ekiti State University's animal house in Ado-Ekiti and acclimated there under controlled conditions before the experiment began. All test animals will have unlimited access to drinking water and rat pellets to eat for 4 weeks.

### Animal Grouping

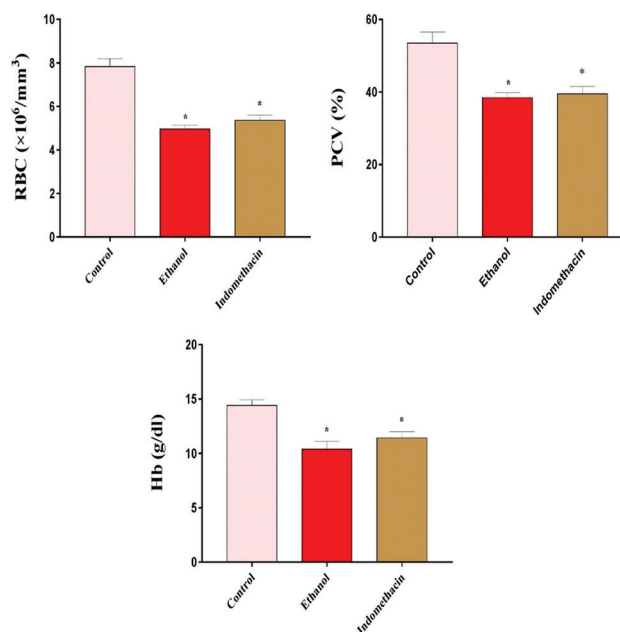
The animals were divided into three groups, each consisting of six albino rats, at random:

Group A acted as the standard control group and received saline as usual for 15 days. For 15 days, Group B received 0.2 mL of ethanol orally each day. Indomethacin 10 mg/kg/day was given orally to Group C once daily for 15 days.

After 15 days, 5 mL of blood samples from the rat hearts were taken, and 1 mL of the sera was made by centrifuging it for 10 min at 3000 rpm before putting it in the freezer until it was time to be analyzed. Hematological parameters were assessed using an automated hemo-analyzer, and serum levels



**Figure 1:** Effect of ethanol and indomethacin on; A. WBC, B. LYM, and C. NEUT in heat-stress male Wistar rats. Values are mean  $\pm$  S D of 5 replicates. \* P < 0.05 vs control, and # P < 0.05 vs ethanol.



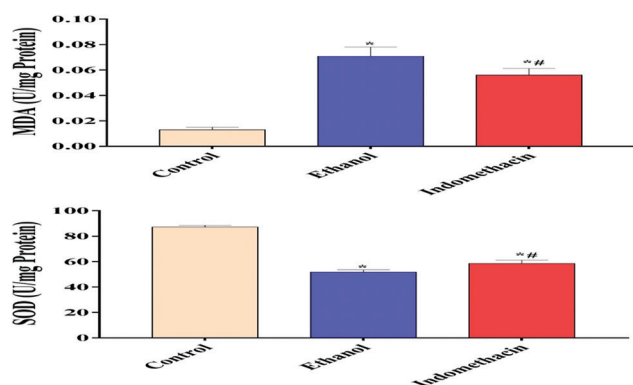
**Figure 2:** Effect of ethanol and indomethacin on; A. RBC, B. PCV, and C. HB in heat-stress male Wistar rats. Values are mean  $\pm$  S D of 5 replicates. \* P < 0.05 vs control

of catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), malondialdehyde (MDA), and glutathione peroxidase (GPX) were determined using a

spectrophotometric kit. From several groups, stomach tissues were taken and preserved in 10% of buffered formalin solution. The tissues were prepared, paraffin-embedded, and slices with a thickness of 5 m were produced. Hematoxylin and eosin were used to stain the sections, then viewed under a light microscope.

### Data Analysis

The Statistical Package for the Social Sciences (SPSS, Evaluation Version 16.0, SPSS Inc., Chicago, IL, USA) was used to analyze all data. If necessary, the Duncan multiple range test was used after the one-way analysis of variance test. Differences were

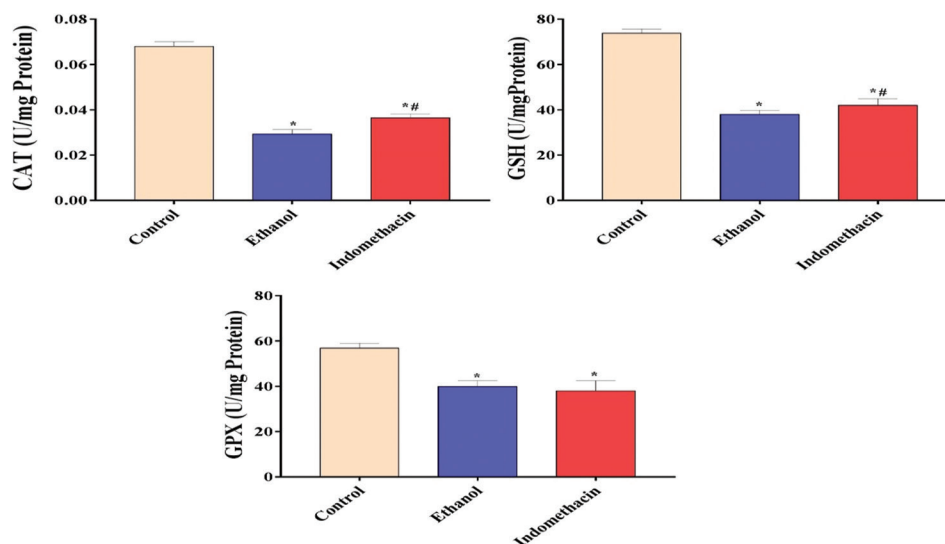


**Figure 3 :** Effect of ethanol and indomethacin on A. MDA and B. SOD in heat-stress male Wistar rats. Values are mean  $\pm$  S D of 5 replicates. \*  $P < 0.05$  vs control, and #  $P < 0.05$  vs ethanol.

deemed significant at  $P=0.05$ . The graphical analyses were done using the GraphPad Prism 5 program (GraphPad Software, San Diego, CA, USA).

### DISCUSSION

This study demonstrates the effects of ethanol and indomethacin on heat-stressed-induced rats regarding hematological, histopathological, and antioxidant effects. The findings demonstrated that ethanol and indomethacin administration significantly reduced the levels of red blood cells, packed cell volume, and hemoglobin in heat-stressed mice compared to the control group [Figure 1]. This is in line with other research that showed ethanol and indomethacin negatively affected rats' hematological parameters.<sup>[4,5]</sup> White blood cell (WBC), lymphocyte, and neutrophil (NEUT) levels also significantly changed as a result of the addition of ethanol and indomethacin [Figure 2]. Compared to ethanol administration, the administration of indomethacin led to notable increases in WBC and NEUT. This result also aligns with earlier research that found indomethacin to have immunosuppressive properties.<sup>[6,7]</sup> In addition, rats fed with ethanol and indomethacin had significantly higher and lower levels of MDA and SOD, respectively, as compared to the heat stress control group [Figures 3 and 4]. This suggests supplementing



**Figure 4:** Effect of ethanol and indomethacin on; A. CAT, B. GSH, and C. GPX in heat-stress male Wistar rats. Values are mean  $\pm$  S D of 5 replicates. \*  $P < 0.05$  vs control, and #  $P < 0.05$  vs ethanol.

rats under heat stress with ethanol and indomethacin may lead to oxidative damage.<sup>[8,9]</sup> Finally, there were significant reductions in CAT, GSH, and GPX levels in rats given ethanol and indomethacin [Figure 5]. Similar results have been reported in earlier studies examining ethanol and indomethacin's effects on oxidative stress parameters. This aligns with earlier research that showed how ethanol and indomethacin negatively affected antioxidant enzymes.<sup>[10]</sup> The stomachs of rats given indomethacin and ethanol treatment underwent histological analysis, which revealed varying degrees of architectural deformities. These imply that indomethacin and ethanol supplementation may negatively affect the gastrointestinal tract, according to Sodipo *et al.*<sup>[10]</sup> As a result, this research shows that supplementing rats under heat stress with ethanol and indomethacin may adversely impact their hematological, histological, and antioxidant parameters. These findings have significant ramifications for treating heat stress and other situations that generate oxidative stress using ethanol and indomethacin.

## CONCLUSION

In Conclusion, this study provides evidence that ethanol and indomethacin supplementation may have negative effects on hematological, histological, and antioxidant parameters in heat-stressed rats. These findings have important implications for the use of ethanol and indomethacin in the management of heat stress and other conditions that cause oxidative stress.

## CONFLICTS OF INTEREST

No known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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