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G-6PDH, GPx activities and TBARS level in the stomach of adult male Wistar rats following the administration of ethanol and ethanolic extracts of neem (*Azadirachta indica*) leaves

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ABSTRACT: The present investigation studied the effects of ethanolic extracts of neem on the activities of glucose-6-phosphate dehydrogenase (G6PDH), glutathione peroxidase (GSH-Px), and the level of thiobarbituric acid test (TBARS), in the stomach, following ethanol-induced lesion in Wistar rats. 24 adult male Wistar rats used in the study were divided into 3 groups; Group A (n=8) received oral phosphate buffered saline and served as control; group B (n=8) received 1.0ml of 50% ethanol orally every 12 hours and group C (n=8) received Neem extract (500 mg/kg) orally and 12 hours later 1.0 ml of 50% ethanol. The experiment lasted for 21 consecutive days, after which the animals were sacrificed by cervical dislocation and the stomach excised. Their stomach was assayed spectrophotometrically for the activities of G-6PDH and GPx and level of TBARS. There was significant ($p = 0.05$) change in G-6-PDH and GPx activities and TBARS level, in the control, ethanol and ethanol and neem administered groups. The results indicate that extracts of neem which is widely consumed for a variety of ailments alters carbohydrate metabolism and antioxidant mechanisms in the stomach tissue.

Key words: Neem, stomach, glucose-6-phosphate dehydrogenase (G6PDH), glutathione peroxidase (GPx), thiobarbituric acid test (TBARS).

Introduction

It has been reported that aqueous neem extract has gastroprotective potentials at 300 mg/kg body weight dose given 12hours before the administration of 1ml of 50% ethanol for seven consecutive days (Ofusori *et al,* 2008). It is also reported that the cytoprotective mechanism of neem extract (300 mg/kg) against ethanol-induced mucosal injury in rats may be due to a reduction in the total acidity as well as an increase in gastric wall mucus. The gastric mucus plays an important role in protecting gastric mucosa against ulcerogens and facilitates the repair of the damaged gastric epithelium. The mucus gel adhering to the gastric mucosal surface protects the underlying epithelium against acid and necrotizing agents, like ethanol (Ofusori *et al,* 2008).

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The lipid peroxidation mediated by reactive oxygen species (ROS) is an important cause of destruction and damage to cell membranes and it is involved in the pathogenesis of acute mucosal injury induced by ethanol, ischemia reperfusion, and indomethacin (Rodr´ıguez *et al*, 2007). In addition, glutathione (GSH) is an important constituent of intracellular protective mechanism against a number of noxious stimuli, and it is known as a major low molecular weight scavenger of free radicals in cytoplasm. Sulphydryl (SH) containing compounds, and also agents that modify SH groups, prevents the acute hemorrhagic erosions caused by ethanol, nonsteroidal anti-inflammatory drugs (NSAIDs), or stress in animal models (Rodr´ıguez *et al*, 2007). In the same way, various antioxidant enzymes such as superoxide dismutase (SOD), an important radical superoxide scavenger, and Glutathione peroxidase (GSH-Px), an enzyme involved in the elimination of hydrogen peroxide and lipid hydroperoxides, play an important role in cell protection (Rodr´ıguez *et al*, 2007). The present study was therefore designed to evaluate the effects of ethanolic extracts of neem leaves on glucose-6-phosphate dehydrogenase (G-6PDH), glutathione peroxidase (GPx), thiobarbituric acid (TBARS) Levels in the stomach of adult male wistar rats.

Materials and Methods

Plant material and extract preparation

The leaves of *Azadirachta indica* were collected within the premises of College of Health Sciences, University of Ilorin, Kwara State, Nigeria. Identification of plant was carried out in the department of botany, University of Ilorin. A large quantity of neem leaves were collected, washed clean with water, air dried and reduced to powder using LAO-style mortar and pestle. The powdered leaves of the neem plant weighing 300g was mixed with 3litre of 70% ethanol and the mixture was left for 24hours, mixture was filtered and the filtrate was concentrated in a water bath (maintained at 60±0.2°c) to yield a green solid extract. It was weighed to be 25g using GALLENKAMP (FA2104A, ENGLAND) the solid extract was stored in a refrigerator.

Experimental animals

Twenty-four adult male rats weighing (200±10g) were bred in the animal house of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin. The rats were housed under standard and good laboratory conditions (light, temperature, humidity and ventilation). They were fed standard rat diet, purchase from Bendel Feeds Limited Ilorin, to avoid changes in dietary compositions. Water was given *ad libitum*. All animals were handled in conformity with the rules and guidelines of the animal rights committee of the College of Health Sciences, University of Ilorin. The study protocol was approved by the same committee. They were evaluated and judged presumably healthy and fit for use in the Study. The rats were randomly divided in to three (3) groups, A, B and C, of eight (8) rats each. Group A (n=8) received oral phosphate buffered saline and served as control; group B (n=8) received 1.0ml of 50% ethanol orally every 12 hours and group C (n=8) received Neem extract (500 mg/kg) orally and 12 hours later 1.0 ml of 50% ethanol for 21 consecutive days, 24 hours after the last administration the animals were sacrificed by cervical dislocation and the stomach were immediately blotted dry, weighed, immediately transferred to 0.25 M sucrose solution, homogenized, centrifuged at 5000 rpm for 10 min (David *et al*, 2009). The supernatants were immediately stored in the freezer (-20°C) and assayed within 48hours. The level of thiobarbituric acid (TBARS), and activities of glutathione peroxidise (GPx) and Glucose – 6 – Phosphate Dehydrogenase (G-6PDH), were estimated according to the methods of Ohkawa et al. (1979), Rotruck et al. (1973) and Korenberg and Horecker (1995) respectively using MDA, GPx and G-6-PDH kits from (Randox Laboratories, ltd. United Kingdom). The enzyme activity was read spectrophotometrically.

Statistical analysis

Values were reported as mean ± S.E.M and data were analyzed using students t-test with the statistical software SPSS version 14 at 95% confidence interval. A $p<0.05$ was considered statistically Significant.

Results and Discussion

Table 1. GPx, G-6PDH activities and TBARS level in the control and experimental groups.

ENZYME	CONTROL	ETHANOL	ETHANOL+NEEM
GPx	61.01±0.020	58.74±0.885 ^{ANS}	64.66±0.400 $^{ANS, B*}$
G-6PDH	68.67±1.145	75.17±0.515^{A*}	58.37±7.630^{ANS,B*}
TBARS	0.201±0.005	0.242±0.007 ^{A*}	0.165±0.015 ^{ANS,B*}

Values are mean ±SEM. A=compared with Control; B=compared with group Ethanol; *p<0.05; NS=not significant.

Glucose - 6 - phosphate dehydrogenase (G6PDH) activity in the stomach

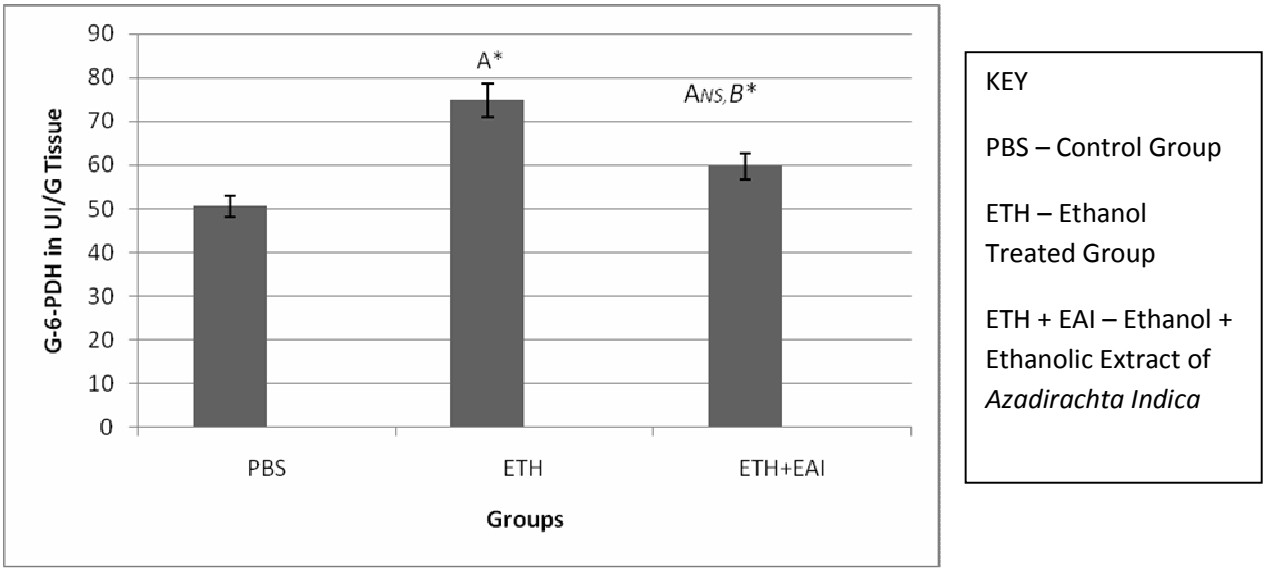
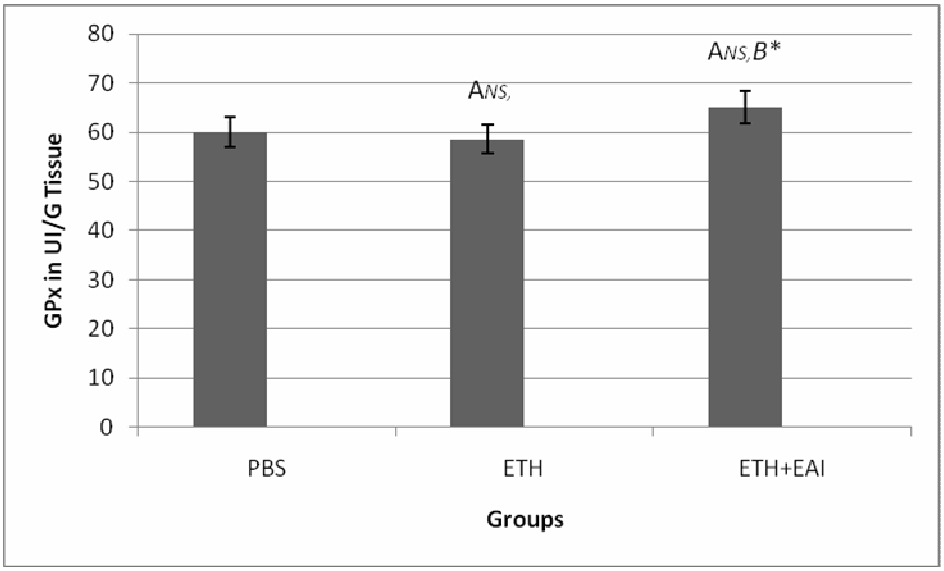


FIGURE 1: Glucose- 6- phosphate dehydrogenase (G6PDH) activity in gastric mucosal damage induced by ethanol. The results are the means in (UI/g tissue). Values are expressed as Mean ± SD of four rats each group. A=compared with group PBS; B=compared with group Ethanol; *p<0.05; NS=not significant.

Glutathion peroxidise activity in the stomach



KEY

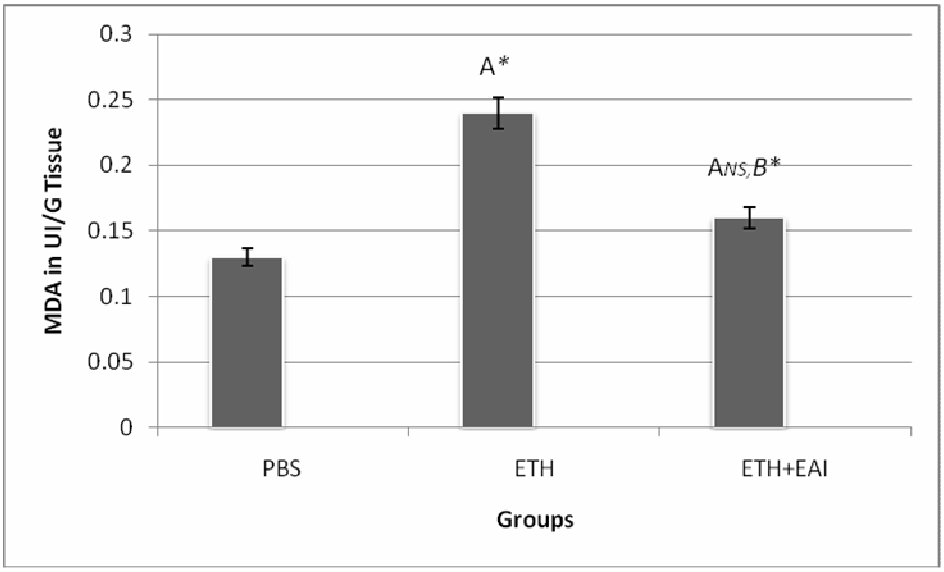
PBS – Control Group

ETH – Ethanol Treated Group

ETH + EAI – Ethanol + Ethanolic Extract of *Azadirachta Indica*

FIGURE 2: glutathione peroxidase (GSH-Px) activity in gastric mucosal damage induced by ethanol. The results are the means in (UI/g tissue) Values are expressed as Mean ± SEM of four rats in each group. A=compared with group PBS; B=compared with group Ethanol; *p<0.05; NS=not significant.

Thiobarbituric acid activity in the stomach



KEY

PBS – Control Group

ETH – Ethanol Treated Group

ETH + EAI – Ethanol + Ethanolic Extract of *Azadirachta Indica*

FIGURE 3: Thiobarbituric acid test (MDA) activity in gastric mucosal damage induced by ethanol. The results are the means in (UI/g tissue). Values are expressed as Mean ± SEM of four rats in each group. A=compared with group PBS; B=compared with group Ethanol; *p<0.05; NS=not significant.

Enzyme studies

The study evaluated the effects of ethanolic extract of neem leaves on some enzymes (G-6-PDH, MDA, and GPx) activities in the stomach of the experimental animals used. Without enzymes, life would not be possible (Rodwell, 1993). Enzymes are the biocatalyst that regulates the rates at which all physiological processes take place. G-6-PDH is a dehydrogenase which catalyzes the removal of hydrogen. Results obtained in this study showed that ethanolic extract of neem at 500 mg/kg alters the activity of G-6-PDH as seen in FIGURE 1, where there is a significant increase in the ethanol treated group as compared with the PBS treated, there was a significant decrease in the enzyme activity in the Neem treated group as compared with the control, but there was no significant change in the Neem treated group when compared with the PBS control group.

Recent observations have shown that the G6PDH also plays a protective role against reactive oxygen species in eukaryotic cells that possess alternative routes for the production of NADPH and that G6PDH expression is up regulated by oxidants through a mechanism acting mainly on the rate of transcription of this gene (David *et al* 2009). This up regulation by oxidants must have accounted for the increase noticed in the Ethanol treated group and was corrected by the administered Neem extract which brought about a significant reduction in the enzyme.

Activities of enzyme Glutathion peroxidise (GPx) was also altered as shown in FIGURE 2, when ethanol was administered, though the decrease was not significant but signifies the presence of reactive oxygen species within the tissues, the enzyme activity increased significantly in the group treated with ethanol and Neem when compared with the Ethanol only group, but no significant change was noticed when the Neem treated group was compared with the PBS treated group. The antioxidant enzyme scavenges free radicals produced through ethanol presence and provides the primary defence against cytotoxic oxygen radical (Gunassekaran *et al*, 2010).

FIGURE 3 shows the level of thiobabituric acid (TBARS) which increased significantly in the Ethanol only group when compared with the PBS treated group. The enzyme activity decreased significantly in the Neem treated group when compared with the Ethanol only group but has no significant difference when compared with the PBS treated group. Lipid peroxidation is an important cause of cell membrane damage since it has been shown that lipid peroxidation degrades the poly unsaturated fatty acid of cell membrane with consequent disruption of membrane integrity (Niki, 1987; Fridorich, 1986). Lipids are modified by ROS and visualized as a thiobarbituric acid reactive substance (TBARS). We measured the TBARS which serve as an indicator for intracellular oxidation in gastric mucosa. The increase in lipid peroxide may suggest a possible mechanism of tissue injury by reactive oxygen intermediates. Increase in TBARS level seen in the ethanol treated group indicates stressed tissue due to presence of reactive oxygen species released by ethanol, while the reduction seen in the Neem treated group indicates Neem has effect and may have attempted to mop up the reactive oxygen species present in the tissue and help provide a safer tissue environment.

Flavonoid in *Azadirachta indica* is highly rich in antioxidants. The mechanism of action of *Azadirachta indica* is believed to be triggered by the presence of these antioxidants. Antioxidants have been known to mop up free radicals in living tissues. The antioxidant properties of *Azadirachta indica* may have assisted in scavenging the free radicals generated by the presence of ethanol (Ofusori *et al*, 2008).

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