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# INGESTION OF ALCOHOL, CALABASH CHALK (NZU), CIGARETTE AND OPIOID ANALGESICS: EFFECT ON OXIDATIVE STRESS MARKERS

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**ABSTRACT:** The intake of psychoactive substances is prevalent among youths today. To further heighten their experience, they experiment with the mixture of these addictive substances. This study was done to determine the effect of a mixture of alcohol, calabash chalk, tobacco, codeine and tramadol on the oxidative stress markers. The subjects were 80 young men of an average age of 25 years who were grouped into four of twenty persons each. Two groups comprised those who were addicts of the said mixture for over one year and two groups were those who were non-addicts. The following oxidative stress markers were assayed: Malondialdehyde (MDA) for lipid peroxidation, reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione-S-transferase (GST), Superoxide dismutase (SOD) and catalase (CAT). The result of the assay revealed an increase in product of lipid peroxidation being MDA in both batches of addicts subjects compared to the non-addicts subjects. A statistically significant decrease at $P<0.05$ was observed in GSH, CAT and SOD levels of the batch A addicts subjects when compared to the non-addict subjects, same trend was observed for GSH, GST and CAT in batch B. The levels of GPx and GST in batch A addict subjects, as well as GPx and SOD in batch B addict subjects were seen to decrease, however, not statistically significant. This study therefore suggests that the synergistic intake of alcohol, calabash chalk, cigarette, codeine and tramadol increases oxidative stress which predisposes humans to disease conditions.

**Keywords:** Alcohol, calabash chalk, cigarette, codeine. Tramadol, oxidative stress.

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## 1.INTRODUCTION

Substance abuse is on the increase among young people in Nigeria and they usually experiment by mixing several substances to get a better 'high' at a cheaper cost [1]. The mixture of alcohol, calabash chalk, tobacco, codeine and tramadol is a practice by some youths in Nigeria. However some of these substances have been found to create oxidative stress in the body. Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (ROS, free radicals) and antioxidant defenses [2]. Free radicals are independent molecular species that contain an unpaired electron, are unstable and highly reactive, and can either donate or accept an electron from other molecules. Examples such as superoxide anion radical, hydroxyl radical, hydrogen peroxide oxygen singlet, hypochlorite, nitric oxide radical and peroxynitrite radical are capable of causing disease [3]. These free radicals are basically nasty little chemicals that go around stealing electrons off other molecules in the body that causes damage in the body. Antioxidants are compounds which dispose, scavenge and suppress the formation of Reactive Oxygen Species (ROS) or oppose their actions [4]. They act by donating an electron to free radical without making themselves unstable, prevent the oxidation of the cells of the body that may lead to diseases by getting itself oxidized in place of the cell [5] Alcohols have been shown to promote the formation of several free radical intermediates as well as cause increased lipid peroxidation in the liver [6][7]. Studies have also shown that rats fed ethanol with diets rich in unsaturated fatty acids showed increase in the hepatic content of lipid peroxidation as well as a decrease in antioxidant levels such as Copper-zinc superoxide dismutase, Glutathione peroxidase and catalase which was associated with liver injury [8] The consumption of calabash chalk (a geophagic material) popularly known as nzu (Igbo language) is practiced in Nigeria for pleasure and by pregnant women as a cure for nausea [9]. Aluminium silicate hydroxide is the major component of calabash chalk. Multi-elemental analysis of calabash chalk has actually shown that it contains 22 elements including lead at a mean concentration of about 40mg/kg [10]. The intake of calabash chalk has been associated with negative effects such as nervous and brain damages in humans and animals [11]. However there is paucity of information on its contribution to oxidative stress. Studies have shown that cigarette smoke is a significant generator of reactive oxygen species (ROS) [12]. Cigarette smoke has been reported to be a major exogenous source of free radicals and the resulting oxidative stress is a major cause of certain diseases [13]. According to Irish cancer society in 2019, cigarette consists of the following chemical substances: tar, nicotine, carbon monoxide, toluene, ammonia, nitrosamines, poly nuclear

aromatic hydrocarbons (PAHs), chlorinated dioxins and furans. However, a study done on rats has shown that low-dose nicotine reduces oxidative stress by increasing levels of glutathione peroxidase and superoxide dismutase in animal model of Parkinson's disease [14]. Chronic administration of codeine in rabbit has been seen to cause a marked rise in oxidative stress markers with a decline in testicular enzymatic antioxidants and oxidative DNA damage amongst other mechanisms leading to testicular atrophy, alterations in testicular histomorphology and drop in testicular testosterone levels [15]. Tramadol has been seen to induce oxidative stress when administered repeatedly to mice. This causes nitric oxide overproduction and increase in malondialdehyde in brain. There is also a decrease in brain intracellular reduced glutathione level and glutathione peroxidase activity induced by the repeated exposure to tramadol [16]. Based on the findings above, this study assessed the impact of the synergistic intake of alcohol, calabash chalk, tobacco, codeine and tramadol on the oxidative stress markers on adult male humans as studies on the combined use of these addictive drugs has not been carried out in the past.

## 2. MATERIALS AND METHODS

The research design was descriptive and analytical. Ethical approval for the study was obtained from the Rivers State ministry of health, Rivers State health research ethics committee and Rivers State hospital management board, Port Harcourt. All test and control human subjects for the study duly gave their consent by filling the questionnaire administered to them.

The study involved 80 male humans of 18-25 years resident in Obio/Akpor local government area of Rivers State, Nigeria. They were divided into two batches:

Batch A: 20 Addicts and 20 Non-Addicts

Batch B: 20 Addicts and 20 Non-Addicts

The addicts included 40 male humans of average age 25 years that were addicts, who regularly ingested a mixture of Alcohol, Calabash chalk, tobacco, codeine and tramadol for over one year. This group was taken as the test group.

The non-Addicts included 40 male humans of average age 25 that are non addicts, who did not engage and had never been engaged or exposed to these substances. This group was taken as the control group.

Addicts and non-addicts who had health issues and comorbidities such as asthma and Human Immuno-Deficiency Virus were excluded.

### Analysis of oxidative stress markers

Five milliliters of blood was collected from the cubital fossa of each respondent and placed in a plain bottle, screened and analyzed in the laboratory for estimation of the oxidative stress markers: Reduced glutathione (GSH), Glutathione-S-transferase (GST), Glutathione peroxidase (GPx), Catalase (CAT), Superoxide dismutase (SOD), and Malonyldialdehyde (MDA) using standard "Randox" antioxidant reagent kit (Randox Laboratories Ltd. London, 140 London Wall, EC2Y

**Statistical Analysis**

All data generated were analyzed using one way Analysis of Variance (ANOVA) with the aid of Statistical Package for Social Sciences (SPSS) version 20 running on Windows PC. Data for each parameter were expressed as mean value ± standard deviation. While the significant differences between the test means and control means were determined at $p<0.05$ confidence level.

## 3. RESULTS AND DISCUSSION

The result obtained from the study revealed a statistically significant increase in lipid peroxidation in the addict subjects of both Batch A and B when compared to the non-addict. A general reduction in the antioxidant markers of the addict subject was observed in all batches when compared to the non-addict subjects.

**Table 1: Effect of a synergistic intake of alcohol, calabash chalk, tobacco, codeine and tramadol on the oxidative stress markers of Batch A subjects.**

| Biomarkers(nmol/g) | Addicts(n=20) | Non-addicts(n=20) |
|---|---|---|
| **MDA** | $0.74^{b}$ ±0.07 | $0.29^{a}$ ±0.09 |
| **GSH** | $0.46^{a}$±0.05 | $1.01^{b}$±0.26 |
| **GPx** | $0.06^{a}$ ±0.06 | $0.10^{a}$ ±0.05 |
| **GST** | $0.09^{a}$ ±0.02 | $0.12^{a}$ ±0.05 |
| **CAT** | $20.08^{a}$ ±1.76 | $23.04^{ab}$ ±3.24 |
| **SOD** | $0.18^{a}$ ±0.08 | $0.26^{b}$ ±0.10 |

- Values are expressed as mean ± standard deviation.
- Values with different superscripts show significant difference at the 0.05 level

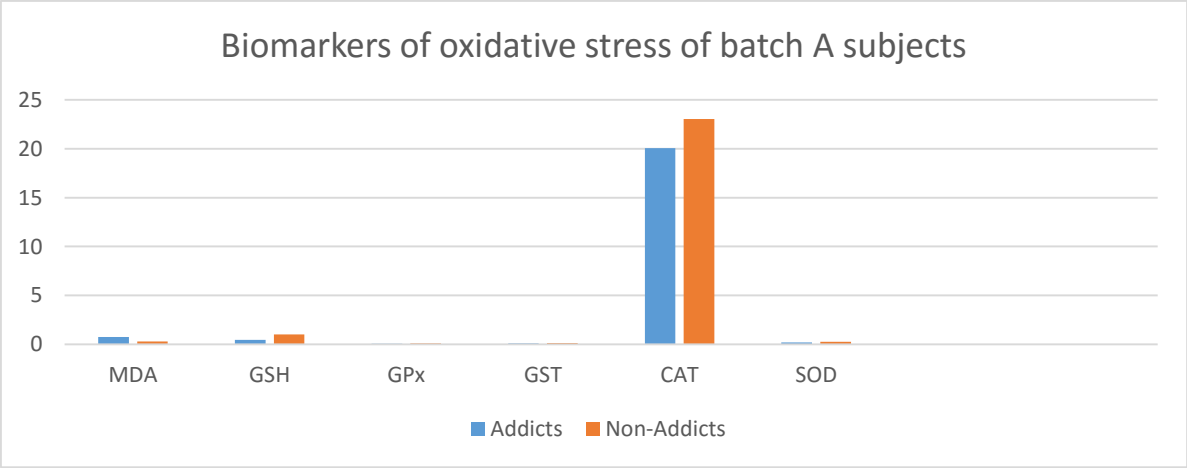


**Fig. 1: Bar chart showing the levels of oxidative stress biomarkers among Addicts and non-Addicts in Batch A subjects**

**Table 2: Effect of synergistic intake of alcohol, calabash chalk, tobacco, codeine and tramadol on the oxidative stress markers of Batch B subjects.**

| Biomarkers(nmol/g) | Addicts(n=20) | Non-addicts(n=20) |
|---|---|---|
| **MDA** | $0.70^{b}$ ±0.19 | $0.39^{a}$ ±0.18 |
| **GSH** | $0.71^{ab}$±0.17 | $1.03^{b}$±0.37 |
| **GPx** | $0.10^{a}$ ±0.10 | $0.11^{a}$ ±0.03 |
| **GST** | $0.10^{b}$ ±0.05 | $0.14^{a}$ ±0.04 |
| **CAT** | $22.64^{ab}$ ±3.25 | $25.76^{b}$ ±0.05 |
| **SOD** | $0.16^{a}$ ±0.06 | $0.21^{a}$ ±0.18 |

- Values are expressed as mean ± standard deviation.
- Values with different superscripts show significant difference at the 0.05 level

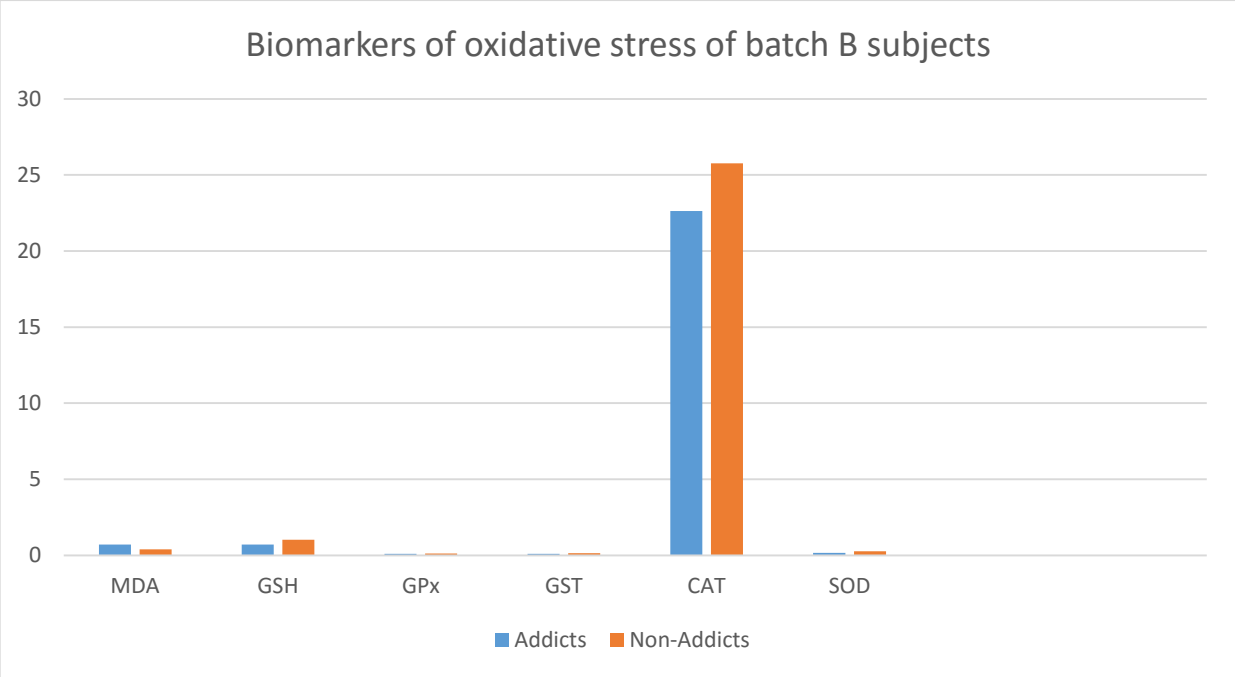


**Fig. 2: Bar chart showing the levels of oxidative biomarkers among Addicts and non-Addicts in Batch B subjects.**

## DISCUSSION

The results reflected the antioxidant status of male humans who ingested a mixture of alcohol, calabash chalk, cigarette, tramadol and codeine for over one year. There was an increase in the product of lipid peroxidation as seen in the increase in the MDA level in both batches (table 1 and 2) of addicts compared to the control which was statistically significant. This could be as a result of the intake of alcohol and tramadol as seen in previous studies by Polvarapu and Ahmed [8] [16]

A statistical significant decrease at $p<0.05$was observed in the CAT, SOD and GSH levels in the

addict groups of batch A when compared with the non-addict group while GPx and GST were not significantly decreased as seen in table 1. The fall in antioxidant levels may be attributed to the intake of alcohol and tramadol [7] [16]. The decrease in antioxidants supports the report on a reduction in the antioxidant status following administration of codeine by Akhigbe [15] However, this decrease disagrees with the findings of Alin that showed increase in antioxidants with intake of low-dose nicotine. [14] Table 2 revealed significant decrease in GSH, GST and CAT levels while GPx and SOD were also decreased but not significant in the addict subjects in comparison with the non-addict subjects. The reduction in antioxidants biomarkers as seen in both batches of addicts in comparison to the controls potentiates the occurrence of oxidative stress predisposing the subjects to chronic degenerative illnesses such as cancer, autoimmune disorders, ageing, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases [17] This study was however limited by not being specific on the concentration of each substance, and which component of the mixtures was producing the effects as seen in the result. Further research should explore the effect of calabash chalk (nzu) on the antioxidant status of humans.

## 4. CONCLUSION

The ingestion of psychoactive substances is prevalent among young people and the mixture of alcohol, calabash chalk, cigarette, tramadol and codeine is also an occurrence among them in Nigeria. The result of this intake results in the increase in lipid peroxidation and a fall in antioxidants status, leading to an increase in oxidative stress which can predispose to diseases in the long term. It is therefore advisable to desist from such harmful habits.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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## CONFLICT OF INTEREST

The authors have no conflict of interest

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