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Protective effect of pineapple (*Ananas cosmosus*) peel extract on alcohol-induced oxidative stress in brain tissues of male albino rats

Ochuko L Erukainure^{1*}, John A Ajiboye², Rachael O Adejobi², Oluwatoyin Y Okafor¹, Sunday O Adenekan³¹Food Technology Division, Federal Institute of Industrial Research, Oshodi, Nigeria²College of Natural and Applied Sciences, Bells University of Technology, Ota, Nigeria³Biochemistry Department, College of Medicine, University of Lagos, Lagos, Nigeria

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ABSTRACT

Objective: To investigate the ability of pineapple peels to protect against alcohol-induced oxidative stress in brain tissues using male albino rat models. **Methods:** Response surface methodology (RSM) was used to design a series of experiments to optimize treatment conditions with the aim of investigating the protective effect of pineapple peel extract on alcohol-induced oxidative stress in brain tissues. Oxidative stress was induced by oral administration of ethanol (20% w/v) at a dosage of 5 mL/kg bw. The treatment lasted for 28 days. At the end of the treatment, the rats were fasted overnight and sacrificed by cervical dislocation. Tissue homogenates were used for the assessment of protein concentration, reduced glutathione (GSH) content, catalase, and SOD. **Results:** Alcohol administration caused a significant decrease ($P > 0.05$) in GSH level in the group which was only fed alcohol. Treatment with pineapple peel extracts caused increase in GSH level in alcohol fed groups. No significant difference ($P < 0.05$) was observed in SOD levels of the negative control and group fed on only pineapple peel extract. Elevated level of catalase was observed in the negative control but pineapple peel extract significantly reduced the levels. **Conclusions:** This study indicates the protective effect of pineapple peel against alcohol-induced oxidative stress in brain tissues.

1. Introduction

Several studies have shown that chronic alcohol consumption leads to several metabolic disorders including hepatic and extra hepatic diseases, which are instigated by reactive oxygen species (ROS) generation as found in liver, heart and brain, leading to cellular damage[1–4]. Oxidative stress sets in when an imbalance occurs between oxidants (ROS) and antioxidants in favour of the oxidants. ROS generation induced by alcohol is believed to be specific to alcohol metabolism by cytochrome P450–2E1 (CYP2E1), which produces H₂O₂ in addition to acetaldehyde (Ach), while alcohol dehydrogenase (ADH) mediated produces only Ach[5]. Interaction of H₂O₂ with copper/iron produces ROS during alcohol oxidation by alcohol-inducible liver microsomal cytochromes P–450 enzymes[5]. Metabolism of alcohol by CYP2E1 causes oxidative liver damage by increased ROS levels and by reduction of glutathione

and superoxide dismutase activity[6,7]. ROS increases the permeability of blood brain barrier, as well as inhibit the mitochondrial respiration[8]. Free radicals generated in the brain have also been reported to influence gene expression, subsequently affecting apoptosis and neuronal death[9]. All of which is intimately linked to the degenerative processes in most neurological diseases.

Several plants have been reported to exhibit antioxidant activities, which protect against the damage caused by reactive oxygen species (ROS)[10]. This antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins[11].

Pineapple (*Ananas cosmosus*) is a popular fruit which grows in the tropics and sub-tropics. It is native to Central and Southern America and belongs to the *bromeliaceae*. Its nutritional and medicinal properties have been widely studied. It is rich in the antioxidants namely flavonoids, vitamin A and C[12]. The peels are well known ingredients in ethnomedicine. Correia *et al.* established a relationship between antioxidant activity, β -glucosidase and total phenolic content in pineapple peel/soy flour extracts[13].

This study aims at investigating the ability of pineapple peels to protect against alcohol-induced oxidative stress in

*Corresponding author: Ochuko L Erukainure, Food Technology Division, Federal Institute of Industrial Research, Oshodi, Nigeria.

Tel: +2348062179388

E-mail: loreks@yahoo.co.uk

brain tissues using male albino rat models.

2. Materials and methods

2.1. Plant materials

Pineapple peels were collected from a fruit seller at Oshodi, Lagos, Nigeria. They were rinsed in distilled water to remove dirt. They were air dried and grounded to fine texture with laboratory mill and were extracted with methanol for 8–10 hours, distilled and concentrated using steam bath and then stored for subsequent use.

2.2. Design of the experiments to optimize the treatment conditions

Prior to feeding trials and analysis, a series of experiments were designed by response surface methodology (RSM) using Design Expert V7.1 software to optimize the treatment conditions. The main factors selected were treatment with distilled water, alcohol and pineapple extract.

2.3. Animals

Thirty male albino rats of weighing 95–120 g were used for the present investigation. They were reared at the Animal House of the Biochemistry Department of Bells University of Technology, Ota, Nigeria.

They were acclimatized for two weeks on normal diet of pelletized mouse chow, with water given *ad libitum* at room temperature with a 12-hour light and dark cycle before the commencement of the experiment. Based on RSM design, the rats were divided into six groups, each consisting of five animals. Group 1: Distilled water only [5 mL/kg body weight (bw)] (Control). Group 2: Ethanol only (Negative control). Group 3: Ethanol + Pineapple peel extract (2.5 mL/kg bw). Group 4: Ethanol + Pineapple peel extract (5 mL/kg bw). Group 5: Pineapple peel extract only (5 mL/kg bw). Group 6: Ethanol + Distilled water (5 mL/kg bw).

Oxidative stress was induced by oral administration of ethanol (20% w/v) at a dose of 5 mL/kg bw. The treatment lasted for 28 days. The dose of ethanol, pineapple peel extract and the period of treatment were selected on the basis of previous studies by Faremi *et al.*[14].

At the end of the treatment, the rats were fasted overnight and sacrificed by cervical dislocation.

The animals used in the present study were maintained in accordance with the approval of the Animal Ethical Committee, Bells University of Technology, Ota, Nigeria.

2.4. Preparation of tissue homogenates

The organs (brain) were removed, rinsed in the ice-cold 1.15% KCl solution to wash off excess blood, blotted dried with filter paper, and weighed. The organs were homogenized in four parts of homogenizing buffer (*i.e.* 1g of organ in 4mL

of buffer) and centrifuged at 10 000 rpm for 15 minutes in an ultracentrifuge at <2 °C to get the mitochondrial fraction. The supernatant (post mitochondrial fraction) was decanted and stored at <4 °C for subsequent analysis. Each time the supernatant was outside the freezer and was kept in ice bags. The protein content of the tissue fractions of the organs were determined by Lowry's method using bovine serum albumin (BSA) as standard[15].

2.5. Determination of oxidative stress parameters

The tissue homogenate was used for the assessment of reduced glutathione (GSH) content, catalase, and SOD[16–18].

2.6. Statistical analysis

Statistical significance was established using One-Way analysis of variance (ANOVA) and data were reported as mean \pm standard deviation. Statistical analyses were carried out using SPSS for Windows, version 14.0 (SPSS Inc. Chicago, IL,USA).

3. Results

3.1. Effect of pineapple peel extract

Feeding on alcohol caused a significant decrease ($P>0.05$) in GSH level in the group fed alcohol only (negative control) (Figure 1). Treatment with pineapple peel extract at 2.5 and 5.0 mL/kg bw caused 58.98% and 2.69% increase in GSH level, respectively in alcohol fed groups.

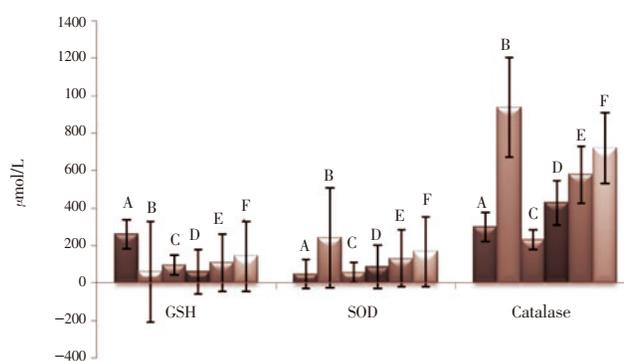


Figure 1. GSH, SOD and catalase activities of pineapple peel extract on brain tissues. Values=mean \pm SEM, n=5, A=group 1; B=group 2; C=group 3; D=group 4; E=group 5; F=group 6.

A significant increase ($P<0.05$) in the SOD level was observed in groups fed alcohol only as compared with the control. Treatment with pineapple extract at 2.5 and 5.0 mL/kg bw caused 72.50% and 25.03% reduction in the SOD level, respectively in alcohol fed rats.

Elevated level of catalase activity was observed in alcohol fed group. Treatment with pineapple peel extracts (2.5 and 5.0 mL/kg bw) caused a significant reduction in catalase level in the alcohol fed groups.

Effect of pineapple peel extract on brain tissue protein concentration was shown in Figure 2. No significant change was observed in protein concentration of the group fed alcohol only as compared with the control. Treatment with pineapple peel extract at 5.0 mL/kg bw significantly ($P<0.05$) increased protein concentration of alcohol fed rats.

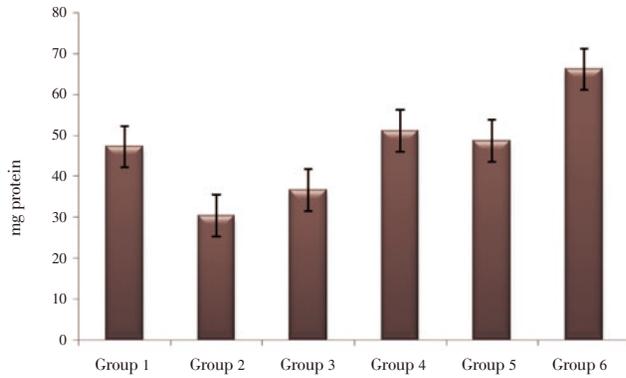


Figure 2. Effect of pineapple peel extract on protein concentration of brain tissues. Values=mean±SEM, n=5.

3.2. Response surface plotting and optimization of treatment conditions

In the present study, the relationship between the studied antioxidant markers namely GSH, SOD, catalase and protein and the three selected treatments which were the controllable factors namely distilled water, alcohol and pineapple peel extract were studied (Table 1).

The models as fitted (Table 2) in terms of the coded factors corresponded to:

$$\begin{aligned} \text{GSH} &= 120.05 + 40.65 \times A - 41.04 \times B + 0.79 \times C - 16.95 \times AB + 4.55 \times C^2 \\ \text{SOD} &= -10.59 - 78.35 \times A + 18.08 \times B + 13.89 \times C + 40.67 \times AB + 26.36 \times C^2 \\ \text{Catalase} &= 38.74 - 252.17 \times A + 67.46 \times B + 98.63 \times C + 142.46 \times AB + 112.97 \times C^2 \\ \text{Protein} &= 55.32 + 13.77 \times A + 5.36 \times B + 7.26 \times C + 4.11 \times AB + 1.04 \times C^2 \end{aligned}$$

In the contour plot it was observed that GSH activities decreased at a constant dose of pineapple peel extract (3.75 mL/kg) as alcohol dosage increases, however its activities increased with increase in distilled water dose as alcohol dosage increases (Figure 3a). SOD activities were observed to increase from -17.45 to -4.42 $\mu\text{mol/L}$ as pineapple peel

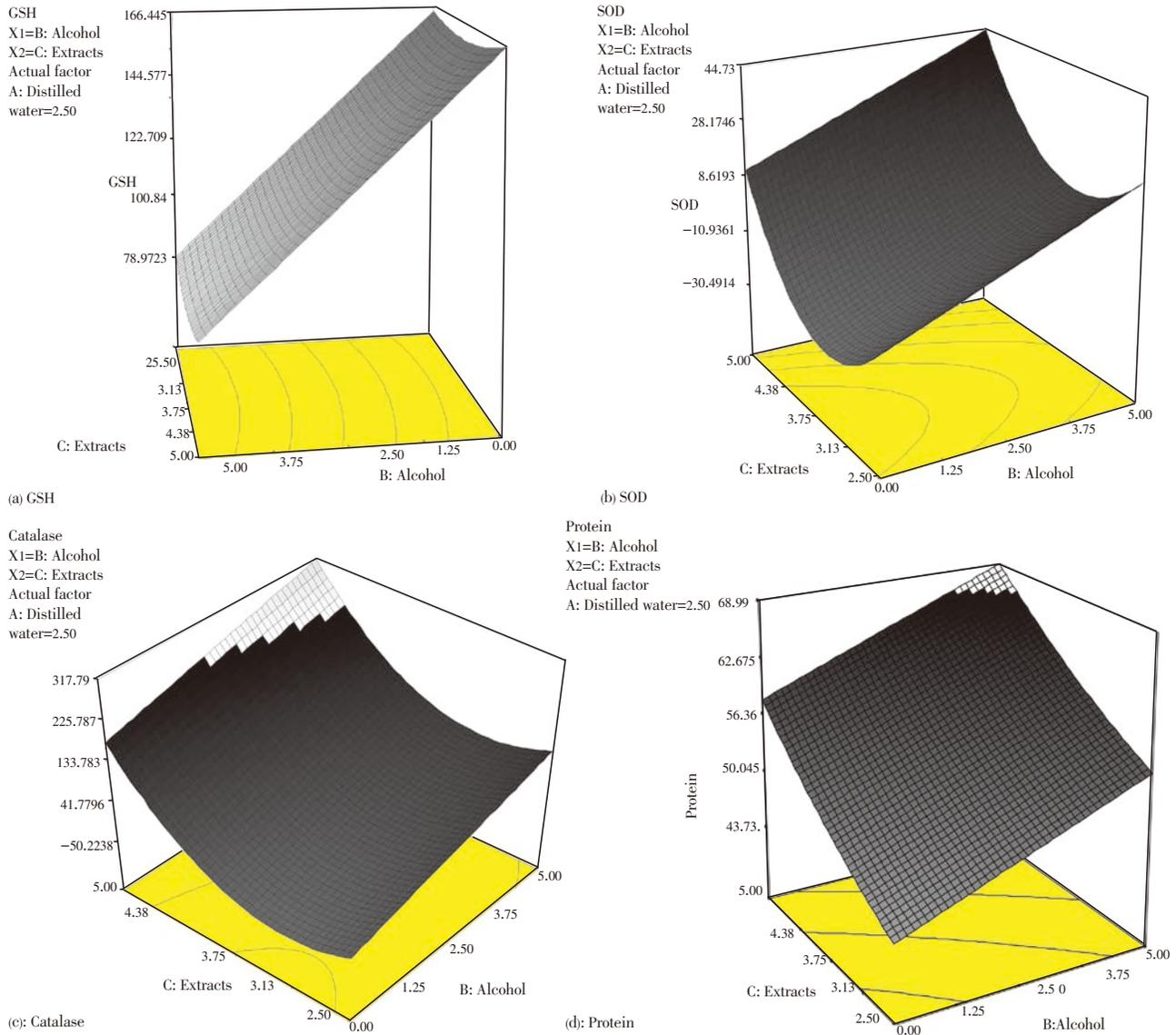


Figure 3. Response surface curve for (a) GSH, (b) SOD, (c) Catalase, and (d) Protein at different dose of co-administration of alcohol and pineapple extract.

Table 1
Design summary.

Parameters	Type	Low actual	High actual	Low coded	High coded
Distilled water (mL/Kg bw)	Numeric	0.00	5.00	-1.00	1.00
Alcohol (mL/Kg bw)	Numeric	0.00	5.00	-1.00	1.00
Extracts (mL/Kg bw)	Numeric	2.50	5.00	-1.00	1.00
	Obs	Minimum	Maximum	Mean	SD
GSH (μ mol/L)	6	59.07	257.29	120.18	67.50
SOD (μ mol/L)	6	47.88	240.73	121.27	67.11
Catalase (μ mol/L)	6	230.25	936.75	531.13	243.99
Protein (μ mol/L)	6	30.41	66.15	46.68	11.32

Study type: Response surface; Runs: 13; Initial design: Box–behken; Blocks: No blocks; Design model: Quadratic.

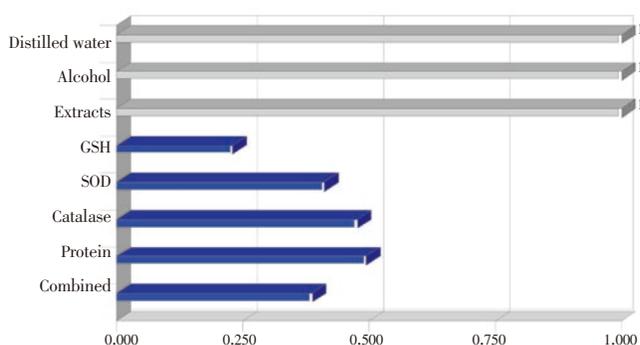
extract dose increased from 3.13 to 3.75 mL/kg between alcohol dose of 1.25–3.00 mL/kg, the activity however remained constant (8.62 μ mol/L) at 2.5 and 5.00 mL/kg, respectively with increased alcohol dose (Figure 3b). Catalase activities were observed to increase with increase in alcohol dose as pineapple peel extract increased, however the activities was observed to reduce with increase in alcohol dosage as distilled water increases (Figure 3c). Protein concentration was observed to increase with increased pineapple peel extract as alcohol dosage increased (Figure 3d).

Table 2
Coefficient estimates of responses.

Factor	GSH	SOD	Catalase	Protein
Intercept	120.05	-10.59	38.74	55.32
A	40.65	-78.35	-252.17	13.77
B	-41.04	18.08	67.46	5.36
C	0.79	13.89	98.63	7.26

A: Distilled water; B: Alcohol; C: Extracts.

Eighteen solutions were obtained when the method of desirability function was applied. The optimal value for considered factors can be one of these eighteen solutions. The final decision of the optimal conditions depends on the effect of the treatments on the antioxidant activities in the brain tissues. Solution one was selected to obtain a combined desirability of 0.41 for the antioxidant activities (Figure 4).

**Figure 4.** Numerical optimization of GSH, SOD, catalase, and protein at different doses of alcohol and pineapple peel extract.

4. Discussion

The detrimental effects of alcohol on the central nervous system are well documented. Chronic alcohol consumption has been shown to cause degenerative changes in the brain^[19]. The mechanism, by which ethanol induces alterations in the brain, may be directly due to ethanol or its oxidation products as it is now generally accepted that oxidative stress plays an important role in the pathogenesis of ethanol toxicity^[20]. This study reports the protective potentials of pineapple peel extract against alcohol-induced oxidative stress in brain tissues.

GSH is a major endogenous antioxidant, which counteracts free-radical-mediated damage^[21] and a marker of oxidative stress. It forms an important substrate for other enzymes, which is involved in the free-radical scavenging^[22]. Replenishment of the GSH level will therefore be a remedy in reverting alcohol-induced oxidative stress. The increased GSH level observed in treatments with pineapple peel extract indicates the antioxidant potentials of the peels.

SOD plays an important role in protecting the cells from oxidative damage by converting superoxide radicals into hydrogen peroxide, which is further metabolized by catalase to molecular oxygen and water^[23–25]. SOD and catalase enzymes are induced in response to oxidative stress^[24]. SOD is the first enzyme of the scavenger enzyme series to ameliorate the damage caused to cells by free radicals^[25], while catalase is one of the several cellular antioxidant enzymes that provide a defense system for the scavenging of reactive oxygen metabolites^[23]. In this study there was an increase in the activities of SOD and catalase in alcohol fed rats. It contradicted the reports by Thenmozhi and Subramanian^[23] that their activities were decreased in alcohol fed rats. The observed increase in SOD and catalase activities on feeding alcohol could be attributed to their increased synthesis due to induction^[28]. The significant reduction observed in treatment with pineapple peel extract is an indication of its antioxidant potentials.

The observed reduction in protein concentration in alcohol-fed rats can also be attributed to production of MDA-protein adduct due to lipid peroxidation.

The relationship between factors and response can best be understood by exploring the series of contour plots generated by holding one factor constant and plotting response as a

function of two other factors^[27]. Therefore, to visualize the combined effects of the factors on the response, the response surface and contour plots were generated for each of the fitted models.

Based on results from this study, it can be concluded that the methanolic extract of pineapple peel protects and exhibits antioxidant activities against alcohol-induced oxidative stress in brain tissues. This effect may be attributed to its bromelin and phytochemical constituents, which might include fiber, phenolics, flavonoids, anthocyanins, and trace minerals. The response surface methodology could be effective in determining the optimum antioxidant activities of the pineapple peel extract. This approach might be useful in determining an acceptable treatment conditions by plant extracts in the management of oxidative stress.

Conflict of interest statement

We declare that we have no conflict of interest.

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