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EFFECTS OF ALCOHOL-GRADED CONCENTRATIONS ON TOTAL THIOLS AND SOME THIOL UTILIZING ENZYMES

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ABSTRACT
Excessive intake of alcohol has been documented to initiate different pathological conditions. Although various researchers have reported these associations, the modulatory effects on endogenous thiols are not well studied. This study investigated the effects of alcohol-graded concentrations on some thiol utilizing enzymes in rats. Adults' male rats were divided into four main groups and treated with distilled water, 30 %, 40 % and 50 % alcohol (3.20 g / Kg body weight). Five rats from each group were sacrificed at the end of 1, 7, 14, 21, and 28 day(s) of the experiment. Assay of glutathione peroxidase and glutathione S-transferase specific activities along with total thiols levels were carried out. Alcohol administration resulted in an upregulation of the activities of glutathione peroxidase and glutathione S-transferase with concomitant depletion of total thiols concentrations. Conclusively, this study affirms that graded dosages of alcohol administration to rats induced perturbations in the thiol utilizing system in a non-time dependent consistent manner.
Keywords: Alcohol; total thiols; thiol utilizing enzymes; grade concentrations; rats

INTRODUCTION

Alcohol (ethanol) is the most socially consumed addictive substance worldwide (Ighodaro *et al*., 2010; Guo and Ren, 2010). Intake of alcoholic beverages is an endorsement in most social parties (Ighodaro *et al*., 2010; Guo and Ren, 2010). However, the intake of excessive alcoholic beverages poses worrisome to health and economics (Room *et al*., 2005; Gupta *et al*., 2005; Guo and Ren, 2010). Several pathological conditions have been linked to chronic alcohol consumption (Room *et al*., 2005; Obad *et al*., 2018; Nowak and Relja 2020). These conditions range from straightforward drunkenness to critical pathological disorders (Molina *et al*., 2003; Room *et al*., 2005; Gupta *et al*., 2005; Das and Vasudevan, 2007; Ighodaro *et al*., 2010; Guo and Ren, 2010; Obad *et al*., 2018; Nowak and Relja 2020).

Alcohol, which enters into the bloodstream after ingestion via the gastrointestinal tract (GIT), is predominantly catabolized in the liver via two major pathways: the alcohol dehydrogenase (ADH) pathway and the microsomal ethanol oxidizing system (MEOS) pathway (Gupta *et al*., 2005; Das and Vasudevan, 2007; Ighodaro *et al*., 2010; Guo and Ren, 2010; Galicia-Moreno and Gutiérrez-Reyes, 2014). These pathways lead to hazardous by-products like acetaldehyde and reactive species (RS) capable of attacking cell membranes and biomolecules, causing oxidative stress (Gupta *et al*., 2005; Albano, 2006; Das and Vasudevan, 2007; Ighodaro *et al*., 2010). Because these metabolic pathways and their deleterious consequences occur in the liver and given the high levels of portal blood alcohol (following absorption), the liver is especially susceptible to alcohol-induced oxidative damage (Albano, 2006; Das and Vasudevan, 2007; Guo and Ren, 2010; Galicia-Moreno and Gutiérrez-Reyes, 2014). In addition, perturbations between pro-and antioxidant species caused by excessive ethanol consumption led to cell injury, which ensued from oxidative damage of lipids, proteins, DNA and some other cell biomolecules (Molina *et al*., 2003; Gupta *et al*., 2005; Albano, 2006; Das and Vasudevan, 2007; Guo and Ren, 2010).

The development and progression of alcoholic liver disease have been linked to this RS production and subsequent oxidative stress in liver cells (Molina *et al*., 2003; Gupta *et al*., 2005). Further, oxidative stress presents a mechanism linking excessive alcohol intake to hepatic inflammation and fibrosis (Gupta *et al*., 2005; Albano, 2006; Das and Vasudevan, 2007; Guo and Ren, 2010).

Cells encompass many antioxidants to thwart or repair RS-provoked damages and regulate redox-sensitive signaling pathways (Gupta *et al*., 2005; Weydert and Cullen, 2010; Birben *et al*., 2012). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are the mammalian primary antioxidant enzymes thought to be necessary for life in all oxygen-dependent cells (Weydert and Cullen 2010; Birben *et al*., 2012). SOD is responsible for the dismutation of superoxide radicals into H_2O_2 and molecular oxygen (O_2), while CAT and GPx detoxify the hydrogen peroxide (H_2O_2) produced. Superoxide and hydrogen peroxide that are deleterious to the cell is converted to a harmless substance (water and oxygen) by these enzymes (Weydert and Cullen, 2010). Other vital non-enzymatic antioxidants are the thiol group (-SH), generally referred to as total thiols (TSH), which are mainly involved in the antioxidant response, and serve as a sensitive indicator of oxidative stress (Bourgonje *et al*., 2020; He *et al*., 2020). Thus, Plasma total thiols have been an integral and vital part of the antioxidant mechanism. Thiol groups comprise either the sulfhydryl groups bound of protein or the free form (glutathione - GSH), with other sulfur-containing compounds like α-lipoic acid, cysteinylgycine, homocysteine, and cysteine. (Das and Vasudevan, 2007; Birben *et al*., 2012; Baskol *et al*., 2014; Bourgonje *et al*., 2020; He *et al*., 2020). Glutathione S-transferase (GST) is a detoxifying enzyme that acts as an antioxidant enzyme by utilizing reduced glutathione in the inactivation of metabolites, like unsaturated aldehydes, epoxides, and hydroperoxides (Gupta *et al*., 2005; Birben *et al*., 2012).

Since reactive oxygen species (ROS) can attach and modify almost all biomolecules, and one of the alcohol toxicities mechanisms is the generations of reactive species, information on the role of free radicals in alcohol-induced tissue injury could serve as essential areas of research that may help to prevent or ameliorate the toxic effects of excessive alcohol intake (Das and Vasudevan, 2007). Although, several authors have reported alcohol toxicity, Akinloye *et al*. (2021) also validated the disruption of lipid homeostasis and induction of tissue dysfunctions by different concentrations of alcohol (30 %, 40 % and 50 %) administration. However, there is a dearth of information on the effects of graded alcohol concentrations over a certain period on thiols utilization and perturbation status in animal models. Therefore, this study was undertaken to evaluate the time course and biochemical effects of graded concentrations of ethanol on thiol groups and thiol utilization enzymes in male Wistar rats.

MATERIALS AND METHODS

Chemicals

All chemicals were obtained from Sigma- Aldrich Chemical (Missouri, USA) and analytical grade, with at least 99% purity.

Experimental animals and design

Male albino rats (160 ± 10 g) were procured from the Department of Physiology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria. They were housed and allowed free access to water and animal feed in the animal care unit of the Department of Pure and Applied Zoology, Federal University of Agriculture, Abeokuta, throughout the experiment with adequate lighting and ventilation. Acclimatization of the animals was done for two weeks before the experiment and observed daily throughout the experiment.

The grouping of the experimental animals was done following the experimental design of Akinloye *et al*. (2021). After acclimatization, five rats were sacrificed for baseline studies; the others were randomly divided into four groups – Control (distilled water), 30 %, 40 % and 50 % alcohol (3.20 g / Kg body weight) – of twenty-five rats each. All the animals were orally administered with their respective group treatment. The administrations were done once daily (every morning) to simulate the traditional form of alcohol consumption using oral gavage (Tiwari *et al* 2009)**.** After twelve hours fasting, twenty rats (five from each group) were sacrificed, starting from the first day and every seven days till the 28^{th} day (on day(s) 1, 7, 14, 21, and 28.). Blood, collected from the animals through the ocular puncture into plain tubes, was allowed to clot, from which the serum was obtained following centrifugation at 3,000 rpm. The liver was quickly excised, cleaned with ice-cold saline, blotted dry, and a 10 % homogenate prepared with 10 mM phosphate buffer saline (pH 7.4). The resulting homogenate was centrifuged at 3,000 rpm for 10 minutes to separate the supernatant. Some biochemical parameters were estimated in the liver homogenates and serum.

Biochemical analyses

Vitamin C content was estimated as described by Marakala *et al*. (2012). The total thiols (TSH) contents were measured following the methods described by Baskol (2014). Assay of glutathione peroxidase (GPx, EC 1.11.1.9) and superoxide dismutase (EC 1.15.1.1) activities were done as described by Sajeeth *et al*. (2011). Ighodaro *et al*. (2010) described protocol was employed for glutathione S-transferase (GST; EC 2.5.1.18) assay.

Statistical analysis

Data generated were analyzed with one-way analysis of variance (ANOVA) and the results are presented as mean ± standard error mean. The significance level was set at $p<0.05$, using Duncan multiple range tests (DMRT) of Statistical Package for Social Sciences version 16.0.

RESULTS

Figure 1A shows vitamin C concentration (mg/dl) in the serum. Generally, the trend of vitamin C concentrations were of almost the same patterns with exception of the concentrations on day 21. Treatment with alcohol at 30 %, 40 % and 50 % concentration after day 21 decreased vitamin C concentration by 17 %, 24 % and 19 % respectively.

Figure 1B shows the concentration of total thiols (TSH) (µmole/g protein) in serum. Generally, total thiols concentrations trends were of different patterns. The concentration of serum TSH was depleted after day one administration by 7 % at 30 % and 50 % alcohol concentrations and by 28 % at 40 % alcohol concentrations compared with the control. After 7 days of administration TSH concentration decreased by 50 %, 49 % and 15 % at 30 %, 40 % and 50 % alcohol concentrations respectively. Similarly, the TSH concentration decreased by 67 %, 49 % and 75 % after day 14 of administration at 30 %, 40 % and 50 % alcohol concentrations compared to the control, respectively. While 40 % and 50 % alcohol concentrations decreased TSH concentration by 36 %, the 30 % alcohol decreased TSH concentration by 42 % compared with the control after 21 days. On day 28 of administration, the TSH concentration decreased by 8 %, 28 % and 40 % for 30 %, 40 % and 50 % alcohol concentrations, respectively, compared with the control.

Figure 1C represents the total thiols (TSH) concentration (µmole/g protein) in the liver. Trends of TSH levels were of the same patterns in all the groups with control groups having the highest concentrations throughout the periods. The TSH level in the alcohol-treated groups decreased by 8.3 %, 26 % and 56 % (at 30 %, 40 % and 50 % alcohol concentrations, respectively) compared with the control after day 1 of administration. In addition, there was a decrease in TSH concentration by 25 %, 36 %, and 41 % at 30, 40 and 50 % alcohol concentration, respectively, compared to the control after administration for seven days. After 28 days of the administration, TSH concentration in the liver decreased by 25 %, 36 % and 32 % at 30 %, 40 % and 50 % alcohol concentrations compared with the control, respectively.

Figure 2A shows the effect of alcohol on GST specific activity (U/g protein) in the serum. Alcohol administration at 30 %, 40 % and 50 % concentrations increased GST specific activity after 7 days by 55 %, 95 % and 1.8 folds respectively. There was an increase in the specific activity of GST by 2.2, 2.7 and 3.1 folds at 30 %, 40 % and 50 % alcohol concentrations, respectively, compared with the control after day 14 of administration. Similarly, after day 28, GST specific activity increased by 2.6, 2.3 and 5.6 folds for 30 %, 40 % and 50 % alcohol concentrations, respectively.

Figure 2B shows GPx specific activity (U/g protein) in serum. There was an increase in GPx specific activity at 30 %, 40 % and 50 % concentrations (81 %, 86 % and 149 % respectively) when compared to the control. After day 7 of administration GPx specific activity increased by 40 %, 22 % and 80 % at 30 %, 40% and 50 % concentrations respectively. Also, at 14 days of administration, GPx specific activity increased by 45 %, 86 % and 46 % at 30 %, 40 % and 50 % concentrations respectively, when compared to the control.

Figure 2C shows the effect of alcohol on SOD specific activity (U/g protein) in serum. SOD specific activity showed an increase on day 1 of administration by 67 %, 89 % and 2.8 folds at 30 % and 40 % and 50 % alcohol concentrations respectively when compared to the control. On day 7, an increase in SOD specific activity was also observed at 30 %, 40 % and 50 % alcohol concentrations (44 %, 1.7 folds, and one-fold, respectively). Elevation of SOD specific activity as a result of 30 % alcohol concentration were observed to be in time-dependent manner up to day 21 of administration.

Figure 3A shows the GST specific activity (U/g protein) in the liver. After 7 days, 30 %, 40 % and 50 % alcohol increased GST specific activity by 177 %, 120 % and 99 % alcohol respectively. After 21 days of the administration, GST specific activity increased by 65 %, two folds and 5.7 folds at 30 %, 40 % and 50 % alcohol. Also, treatment with 30 %, 40 % and 50 % alcohol concentrations increased the

specific activity of GST after 28 days of administration by 8-, 14- and 17- folds respectively, when compared to the control. Figure 3B shows the GPx specific activity (U/g protein) in the liver. The specific activity of GPx increased by 1.5 folds, 2.4 folds and 69 % at 30 %, 40 % and 50 % alcohol concentrations, respectively, after day 7 of administration. After 21 days, GPx specific activity increased by 96 %, 132 %, and 117 % at 30 %, 40 %, and 50 % alcohol concentrations, respectively, compared to the control.

Figure 3C shows the effect of alcohol on SOD specific activity (U/g protein) in the liver. After day 1 of administration, SOD specific activity increased by 1.7, 1.6 and 1.1 folds at 30 %, 40 % and 50 % concentrations compared to the control, respectively. SOD specific activity also increased on day 14 of administration by 75 %, 15 %, and 39 % at 30 %, 40 % and 50 % alcohol concentrations when compared to the control, respectively. When compared to the control, administration of alcohol at 30 %, 40 % and 50 % concentrations for 21 days increased SOD specific activity by 2.9, 1.5 and 2.2 folds, respectively. The alcohol-induced elevations in liver SOD activity were also observed to be time-dependent.

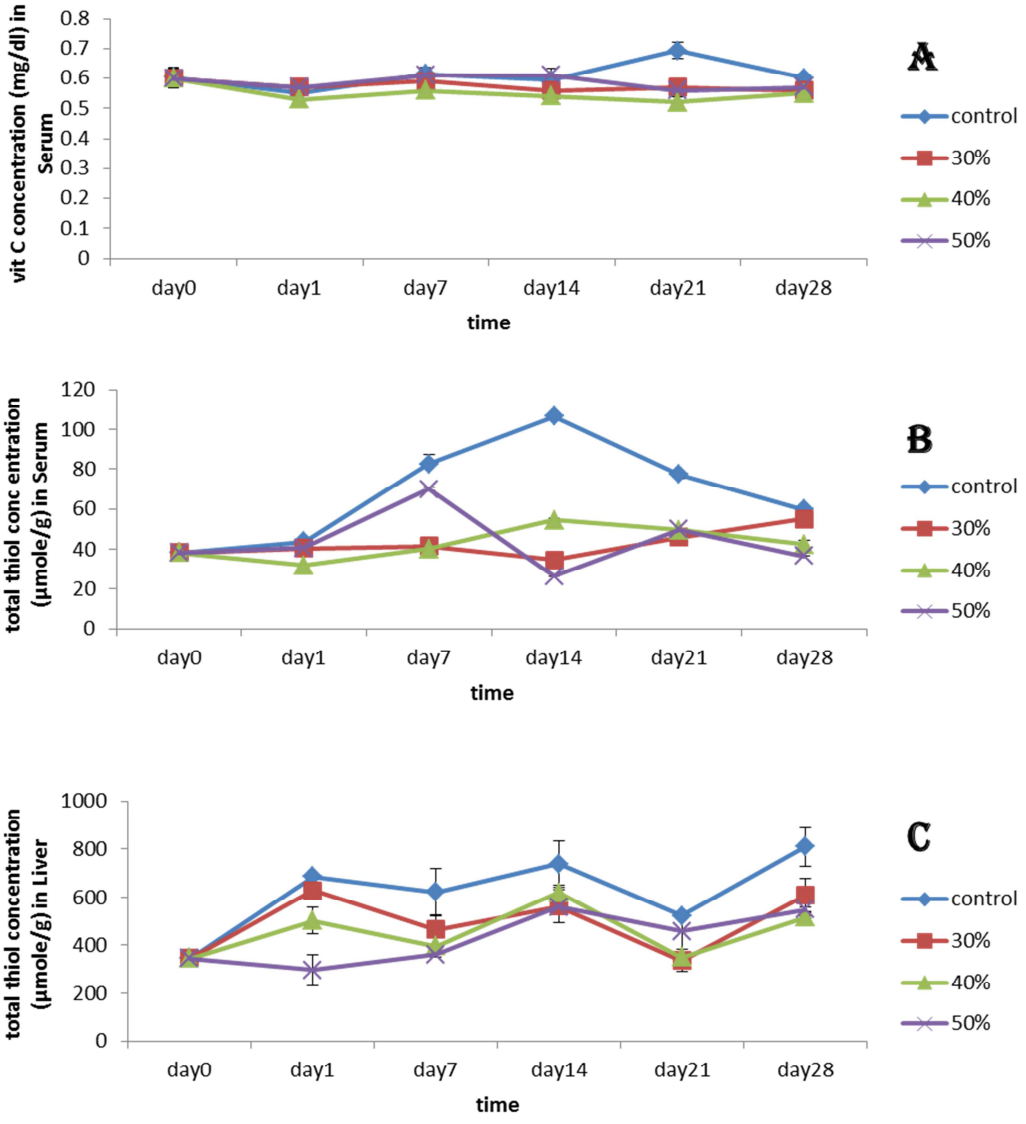


Figure 1: Time-course effects of graded concentrations of alcohol on concentrations of vitamin C and total thiols in control and experimental rats. **A** - Serum Vitamin C, **B** - Serum total thiol and **C** - Liver total thiol. Data were expressed as mean ± SEM. Significant difference was indicated at $p < 0.05$.

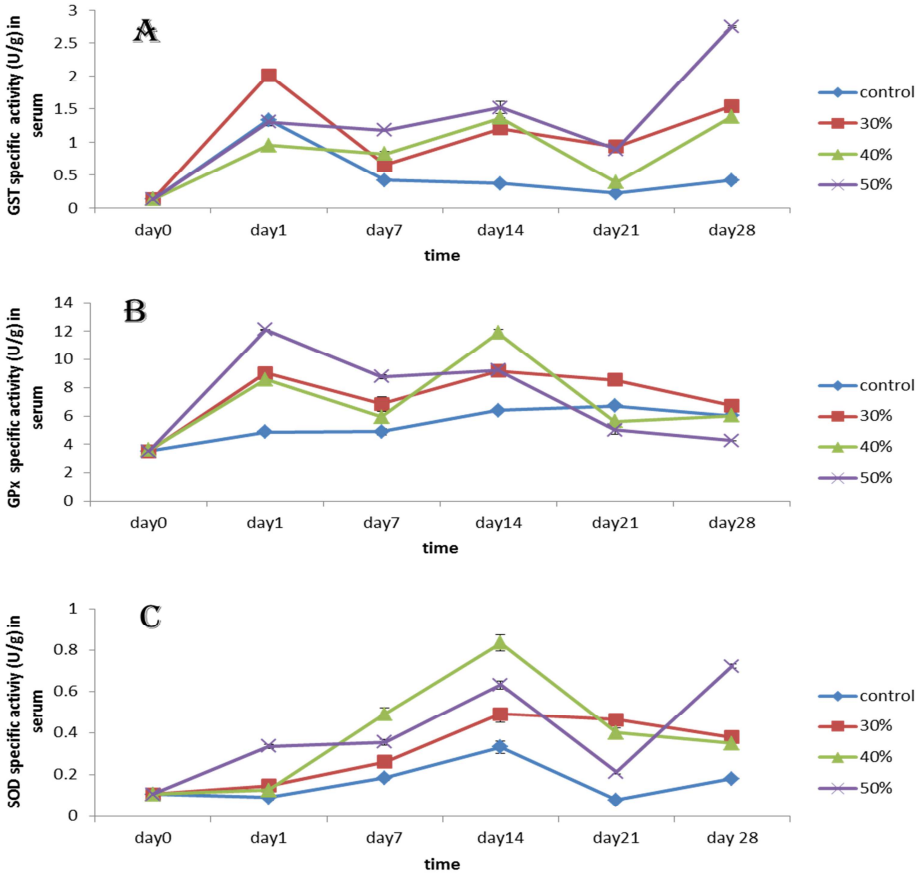
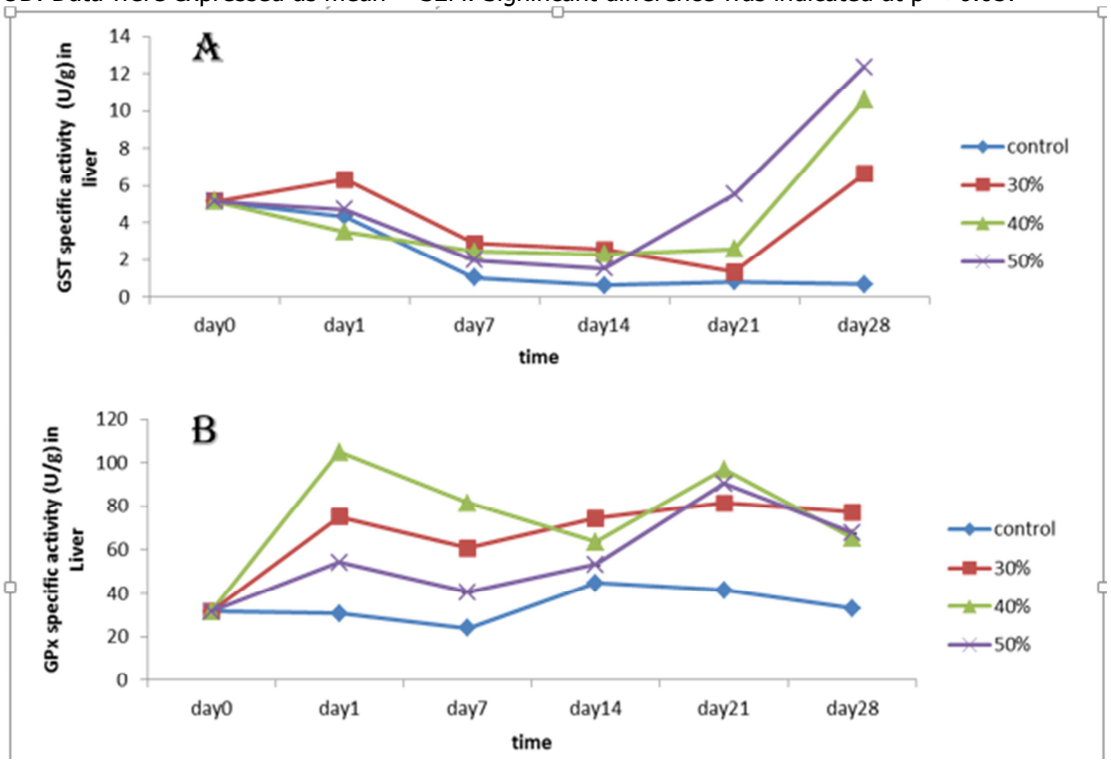


Figure 2: Time-course effects of graded concentrations of alcohol on specific activities of detoxifying and some antioxidant enzymes in serum of control and experimental rats. **A** - GST, **B** – GPx and **C** - SOD. Data were expressed as mean ± SEM. Significant difference was indicated at $p < 0.05$.



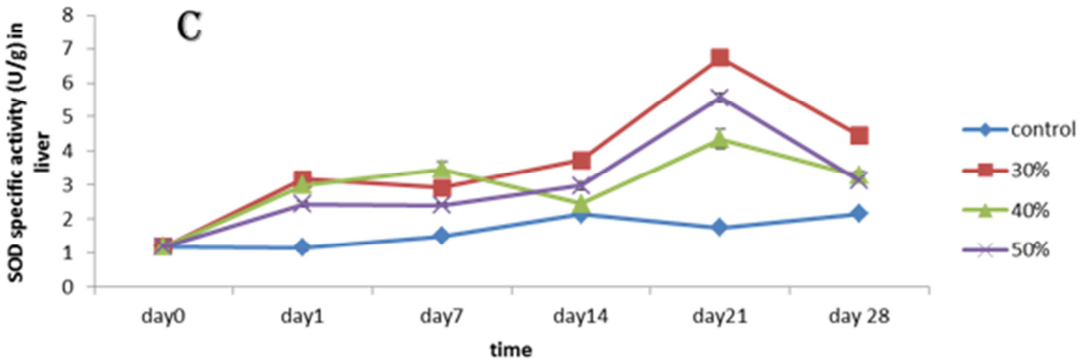


Figure 3: Time-course effects of graded concentrations of alcohol on specific activities of detoxifying and some antioxidant enzymes in liver of control and experimental rats. **A** – GST, **B** - GPx and **C** - SOD. Data were expressed as mean ± SEM. Significant difference was indicated at $p < 0.05$.

DISCUSSION

Excessive alcohol intake is associated with several changes in cell function and the oxidant-antioxidant bio-system (Das and Vasudevan, 2005; Das and Vasudevan, 2007; Albano, 2006; Hansel *et al.,* 2010). The antioxidants system involves the action of certain enzymes, vitamins, and thiols in biological system (Das and Vasudevan, 2005; Birben *et al*., 2012). These antioxidants yield non-radical products on reacting directly with the oxidizing radicals that may otherwise result in the loss of balance between the ROS (reactive oxygen species) production and antioxidant defence leading to "oxidative stress" causing various pathological conditions (Das and Vasudevan, 2005; Foerster *et al*., 2009; Wakabayashi, 2010; Birben *et al*., 2012). In this study, we examined the time-course effects of graded alcohol concentrations (30 %, 40 % and 50 %) on selected markers of thiols perturbations.

Vitamin C (a water-soluble vitamin) is a well known free radicals scavenger (Birben *et al*., 2012; Marakala *et al*., 2012). Thus, it possess ability to impede the detrimental chain reactions triggered by the actions of excessive free radicals generations and thereby preventing oxidation of thiol-containing molecules in the biological system (Marakala *et al*., 2012; Bourgonje *et al*., 2020; He *et al*., 2020). Hence, ethanol-induced toxicity is protected by vitamin C (Das and Vasudevan, 2005). However, administration of graded concentrations of alcohol for 21 days and 28 days decreased vitamin C concentration in a concentration and time-dependent manner, with 40 % alcohol concentration showing consistent reduction effects. This vitamin C depletion might ensue from being used up in the protection against alcohol-induced oxidative stress, which is in agreement with earlier reports that concentrations of vitamin C in the physiological system are reduced as a result of protection against alcohol-induced toxicity (Das and Vasudevan, 2005; Janani and Surapaneni, 2010).

Total thiols (TSH) play significant roles in the detoxification of xenobiotics and maintenance of the redox status of the cells as the most detoxifying enzyme uses it for the neutralization of xenobiotics and reactive oxygen species (Ighodaro *et al*.,2010; Birben *et al*., 2012; Baskol *et al*., 2014; Bourgonje *et al*., 2020; He *et al*., 2020). TSH is considered to be a significant component of the antioxidant defence system which can be viewed as a systemic redox buffer, therefore, decreased levels of total thiols (such as GSH) are indicative of oxidative stress (Albano, 2006; Singh *et al*., 2013; Bourgonje *et al*., 2020; He *et al*., 2020). Reductions in serum and liver TSH levels ensued from respective concentrations of alcohol administrations in this work could be linked to alcohol-induced oxidative stress that overwhelmed TSH concentrations through the generation of toxic acetaldehyde and other reactive molecules during alcohol metabolism in the cell. This finding corroborated Ighodaro *et al*. (2010) report that GSH as a free thiol group help in neutralizing toxic reactive species (like acetaldehyde) generated by alcohol toxicity. Also, Galicia-Moreno and Gutiérrez-Reyes (2014) reported increased cell damage from the high formation of acetaldehyde by alcohol dehydrogenase in the cytosol.

Furthermore, Gupta *et al*. (2005) reported that reduced glutathione (GSH), a chief constituent of total thiols pool, was significantly reduced in alcoholics with a strong significant positive correlation with total thiols (TSH). Moreover, this reduction in the total thiols could result from their utilization by GST and GPx in scavenging

the free radicals and detoxification of xenobiotics, including alcohol, hydrogen peroxide and lipid peroxides. This study further discovered that total thiols depletion effects were not wholly time and concentration-dependent. Differences in individual responses to excessive alcohol toxicities could be responsible for the disparity observed in this case. These findings agree with earlier reports that alcohol consumption and its toxic effects may not necessarily depend on the concentrations (Foerster *et al*., 2009; Hansel *et al*., 2010; Wakabayashi, 2010).

Glutathione S-transferase is an enzyme involved in the binding, transport and biotransformation of many endogenous and exogenous toxic compounds, using glutathione as a cofactor (Singh *et al*., 2012; Birben *et al*., 2012). Furthermore, GST possesses glutathione peroxidase activity and has been shown to scavenge hydrogen peroxide (H_2O_2) (Birben *et al*., 2012). Alcohol, among other drugs and chemicals, is known to induce GST and other detoxifying enzymes (Singh *et al.,* 2013). Increased GST activity is considered an index of increased load on the hepatocytes in detoxifying toxins (Singh *et al.,* 2012). An increase in liver and serum GST specific activities was also observed in this work. These observations corroborated with reports of Ighodaro *et al.* (2010) and Singh *et al.* (2013) that alcohol toxicity increase the activities of GST. A possible explanation could be due to the defensive response of GST to detoxify the toxic metabolites produced in the course of alcohol metabolism.

Superoxide dismutase is another crucial antioxidant enzyme involved in the dismutation of superoxide anion to hydrogen peroxide (Koch *et al*., 1994; Gupta *et al*., 2005; Janani and Supraneni, 2010). Dose-independent increase in the specific activity of SOD observed in the liver and serum could be attributed to over-expression of SOD as an adaptive response to alcohol-induced oxidative stress.These findings agreed with the report of Koch *et al*. (1994) that an up-regulation of SOD at the mRNA level, in response to chronic ethanol feeding of rats; thereby, suggesting a possible protective response against alcohol toxicity. Glutathione peroxidase is an oxidative stress-inducible enzyme involved in the scavenging of reactive species like lipid peroxides and hydrogen peroxide by utilizing low-molecular-weight thiols (such as GSH) and maintaining the functional integrity of cell membrane (Gupta *et al*., 2005; Janani and Supraneni, 2010; Birben *et al*., 2012). The dose-dependent increase in the liver and serum GPx specific activities observed in this study may be due to the induction of the enzyme to neutralize the effects of the lipid hydroperoxides produced during lipid peroxidation induced by alcohol toxicity.

CONCLUSION

Considering this study's outcome, it could be concluded that consumption of graded concentrations of alcohol may induce an imbalance in the total thiols levels and its utilizing system in concentrations and non-time dependent manner and that these toxic effects of alcohol administered were not consistent at 50 % alcohol concentration.

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