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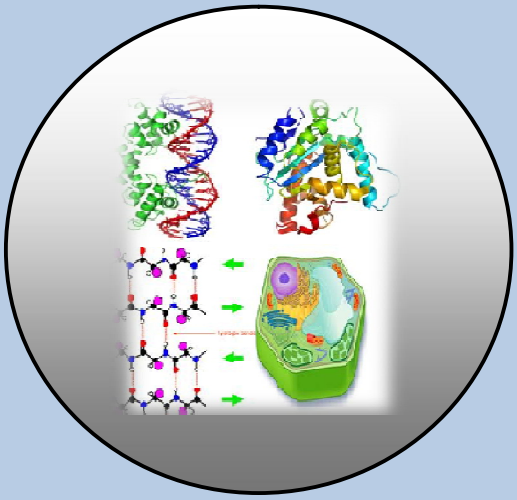

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Effects of Ugandan Common Sachet Alcohol Brands on Liver Catalase Activity

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ABSTRACT

Catalase (CAT) is a vital enzyme found in both animals and plants that detoxifies hydrogen peroxide (H_2O_2); a major toxic by product of various oxidase and superoxide dismutase activities which if left to accumulate causes the oxidation of cellular targets such as DNA, proteins and lipids resulting into mutagenesis and cell death. CAT activity is inhibited by chemicals like cyanide, ethanol, azide, hydroxylamine, aminotriazole and mercaptoethanol. In this study therefore, we examined the effects of ethanol concentrations and duration of bovine liver catalase (BLC) exposure to Ugandan common sachet alcohol brands (CSAB) on its activity as this is similar to that of humans in function. The concentration of H_2O_2 ($[H_2O_2]$) broken down determined by redox titration using acidified potassium permanganate ($KMnO_4$) was used as a measure of the activity of BLC. The activity decreased with increasing ethanol concentrations, that is, 0.4560 ± 0.0000 mol L^{-1} at 0 % ethanol to 0.2303±0.0125 mol L^{-1} at 43% ethanol and r^2 = 94.6%; p = 0.001 ; and hypothesized to cease within 15 minutes at a 95.45% ethanol. BLC activity also decreased (r^2 = 69.9%; p = 0.038) with increasing duration of exposure to 40.0% ethanol CSAB from 0.4560 ± 0.0000 mol L^{-1} at 0 minutes to 0.1475 ± 0.0032 mol L^{-1} at 25 minutes; and stopped within 34.85 minutes. High ethanol concentrations (> 40%) therefore inactivate BLC as well as 40.0% if incubated in it for longer than 35 minutes. We therefore postulate that the Ugandan CSAB of 37-43% ethanol sold on the open markets in Uganda should not be consumed in large quantities continuously for more than 35 minutes as this may be lethal.

Keywords: Catalase, Acetaldehyde, Acidified potassium permanganate, Ethanol, Hydrogen peroxide and Redox titration.

INTRODUCTION

CAT is a common enzyme found nearly in all living organisms exposed to oxygen. It is mainly localized in peroxisomes in all cells capable of generating H_2O_2 with most activity found in the liver and the erythrocytes (Harishekar, 2012). CAT catalyses the decomposition of H_2O_2 to water and molecular oxygen (Vines and Rees, 1972). H_2O_2 is a by-product of various oxidase and superoxide dismutase reactions. It is highly deleterious to the cell and if left to accumulate causes the oxidation of cellular targets like DNA, proteins and lipids resulting into mutagenesis and cell death. The removal of H_2O_2 from the cell by CAT gives protection to it against oxidative damage (www.sigma-aldrich.com). Lysis of H_2O_2 by CAT occurs in two steps in that the first molecule of H_2O_2 oxidizes the heme group to an oxyferryl species whilst the second reduces CAT-ethanol complex to regenerate the resting stage of the enzyme producing a molecule of oxygen and water (Oshino et al, 1973; Worthington Biochemical Corporation, 2012). CAT uses H_2O_2 as a hydrogen acceptor and donor in a 'catalatic' activity as illustrated in equations 1 and 2 below (Oshino et al, 1973).

$$\boldsymbol{CAT + H_2O_2 \rightarrow CAT - H_2O_2\ (Compound\ 1)} \qquad \boldsymbol{(1)}$$

$$CAT - H_2O_2 + H_2O_2 \rightarrow CAT + 2H_2O_2 + O_2 \qquad (2)$$

However, CAT-H_2O_2 is also capable of reacting with other compounds that are hydrogen donors such as phenols, formate and ethanol to form free CAT and water while ethanol is oxidized to acetaldehyde (CH_3CHO) in a 'peroxidative' activity in the peroxisomes (Oshino et al, 1973; EDVOTEK, 1998) as shown in equation 3 below:

$$CAT - H_2O_2 + CH_3CH_2OH \rightarrow CAT + 2H_2O + CH_3CHO \qquad (3)$$

Therefore, increasing the concentration of ethanol (a hydrogen-donor) decreases the concentration of the CAT-H_2O_2 complex (Kremer, 1975) producing a toxic metabolite (CH_3CHO) which accumulates (3) above that is later converted by alcohol dehydrogenase to acetate (CH_3COOH) that is released into blood and oxidised to carbon dioxide, fatty acids and water (Caballeria, 2003). Over 90% of the ethanol consumed by humans is first converted by CAT in the liver to CH_3CHO which is then metabolized steadily to prevent cell damage on interaction with lipids and proteins (Eriksson, 2001) thus preventing ethanol toxicity. However, consumption of large quantities of ethanol by alcoholics prevents CAT activity and destroys liver cells (liver cirrhosis) leading to death (Frezza et al, 1990). Alcohol abuse has negative effects like addiction, emotional problems, obesity, gastritis and ulcers, pancreatitis, cirrhosis, hypoglycemia and diabetes, hepatitis, gout, nerve and brain dysfunction, cancer, nutritional deficiencies, immune suppression, and injury and death from falls and auto accidents (www.globalhealingcenter.com).

WHO (2004) ranked Uganda as the leading consumer of alcohol in the 189 WHO member states with a per capita alcohol consumption of 19.47 litres. Kasirye and Kigozi (1997) reported that 5-10 % of the Ugandan population was abusing alcohol. Kakira Sub County (Jinja district) had more young adults than old abusing alcohol in 2007 of the 11.3% alcohol abusers (http://hdl.handle.net/10570/447). Thus in this study, we determined the effects of the concentrations and exposure duration of the CSAB sold in the open markets in Uganda on the activity of BLC.

MATERIAL AND METHODS

Sample and Reagents

Ten CSAB namely Pombey King (37%), London No.1 (37.5%), Coffee Liquor (37.5%), Coffee Spirit (39.2%), Sky Blue Vodka (40%), King Waragi (40%), Royal Vodka (40%), Empire Cane Spirits (40%), Hunters Gin (40%) and Golden Drop Vodka (43%) with 37% to 43% ethanol concentrations commonly sold in the open markets in Uganda were used. The percentages of these alcohol brands were all certified by the Uganda National Bureau of Standards (UNBS). The CSAB above with a 40% ethanol concentration were used to determine the effects of the duration of alcohol exposure on BLC activity because most of them on average contained about this concentration. Bovine liver (BL) was used because it is easily accessible and affordable. Samples of BL (each 0.5 kg) were randomly purchased from four different butchers, in Nagongera Town Council, Tororo district, Uganda packed in thermos flasks maintained at 37^{o}C and transported to the Biology Laboratory at Busitema University, Nagongera Campus for the study. BL samples were obtained in a similar way for three more consecutive days. Distilled water, H_2O_2, KMnO4, sulphuric acid (H_2SO_4) and sodium hydrogen phosphate buffer (pH 7.4) available at the Busitema University Biology Laboratory were used to determine the effects of ethanol concentration on BLC activity.

Estimation of the effects of ethanol percentage in the CSAB on BLC activity

Distilled water, Pombey King, London No.1, Coffee Liquor, Coffee Spirits, Sky Blue Vodka, King Waragi, Royal Vodka, Empire Cane Spirits, Hunters Gin and Golden Drop Vodka were pipetted (each 25.0 ml) into ten 100 ml beakers labelled 1 to 10 assembled in a rack followed by 2.0 ml of sodium hydrogen phosphate buffer (PH 7.4) and covered with aluminium foils. Four fresh BL samples purchased were cut into numerous 1.0 cm^3 cubes using a razor blade. Two of these cubes were randomly picked using a pair of forceps, immersed into each of the ten beakers above and incubated at 40^{0}C for 15 minutes to oxidize any H_2O_2 that may be present in them. Another set of ten 100 ml beakers each containing 2.0 ml of sodium hydrogen phosphate buffer (PH 7.4) and 50.0 ml of 0.6 M H_2O_2 were placed in a water bath maintained 40^{0}C. After the 15 minutes of incubation above, each set of the two BL cubes were transferred into separate beakers in the water bath at 40^{0}C and incubated for seven minutes (pre-determined duration in which some H_2O_2 will be left unbroken). Then 10.0 ml of 1.0M H_2SO_4 were added to each of the ten beakers in the water bath to stop the reaction. A solution of 25.0 ml was pipetted from each beaker, titrated against 0.2M $KMnO_4$ solution and the titre volume determined as the difference between the final and initial burette readings. The titration procedure was repeated twice to obtain consistent and reliable results. The above procedures were repeated for more three consecutive days using fresh BL samples Purchased during these days. The concentration of hydrogen peroxide remaining after the seven minutes incubation was used to determine the one broken down (oxidized) by BLC as shown below.

Estimation of the amount of H_2O_2 broken down by BLC

$KMnO_4$ solution reacts with H_2O_2 according to equation (4) below:

$$2MnO_{4\,(aq)}^{-} + 5H_2O_{2\,(aq)} + 6H_{(aq)}^{+} \rightarrow 2Mn_{(aq)}^{2+} + 5O_{2(g)} + 8H_2O_{(l)} \quad (4)$$

The average volume of $KMnO_4$ for each day was determined and the mean of the averages of the four days' titrations calculated.

Let *A* represent the mean volume of $KMnO_4$ solution from the four days' titrations. Thus: 1000 cm^3 of solution contained 0.2 mole of MnO_4^-, *A* cm^3 of solution contained $\frac{0.2A}{1000}$ mole of MnO_4^-. Therefore from equation (4), *2* moles of MnO_4^- react with *5* moles of H_2O_2, thus $\frac{0.2A}{1000}$ mole of MnO_4^- reacted with $\frac{5\times0.2A}{2\times1000}$ mole of H_2O_2, Therefore; 25.0 cm^3 of solution contained $\frac{5\times0.2A}{2\times1000}$ mole of H_2O_2 and 1000 cm^3 of solution contained $\frac{5\times0.2A\times1000}{2\times1000\times25.0}$ mole of H_2O_2 = *0.02A* mole of H_2O_2.

The **[***H_2O_2***]** remaining unbroken down is 0.02A mole per litre. The **[***H_2O_2***]** broken down was obtained by subtracting the **[***H_2O_2***]** remaining unbroken down from that of the stock (0.6M) as in equation (5) below:

$$[H_2O_2] = 0.6 - 0.020A \quad (5)$$

Estimation of the effects of CSAB duration of exposure on BLC activity

The five 40% ethanol CSAB were randomly chosen and 25.0 ml pipetted into ten 100 ml beakers (each alcohol brand in two) labelled 1 to 10 followed by 2.0 ml of sodium hydrogen phosphate buffer (PH 7.4) and covered with aluminium foils. Fresh BL was then cut into numerous 1.0 cm^3 cubes using a razor blade. Two BL cubes were randomly picked using a pair of forceps, transferred into each of the ten beakers and divided into five pairs, A, B, C, D and E and incubated in the 40% ethanol CSAB at 40 ^{0}C for 5.0, 10.0, 15.0, 20.0 and 25.0 minutes respectively. Ten beakers each containing 2.0 ml sodium hydrogen phosphate buffer (PH 7.4), 50.0 ml of 0.6M H_2O_2 were prepared and placed in a water bath maintained at 40 ^{0}C. After the incubation of BL cubes in the CSAB for 5.0-25.0 ml above; re-incubation for 7 minutes at 40 ^{0}C and determination of H_2O_2 oxidized was carried out as in the estimation of the effects of ethanol percentage in the CSAB on BLC activity above. Data was analyzed using *Ms Excel* and *MINITAB V. 14*. Regression analyses and one-way ANOVA were performed at *5%* significance level. Regression analyses were performed to determine the relationship between ethanol concentration in the CSAB and the amount of H_2O_2 broken down by BLC; and the relationship between the duration of BLC exposure to 40% ethanol CSAB and the amount of H_2O_2 broken down. One-way ANOVA was performed to determine the significance of the relationships and the difference between the CSAB.

RESULTS

Effects of ethanol percentage in the CSAB on BLC activity

The mean [H_2O_2] broken down was used as a measure of the activity of BLC in the presence of the CSAB. This concentration decreased with the increasing percentage of ethanol in the CSAB from 0.4560 ± 0.0000 mol L^{-1} at 0 % ethanol to 0.2303±0.0125 mol L^{-1} at 43% ethanol (Table 1 and Fig. 1). There was a strong negative correlation between ethanol percentage in the CSAB and the amount of H_2O_2 broken down by BLC (solid line; r^2 = 94.6%, p = 0.001; Fig. 1). There was also a significant difference between the control (0% ethanol) and the rest of the CSAB (p = 0.000, Table 1) thus increasing ethanol percentages decrease the activity of BLC. One-way ANOVA also showed a significant effect of ethanol in the CSAB on BLC activity (F = 62.16, p = 0.000, Table 2). By calculation from the regression equation (6) below, was postulated that BLC will be halted from decomposing H_2O_2 at 95.45% ethanol concentration.

Table 1. Mean [H_2O_2] broken down by BLC in the CSAB and the p– values as compared to the control.

CASB [% Ethanol]	*Mean ± s.e.m of [H_2O_2] broken down (mol L^{-1})*	*P – value*
Distilled water (0.0)	0.4560 ± 0.0000	*0.000*
Pombey King (37.0)	0.3135 ± 0.0100	*0.000*
London No.1, Coffee Liquor (37.5)	0.2893 ± 0.0095	*0.000*
Coffee Spirit (39.2)	0.2663 ± 0.0121	*0.000*
Sky Blue Vodka, King Waragi, Royal Vodka, Empire Cane Spirits, Hunters Gin (40.0)	0.2575 ± 0.0116	*0.000*
Golden Drop Vodka (43.0)	0.2303 ±0.0125	*0.000*

Stock [H_2O_2 solution] = 0.6000M and Volume of pipette used = 25.0 ml.

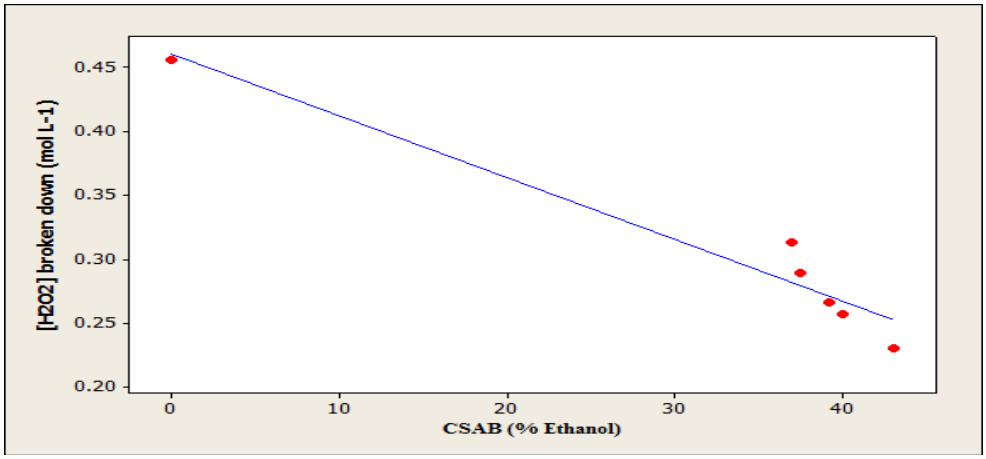

Figure 1. Relationship between [H_2O_2]broken down by BLC and concentration of ethanol in CSAB.

The regression equation is:

$$[H_2O_2]\ (\text{mol}\ L^{-1}) = 0.461 - 0.00483\ CSAB\ (Ethanol\ \%) \quad (6)$$

Table 2. One-way ANOVA to determine the effect of ethanol percentage in the CSAB on [H_2O_2] broken down by BLC.

Source	DF	SS	MS	F	P
ALC	5	0.129668	0.025934	62.16	0.000
Error	18	0.007510	0.000417		
Total	23	0.137179			

Effect of BLC duration of exposure to 40% ethanol CSAB on its activity

The mean [H_2O_2] broken down by BLC decreased with increasing duration of exposure to 40% ethanol CSAB ranging from 0.4560 ± 0.0000 mol L^{-1} at 0 minutes to 0.1475 ± 0.0032 mol L^{-1} at 25 minutes (Table 3 and Fig. 2). There was a strong negative correlation between ethanol exposure duration and the amount of H_2O_2 broken down by BLC (solid line; r^2 = 69.9%, p = 0.038; Fig. 2). There was also a significant difference between the control (at 0 minutes) and the rest of the durations (p = 0.000, Table 3) thus the longer BLC is exposed to 40% ethanol CSAB, the less its activity will be. One-way ANOVA results showed a significant effect of the duration of exposure of BLC to 40% ethanol CSAB on its activity (F = 335.75, p = 0.000, Table 4). By calculation from equation (7), it's postulated that BLC activity will cease after 34.85 minutes if continuously exposed to 40% ethanol CSAB.

Table 3. Mean [H_2O_2] broken down by BLC in 40% ethanol CSAB over time and the p – values as compared to the control.

BLC exposure duration to 40% ethanol CSAB (minutes)	*Mean ± s.e.m of [H_2O_2] broken down (mol L^{-1})*	*P – value*
0	0.4560 ± 0.0000	0.000
5	0.2358 ± 0.0038	0.000
10	0.2048 ± 0.0113	0.000
15	0.1808 ± 0.0062	0.000
20	0.1583 ± 0.0066	0.000
25	0.1475 ± 0.0032	0.000

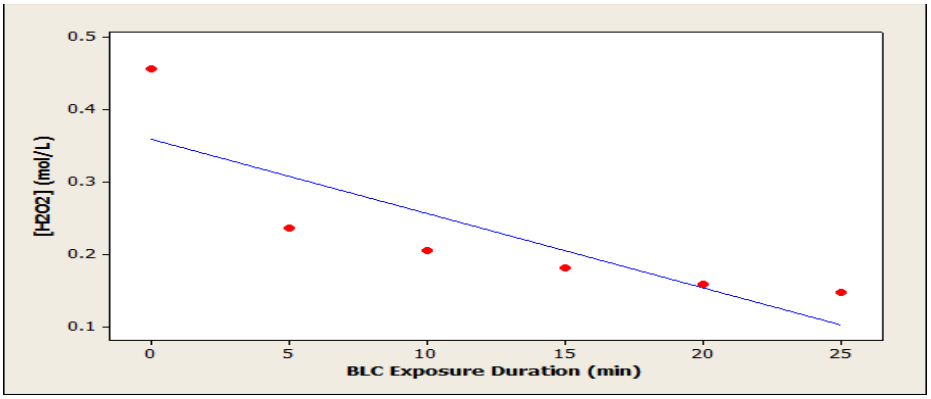

Figure 2. Relationship between [H_2O_2] broken down by BLC and exposure duration to 40% ethanol CBAB.

The regression equation is:

$$[H_2O_2]\ (\text{mol}\ L^{-1}) = 0.359 - 0.0103\ BLC\ Exposure\ Duration\ (min) \quad (7)$$

Table 4. One-way ANOVA to determine the effect of 40% ethanol CSAB on the [H_2O_2] broken down by BLC over time.

Source	DF	SS	MS	F	P
TIME	5	0.264500	0.052900	335.75	0.000
Error	18	0.002836	0.000158		
Total	23	0.267336			

DISCUSSION

The rate of BLC activity was higher in both controls (Fig. 1 and Fig. 2) than in their respective tests. This is because BLC was provided with suitable conditions of temperature (40^0C) and PH of 7.4 for its decomposition of H_2O_2 to water and oxygen. There were no inhibitors such as cyanide, azide, hydroxylamine, aminotriazole and mercaptoethanol known to inhibit CAT activity (Worthington Biochemical Corporation, 2012), ethanol inhibited the rate of the CAT reaction as also reported by Harishekar (2012) that ethanol has a strong negative effect on rat liver CAT activity. BLC activity then decreased rapidly with increasing percentage of ethanol in the CSAB that it was exposed. This may be because ethanol promotes the generation of reactive oxygen species (ROS) that reacted with BLC thus denaturing it. Ethanol metabolism in the liver could have also resulted into the formation of molecules whose further metabolism in the liver cells led to the production of ROS. It could have also stimulated the activity of enzymes known as cytochrome P450s which contributed to further ROS production. Ethanol also reduces the levels of agents that eliminate ROS (antioxidants) and this could have resulted into oxidative stress (Cederbaum, 2001; Bondy, 1992; Nordmann et al, 1992). ROS - induced oxidation could have led to changes in the three- dimensional structure as well as fragmentation, aggregation and cross-linking of BLC. This effect therefore increased with increasing ethanol concentration hence the decreasing activity of BLC with the increasing ethanol percentage in the CSAB. It was also predicted that ethanol concentration of *95.45%* could stop the activity of BLC within 15 minutes. Also 40% ethanol CSAB was predicted to stop BLC activity after *34.85* minutes of exposure to it. This could be attributed to the expected very high production of ROS that could act as already explained above.

CONCLUSION

In conclusion, therefore, this study revealed that high ethanol concentrations (>40%) inactivate BLC as well as 40% if incubated in it for longer than 35 minutes. We thus postulate that the CSAB of 37-43% ethanol sold on the open markets in Uganda should not be consumed in large quantities continuously for more than 35 minutes as this may be lethal. We further recommend *in vivo* studies to ascertain these *in vitro* findings.

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