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Antioxidant Effect of Co-Treatment of *Solanum aethiopicum* and *Ocimum gratissimum* in Potassium Bromate-Induced Toxicity

Okon Victoria Edem[1] & Enene Esu Ukpai[2]
[1]University of Cross River State, Calabar, Nigeria
[2]Federal University Wukari, Taraba State, Nigeria
okonvictoria40@gmail.com

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Abstract

Potassium bromate induces oxidative cellular damage, creating a need to identify plant-derived interventions capable of strengthening endogenous antioxidant defenses. This study investigated the effects of combined *Solanum aethiopicum* and *Ocimum gratissimum* treatment on superoxide dismutase (SOD) and catalase (CAT) activities in potassium bromate-induced toxicity in Wistar rats. Thirty Wistar rats weighing 140–150 g were allocated into five groups of six animals each. Group 1 served as the normal control and received standard rat pellets and 0.2 mL of normal saline, whereas Group 2 received potassium bromate at 50 mg/kg. Groups 3 and 4 received potassium bromate followed by low-dose (150 mg/kg) and high-dose (300 mg/kg) co-treatment with *S. aethiopicum* and *O. gratissimum*, respectively, while Group 5 received potassium bromate followed by vitamin C at 100 mg/kg. Potassium bromate was administered to Groups 3–5 for two weeks before the respective treatments, which were administered daily for 14 days using an oropharyngeal cannula. SOD and CAT activities were assessed as oxidative stress biomarkers, and the resulting data were analyzed using analysis of variance. CAT activity

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increased significantly in the low-dose co-treatment group ($p < .05$), whereas the high-dose group showed a marginal but nonsignificant decrease relative to the control group ($p > .05$). The potassium bromate-only and potassium bromate plus vitamin C groups exhibited significantly reduced CAT activity compared with the control group ($p < .05$). Similarly, SOD activity showed a marginal significant increase in the low-dose co-treatment group but decreased significantly in the high-dose group relative to the control group ($p < .05$). Significant reductions in SOD activity were also observed in the potassium bromate-only and potassium bromate plus vitamin C groups ($p < .05$). These findings indicate that low-dose co-treatment with *S. aethiopicum* and *O. gratissimum* may enhance endogenous antioxidant enzyme activities and mitigate potassium bromate-induced cellular toxicity. However, the inconsistent response observed at the higher dose suggests a dose-dependent effect and highlights the importance of optimizing therapeutic dosage when evaluating these plants as potential antioxidant interventions.

Keywords: Antioxidant Enzymes; Catalase; Oxidative Stress; Potassium Bromate; Superoxide Dismutase

INTRODUCTION

Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, constitutes a fundamental mechanism in the pathogenesis of chemical-induced toxicity[1,2]. The antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) form a crucial primary defense line, with SOD catalyzing the dismutation of superoxide radicals and CAT decomposing hydrogen peroxide into water and oxygen [3,4]. The food-additive and environmental oxidant potassium bromate ($KBrO_3$, a compound classified as a possible human carcinogen, pose significant health risks by inducing a state of oxidative stress that overwhelms these endogenous protective systems [2, 5, 6]. Scientific evidence confirms that potassium bromate exposure precipitates a marked depletion in the activities of SOD and CAT, concurrently elevating markers of oxidative damage like malondialdehyde (MDA) across various biological models, including murine liver and blood cells [2]and plant meristematic cells [7,8] showed that administration of $KBrO_3$ to rats significantly suppressed SOD and CAT activity in brain tissue while increasing malondialdehyde levels, signifying oxidative insult[8]. In parallel, a study in mice reported that $KBrO_3$ exposure led to marked depletion of both

SOD and CAT in liver and serum, corroborating the enzyme-inhibitory effect of $KBrO_3$ induced ROS generation[2]. Given the vulnerability of SOD and CAT to toxic-mediated inactivation, strategies that restore or enhance these enzymes are of significant interest for mitigating $KBrO_3$-induced toxicity. In this context, medicinal plants like *Ocimum gratissimum* and *Solanum aethiopicum*, which are repositories of diverse bioactive compounds, have garnered significant scientific interest for their antioxidative potential. *Ocimum gratissimum* L., a widely used culinary and medicinal herb, is rich in phenolic compounds and flavonoids such as rosmarinic acid, luteolin, apigenin, and gallic acid, which contribute to its documented antioxidant, anti-inflammatory, and hepatoprotective properties [9]. Critically, research has demonstrated that extracts of *Ocimum gratissimum* can effectively reverse toxin-induced oxidative stress by significantly increasing the levels of glutathione (GSH), SOD, and CAT in organs like the spleen and thymus [10]. Similarly, *Solanum aethiopicum* L., the scarlet eggplant, possesses a rich phytochemical profile comprising hydroxycinnamic acids like chlorogenic acid, flavanones, and flavanols, which are responsible for its potent antioxidant activity [11]. Studies on its peel extract have shown it can enhance the expression of endogenous antioxidants, including CAT, in human liver cells, presumably through the activation of the Nrf2 pathway, a master regulator of cellular antioxidant responses [12]. While ascorbic acid (Vitamin C) is a well-established exogenous antioxidant [13],[14]), the multifaceted and synergistic action of the complex phytochemical mixtures found in these plants may offer a superior protective advantage against multi-organ toxicity. Therefore, this study is designed to investigate and compare the combined efficacy of *Solanum aethiopicum* and *Ocimum gratissimum* extracts with that of Vitamin C in mitigating potassium bromate-induced toxicity, with a specific focus on the restoration of the pivotal antioxidant enzymes, superoxide dismutase and catalase.

MATERIALS AND METHODS

Materials Used

ITEMS	MANUFACTURER	SOURCE
Iron cages	Local	Okuku
Water bottle	Local	Okuku
Hand gloves	Neogloves	Okuku
Wistar rats	Local	Physiology department
Animal feed	Vital feed Nig. LTD	Okuku

ITEMS	MANUFACTURER	SOURCE
Plates	Local	Okuku
Digital weighing balance	China	Physiology department
Glucometer	Local	Physiology department
Sample bottles	Local	Physiology department
Centrifuge	USA.	Physiology department
Chloroform	USA	Physiology department
Dissecting set	Local	Ogoja
Saw dust	Local	Okuku
Paper tape	Local	Okuku
Ocimum gratissimum	Local	Okuku
Solanum aethiopicum	Local	Okuku
Disinfectant	Local	Physiology department
Desiccator	USA	Physiology department
Syringe (2ml)	HMA medical Ltd	Okuku
Cotton wool	Local	Okuku
Book, pen and paper	Local	Okuku
Distilled water	Eva water	Okuku
Tissue paper	Local	Okuku
Measuring cylinder	Local	Physiology department
Oropharyngeal cannulas	Local	Physiology department
Lancets	Local	Physiology department
Laboratory coat	Local	Okuku
Methanol	Local	Physiology department
Electronic scale	USA	Physiology department
Potassium bromate	Local	Okuku

Experimental Animals

Thirty (30) Wistar rats of body weight ranging from 140g-150g were used for this research study. The rats were purchased from the animal unit of Physiology Department, Faculty of Basic Medical Sciences, University of Cross River State (UNICROSS) Okuku Campus. The animals were acclimatized for a period of one day, and their body weight were noted before administration. The animal were randomly distributed and housed in cages at temperature (28℃). The animals were fed with standard rat diet and good tap water.

Experimental Design

A total of thirty (30) Wistar rats were used for the experiment. The animals were grouped into 5 groups according to their body weight and each group had one cage (6 rats per cage). The Wistar rats were well fed and given clean water.

The following was the grouping;

Gp1: Control group - control group were given 0.2ml. of normal saline.

Gp2: KBr- Administered with potassium bromate 50mg/kg body weight of the animals

Gp3: KBr + LD Occ + GEL- *Solanum aethiopicum* and *Ocimum gratissimum* extract low dose treated with 150mg/kg body weight of the animals

GP4: KBr+ HD Occ+ GEL - *Solanum aethiopicum* and *Ocimum gratissimum* extract high dose group treated with 300mg/kg body weight of the animals

Gp5: KBr +Vit.C- potassium bromate and vitamin C 100mg/kg body weight of the animals

Group 3, 4 and 5 where pretreated with KBr for 2 weeks after which extract was administered for 2 weeks.

Preparation of *Ocimum gratissimum* and *Solanum aethiopicum*

Fresh leaves of *Ocimum gratissimum and Solanum aethiopicum* were collected from Okuku Market within the vicinity of University of Cross River State, Okuku Campus, located in Yala Local Government Area of Cross River State, Nigeria. The leaves were washed and sprayed under shade within a temperature range of 25-28^{o}C and blended to tiny particles using electric blender. The blended extracts was mixed with ethanol to bring out the actual content. Since the plants is not totally soluble in water, therefore tween 80 was used to dissolve it totally using a sterile plastic container. It was filtered using cheese cloth (sieve) into a plane sample bottle and stored in a refrigerator till use.

Ethical consent

Ethical consent of the research was approved by the Research and Ethics Committee, Faculty of Basic Medical Sciences, Okuku Campus, Cross River State, Nigeria. The approved number was UNICROSS/FBMSEC/2025-HP013

Administration of *Ocimum gratissimum* and *Solanum aethiopicum* leaf extract

1g of the leaves (extract) was dissolved in 40ml of distilled water, and was administered at a dose of 150 mg/kg body weight (use of oral cannula) and 150 mg/kg body weight of extract was administered once a day for 14 days.

Acute toxicity study (LD50) of *Ocimum gratissimum* and *Solanum aethiopicum*

The method of Okon[15] was adopted for acute toxicity testing. Albino Wistar rats were used for the study. Five groups of six (6) rats each weighing (140-150kg) body weight. The animals were observed for physical signs of toxicity and death for 24 hours, after which the number of dead rats were counted in each group and percentage mortality calculated.

Sacrifice of animal

At the end of 28 days of administration of extract, the animals were sacrificed and blood was obtained through cardiac puncture then taken to the laboratory for biochemical analysis

Statistical Analysis

The result was expressed as mean ± standard error of mean (SEM). The results were compared using one-way ANOVA test as appropriate. Probability level of ($p<0.05$) was considered to be statistically significant. Statistical analysis was done with aid of computer software (SPSS 18.0).

Conflict of interest

All authors hereby declare no conflict of interest.

RESULTS

Result on potential role of *Solanum aethiopicum* and *Ocimum gratissimum* on superoxide dismutase (sod) and catalase (cat) in potassium bromate-induced cell toxicity in Wistar rats

Table 1 shows that the catalase (CAT) and superoxide dismutase (SOD) levels varied significantly across the treatment groups. The control group recorded CAT and

SOD values of 20.67 ± 0.37 U/L and 33.00 ± 0.32 U/L, respectively. The potassium bromate (KBr)-treated group showed marked decreases in CAT (16.67 ± 0.18 U/L) and SOD (28.33 ± 0.66 U/L) levels. Administration of the combined *Solanum aethiopicum* and *Ocimum gratissimum* extracts at low dose (KBr + LD Occ + GEL) significantly increased CAT (25.67 ± 0.18 U/L) and SOD (33.67 ± 1.02 U/L) compared to the KBr group. The high-dose extract group (KBr + HD Occ + GEL) also produced elevated CAT (20.33 ± 0.48 U/L) and SOD (31.33 ± 0.37 U/L) levels relative to the KBr group. Conversely, the vitamin C-treated group (KBr + Vit C) exhibited the lowest CAT (14.33 ± 0.18 U/L) and SOD (24.33 ± 0.18 U/L) activities among all groups.

Table 1: Effect of the combined Solanum aethiopicum and occimum gratissimum on superoxide dismutase (SOD) and catalase (CAT) in potassium bromate-induced cell toxicity

Groups	Treatment	CAT (u/l)	SOD (u/l)
1	Control	20.67 ± 0.37^{c}	33.00 ± 0.32^{cd}
2	KBr	16.67 ± 0.18^{b}	28.33 ± 0.66^{b}
3	KBr + LD Occ + GEL	25.67 ± 0.18^{d}	33.67 ± 1.02^{d}
4	KBr+ HD Occ+ GEL	20.33 ± 0.48^{c}	31.33 ± 0.37^{c}
5	KBr +Vit C	14.33 ± 0.18^{a}	24.33 ± 0.18^{a}

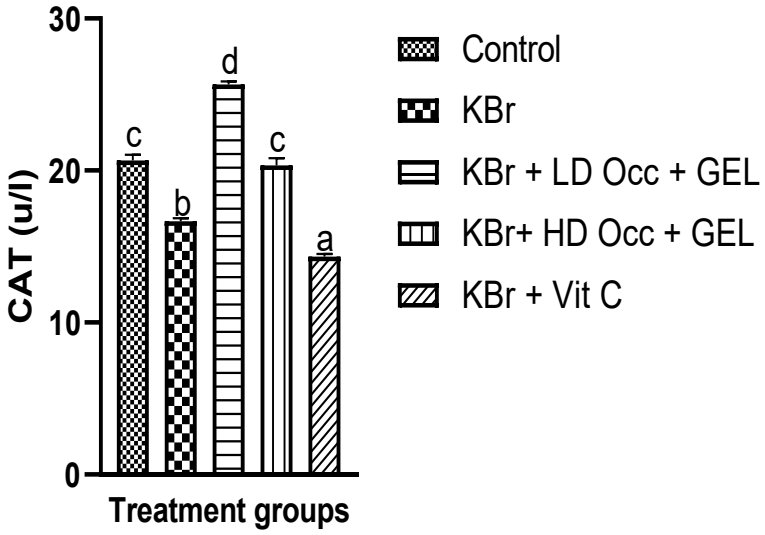


Figure 1: Treatment groups for change in CAT (u/l)

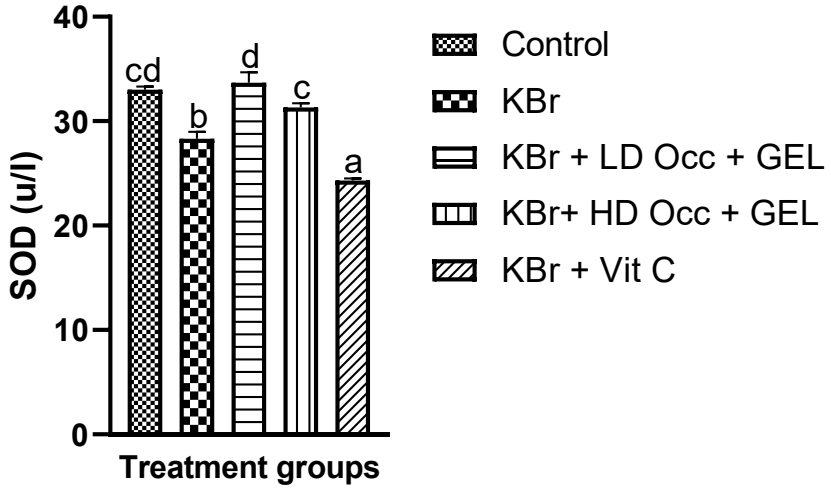


Figure 2: Treatment groups for change in SOD (u/l)

DISCUSSION

The administration of potassium bromate ($KBrO_3$) significantly disrupts the cellular redox balance, primarily through the generation of reactive oxygen species (ROS) [5], [16]). The observed alterations in the activities of key antioxidant enzymes (Superoxide Dismutase (SOD) and Catalase (CAT) in the study offer crucial insights into the oxidative stress induced by $KBrO_3$ and the protective efficacy of the combined extract of *Solanum aethiopicum* and *Ocimum gratissimum*. SOD serves as the first line of defense against oxidative stress by catalyzing the dismutation of the superoxide anion (O_2^-) into hydrogen peroxide (H_2O_2) and oxygen [10]. The significant decrease in SOD activity in the $KBrO_3$-treated group (Group 2) compared to the control is a classical biomarker of oxidative assault. This finding is consistent with previous literature, where $KBrO_3$ exposure was demonstrated to deplete antioxidant enzymes, leading to the accumulation of superoxide radicals and subsequent cellular damage [5]. The restoration of SOD activity in groups co-treated with the combined plant extract (Groups 3 and 4) highlights its potent antioxidative property. This ameliorative effect can be attributed to the rich phytochemical profile of the individual plants. Specifically, *Ocimum gratissimum* has been documented to significantly increase the specific activity of SOD in rat models, enhancing the cellular machinery to neutralize superoxide radicals ([10]). Similarly, *Solanum aethiopicum* is known to upregulate the expression of endogenous antioxidants, presumably through the activation of the Nrf2 pathway, a

master regulator of antioxidant response [11, 12]. The combination of these plants likely provides a synergistic boost to the cellular defense system, effectively countering the $KBrO_3$-induced suppression of SOD. The higher activity in the combined extract group, compared to the Vitamin C group, suggests that the phytocomplex in the herbal formulation may offer a more robust or sustained stimulation of the antioxidant system than a single antioxidant molecule.

Catalase is a crucial enzyme that detoxifies hydrogen peroxide (H_2O_2), produced by SOD, into water and molecular oxygen, thus preventing the formation of the highly reactive hydroxyl radical via the Fenton reaction [17]. The suppression of CAT activity in the $KBrO_3$-treated group (Group 2) indicates an overwhelming oxidative burden that incapacitates this protective enzyme, a phenomenon consistently reported in studies on bromate toxicity. The reversal of this effect in the groups receiving the combined *S. aethiopicum* and *O. gratissimum* extract highlights its capacity to protect and reactivate this essential enzyme. Research on *Ocimum gratissimum* has firmly established its proficiency in elevating CAT activity. For instance, in rats exposed to lead acetate, co-treatment with *O. gratissimum* extract significantly increased CAT activity, mitigating oxidative damage in tissues. Furthermore, the essential oil of *O. gratissimum* has been shown to enhance CAT activity in other biological models [18]. Concurrently, *Solanum aethiopicum* peel extract has demonstrated the ability to improve oxidative stress by modulating the Nrf2 pathway, which governs the expression of CAT, thereby strengthening the cellular antioxidant shield[11]. The combined action of these plants appears to not only scavenge ROS directly but also to fortify the endogenous enzymatic defenses, leading to the observed normalization of CAT levels.

The finding that vitamin C treatment resulted in the poorest recovery of both SOD and CAT is notable, though in other $KBrO_3$ models vitamin C has been shown to partially restore antioxidant enzyme activities [19]. The discrepancy may stem from dosage, timing, or bioavailability differences. The relatively better performance of the low-dose extract over the high dose suggests a possible hormetic or biphasic response: beyond a threshold, excessive phytochemical load may inhibit enzyme induction or overload redox systems. The strong restoration by the combined extract supports the hypothesis of synergism, multiple phytoconstituents working across different mechanisms (direct scavenging,

enzyme gene modulation, metal chelation) to rehabilitate both SOD and CAT simultaneously more effectively than a single antioxidant.

CONCLUSION

The findings of this study demonstrate that the combined administration of *Solanum aethiopicum* and *Ocimum gratissimum* extracts effectively ameliorated potassium bromate–induced oxidative stress by enhancing superoxide dismutase (SOD) and catalase (CAT) activities in Wistar rats. The low-dose combination exhibited superior antioxidant efficacy compared to both the high-dose and vitamin C treatments, suggesting a synergistic interaction between the phytochemicals of the two plants. This indicates that co-administration of *S. aethiopicum* and *O. gratissimum* may serve as a potent natural therapeutic approach for mitigating oxidative damage caused by toxic agents such as potassium bromate. Further studies are recommended to isolate and characterize the specific bioactive compounds responsible for this synergistic antioxidant effect.

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