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Research Article

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Comparative studies on the antioxidant and scavenging activities of *Garcinia kola* extract and vitamin E: Modulatory effects on $KBrO_3$ – induced oxidative stress in rats

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ABSTRACT

The comparative antioxidant and scavenging properties of Garcinia kola seeds extract (GKSE) and Vitamin E (VE) were evaluated in a series of in vitro assays involving free radicals and reactive oxygen species. GKSE and VE exhibited marked scavenging effects (26-68%) on 1, 1 diphenyl 2-picryl hydrazyl (DPPH) radical ion in a concentration dependent manner. But VE showed a better DPPH radical scavenging activity of 68% when compared with the 40% scavenging effect of GKSE. Furthermore, GKSE inhibited the superoxide ($O_2^{\cdot}$) radical more obviously than VE. At 500µg/ml, GKSE and VE scavenged $O_2^{\cdot}$ by 69% and 62% respectively. The scavenging of hydrogen peroxide (H_2O_2) by GKSE was comparable with VE especially at higher concentrations (300- 500 µg/ml). GKSE and VE were also effective at modulating the Malondialdehyde (MDA) formation observed in the kidneys of potassium-bromate ($KBrO_3$) treated rats. A significant increase ($p<0.05$) in MDA levels in kidneys treated with $KBrO_3$ was observed when compared with the control group. GKSE and VE elicited a 82% and 87% decrease in $KBrO_3$-induced increase in MDA formation in the kidney, respectively. The results of the present investigation suggested that GKSE and VE are potent chemopreventive agents and may suppress $KBrO_3$ mediated renal oxidative stress in rats.

Key words: Free radicals, Oxidative stress, Antioxidant, Potassium bromate, *Garcinia Kola*, Vitamin E

INTRODUCTION

Reactive oxygen species (ROS) including oxygen-centered radicals and some non-radical derivatives of oxygen cause oxidative stress to cells. Oxidative stress can be defined as an imbalance between pro-oxidant/free radical production and opposing antioxidant defenses. It has been reported that acute and chronic oxidative stress are implicated in degenerative diseases, such as atherosclerosis, diabetes mellitus, ischemia/reperfusion injury, Alzheimer's disease, inflammatory diseases, carcinogenesis, neurodegenerative diseases, hypertension, ocular diseases, pulmonary diseases and hematological diseases [1, 2]. Potassium bromate ($KBrO_3$) plays an important role as a food addictive in bread making, production of fish paste and in fermented beverages. Moreover, it is an

important potential contaminant in hypochlorite, and it is also a by-product of ozone used as disinfectant in drinking water, because ozonation of drinking water containing bromide may lead to the formation of bromate. In addition, $KBrO_3$ is used in cold-wave hair lotion [3, 4]. In Nigeria, and in many parts of the world, use of potassium bromate as bread improver has been banned; however, some bread makers and bakeries in Nigeria have continued to include potassium bromate in their bread [5]. $KBrO_3$ has been reported to be a potent nephrotoxic agent that can mediate renal oxidative stress, toxicity and tumor response in rats. It also enhances renal lipid peroxidation and hydrogen peroxide formation with reduction in renal antioxidant enzymes [6]. Also, potassium bromate contributes to the cellular redox status and impairment of membrane protein activities in rats [7]. In a recent study, Zhang et al. [8] suggested that $KBrO_3$ induced oxidative stress and genotoxicity in human hepatoma cell line, HepG2 cells, through the mechanisms of lysosomal damage.

The process of lipid peroxidation is initiated by the attack of a free radical on unsaturated lipids, and the resulting chain reaction is terminated by the production of lipid breakdown products such as lipid alcohols, aldehydes or malondialdehyde. There is a cascade of peroxidative reactions which ultimately leads to the destruction of the lipid, and possibly the structure in which it is located [9].

Although about 4000 antioxidants have been identified, the best known are Vitamin E, Vitamin C and the carotenoids. Vitamin E is a generic name used for all eight naturally occurring tocopherols and tocotrienols as well as their derivatives. Of all these, α-tocopherol has been reported as the most biologically active form of Vitamin E. Over the last decades, evidences abound to show that Vitamin E is a chain-breaking anti-oxidant that inhibits lipid peroxidation, as well as a scavenger of free radicals [10,11].The potential of antioxidants from natural substances such as plants, spices and herbs that are consumed as foods or ingredients have been widely investigated for several biochemical and pharmacological properties [12,13].

Garcinia kola Heckel (Guttiferae) seeds, otherwise known as “bitter kola” are known to contain high levels of biflavonoid compounds [14]. Traditional African medicinal uses include: treatment of cough; purgative, anti-parasitic, anti-microbial and anti-inflammatory effects [15,16,17]. GKSE has been reported to modulate the hepatotoxicity of carbon tetrachloride, galactosamine, amanita toxin, paracetamol, thioacetamide and 2-acetyl amino fluorine in various experimental models (18). Similarly, the extract from the seeds has demonstrated the ability for free radical scavenging, anti-lipoperoxidative and ovulation-inhibitory effects [19, 20, 21]. Recently, there had been reports of the protective effects of kolaviron, a biflavonoid *of Garcinia kola*, on reproductive toxicity induced by cadmium and gamma-irradiation, in rats [22, 23].

The present study compares the antioxidative and free radical scavenging effect of GKSE with that of Vitamin E with a view to evaluating their ability to modulate $KBrO_3$-mediated oxidative stress in kidneys of rats.

EXPERIMENTAL SECTION

Chemicals

Butylated hydroxyanisole (BHA), hydrogen peroxide, nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), 1,1, Diphenyl-2-Picryl Hydrazyl (DPPH), Trichloroacetic acid were obtained from Sigma (St. Louis, MO, USA). Other chemicals were of analytical grade and were obtained from BDH (Poole, Dorset, UK).

Extraction of GKSE

The plant material, *Garcinia Kola* seeds were bought from the local market in Ibadan, Nigeria and certified at the herbarium in the Department of Botany, University of Ibadan, Nigeria. Five kilograms of the seeds were cut into pieces and air-dried, after removing their outer coats. The dried seeds were ground to fine powder and extraction done using light petroleum ether (bp 40°-60° C) in a Soxhlet extractor for 24 hours. The defatted, dried marc was repacked and then extracted with methanol. The concentrated extract was diluted to twice its volume with water and extracted with ethyl acetate (6×100ml).

Animal treatment

Male Wistar albino rats weighing 150-200g were used for the experiment. They were acclimatized to housing conditions for one week prior to the commencement of the experiment. Animals were maintained on a 12hr light 12 hr dark cycle and provided with water and food (rat pellets) *ad libitum*. Six experimental groups of five animals each were used. The first group served as controls, while rats in groups two and three received 100mg/kg of body weight of GKSE and Vitamin E respectively. Rats in group 4 were pre-treated with $KBrO_3$ at a dose of 125mg/kg of body

weight/day. The fifth group was treated simultaneously with GKSE (100mg/kg of body weight/day) and $KBrO_3$, while the sixth group was treated with a combination of Vitamin E (100mg/kg of body weight/day) and $KBrO_3$.

Rats were starved overnight and sacrificed by cervical dislocation after 7 days of administration. Kidneys were removed, rinsed in 1.15% KCl and homogenized in aqueous potassium phosphate buffer (0.1M, pH 7.4) and homogenates were centrifuged at 10,000g for 20 minutes to obtain the supernatant fraction. Microsomes were obtained after subsequent centrifugation at 100,000 g for 90 min. The microsomal pellets were re-suspended in 0.25M sucrose solution. Aliquots of this suspension were stored at -80ºC and thawed before use. All procedures were carried out at temperatures between 0 and 4ºC.

Scavenging of 1, 1, Diphenyl-2-Picryl Hydrazyl (DPPH) radical

The quenching activity of *Garcinia Kola* extract against DPPH radical was measured spectrophotometrically. Concentrations of extract or Vitamin E (100-500µg/ml) were added to a methanolic solution (0.5ml) of DPPH radical (final concentration of DPPH was 2.0×10^{-4}M). The mixture was shaken vigorously and left to stand for 30 minutes and the absorbance was measured at 517nm.

Scavenging of superoxide radical

The influence of extracts /Vitamin E on the generation of superoxide radical was achieved by means of spectrophotometric measurement of the product formed on the reduction of nitroblue tetrazolium. Superoxide radical was generated in a non-enzymic (phenazine methosulfate-NADH) system as reported by Robak and Gryglewski [24]. Concentrations (100-500µg/ml) of the extract or Vitamin E in methanol were added to test tubes containing phenazine methosulfate (20µM), NADH (156µM) and Nitroblue tetrazolium (50µM) in phosphate buffer (0.1M, pH7.4). After five minutes of incubation at ambient temperature, the absorbance was determined spectrophotometrically against reagent blanks.

Scavenging of hydrogen peroxide

Hydrogen peroxide was measured by using peroxide based assay systems. A solution (0.04M) of hydrogen peroxide was prepared in phosphate buffer (pH7.4). 100-500µg/ml of extract/Vitamin E in methanol were added to hydrogen peroxide solution (0.6M). Absorbance was determined at 230nm after 10 minutes against a reagent blank solution.

Lipid Hydroperoxide formation

The effect of extract/Vitamin E on the formation of hydroperoxide was determined according to the method of Yen and Hsieh [25]. Briefly, (100-500µg/ml) of extract /Vitamin E in methanol were mixed with 2.5ml of a linoleic acid emulsion(0.02M, pH7.0) in test tubes. The tubes were incubated at 37 ^{0}C. The concentrations of linoleic acid hydroperoxide in each sample was determined before and after incubation by reading the absorbance at 500nm using thiocyanate reagent against distilled water as blank.

Assesment of lipid peroxidation

Lipid peroxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) produced according to the method of Walls et al [26]. Briefly, 1 mg/ml final concentration of microsomal fraction in isotonic phosphate buffer, pH 7.4 was incubated for 6 hrs at 37° C in a shaking water bath with or without 1mM $FeSO_4$, 0.2mM H_2O_2 and 1mM ascorbate. 0.5mL of 0.75% thiobarbituric acid in 0.1M HCl was added to 0.5ml of the incubation mixture already quenched with 0.5ml of 10% Trichloroacetic acid. The mixture was heated at 90°-95° C for 20 minutes and after cooling, centrifuged for 10min at 780g. The supernatant was transferred into acid resistant tubes and centrifuged at 32,000g for 10 minutes. Absorbance was determined at 532nm.

Protein Determination

Protein content was estimated by the method of Lowry et al [27], using Bovine Serum Albumin as standard.

Statistical Analysis

Results were expressed as the mean ± Standard Deviation. Statistical analysis was performed using Student's t-test. P values lower than 0.05 were considered significant.

RESULTS AND DISCUSSION

Comparative free radical scavenging and antioxidant activities of GKSE and VE

The implication of oxidative stress in the etiology of other human diseases has stimulated the interest of many investigators to search for natural and synthetic antioxidant compounds for the treatment of these diseases. Chemopreventers have been found in all classes of food including vegetables, fruits, red wine, tea, apples and onions [28]. In the present study, we used a combination of in vitro and in vivo assays to evaluate the comparative scavenging and antioxidant effects of Garcinia Kola seeds extract and that of Vitamin E.

Figure 1 shows the scavenging effect of GKSE and VE on hydrogen peroxide. GSKE and VE were capable of scavenging hydrogen peroxide in a concentration dependent fashion after 10 minutes of incubation. The scavenging of H_2O_2 by GSKE was comparable with VE especially at higher concentrations (300- 500 µg/ml). In this assay, GKSE and VE were more effective than BHA. The scavenging activity of GKSE and VE on superoxide anion radicals generated non-enzymatically using phenazine methosulfate-NADH system is shown in **Figure 2**. At lower concentrations (100-200 µg/ml), VE exhibited better scavenging effect than GKSE. However, at higher concentrations, the scavenging effect of between 55- 68% was obtained for GKSE, VE and BHA.

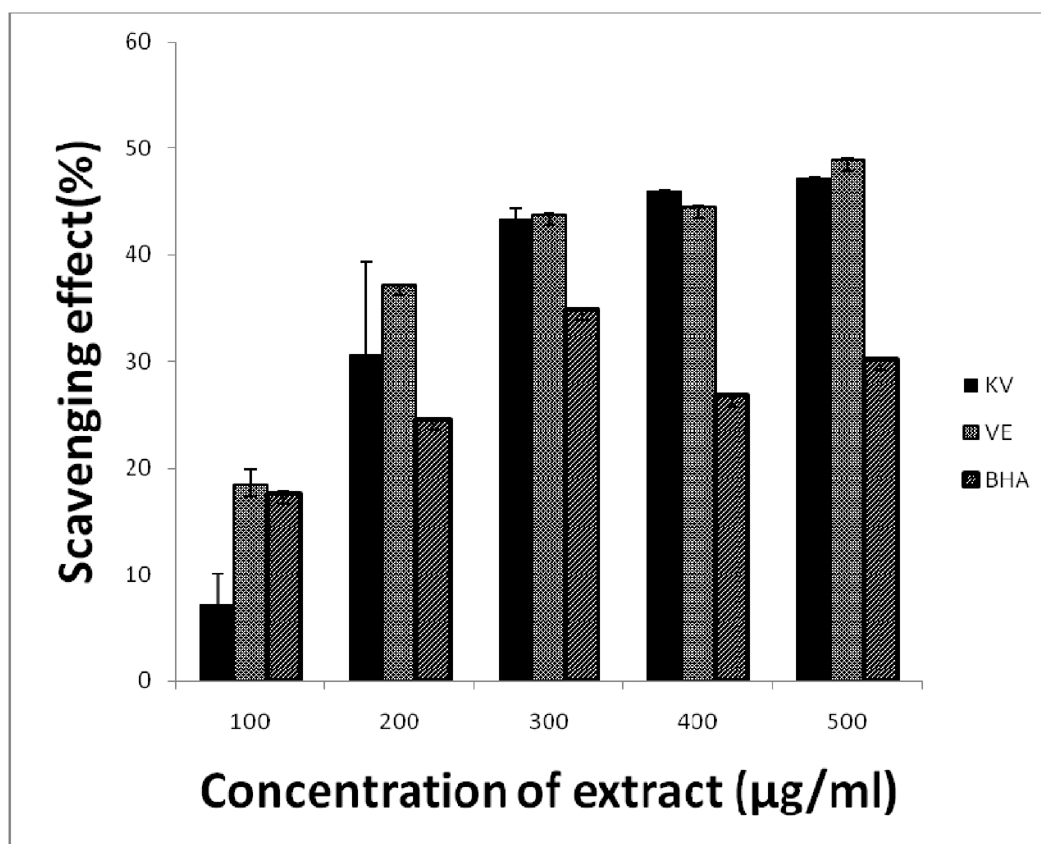

Figure 1: The comparative scavenging effect of *Garcinia Kola* Seed extract (KV) and Vitamin E (VE) on hydrogen peroxide *in vitro*. Values are means± S.D of five determinants.

The scavenging effect on GKSE and VE is depicted in **Figure 3**. At a concentration of 500 µg/ml, VE exhibited a better DPPH radical scavenging activity (68%) than GKSE, which exhibited about 48% scavenging activity. BHA, which served as a control also showed marked scavenging activity.

Peroxide formation is a means of propagating free radical reactions and GKSE and VE exhibited > 50% inhibition of peroxide formation. This shows that their antioxidative activity may also be due to the reduction of hydroperoxide formation, although moderately. This is consistent with the report that phenolic antioxidants and VE protect cultured

human endothelial cells against linoleic acid hydroperoxide-induced cytotoxicity resulting from hydro-peroxide induced lipid peoxidation[28].

At different concentrations, GKSE and VE elicited antioxidant activities by inhibiting the oxidation of linoleic acid. Both GKSE and VE (500 µg/ml) showed > 50% antioxidant activity, but their comparative activity were not significantly different at p>0.05(Figure 4). GKSE and VE at the tested doses exhibited marked radical scavenging ability on DPPH which is reduced in the presence of the antioxidants to give rise to uncoloured methanol solutions. The decrease in the concentration of DPPH radical due to scavenging ability of GKSE and VE is comparable to the reported value of that of hydro alcoholic extract of plant and vitamin C [29]. This suggests that GKSE and VE possibly act as primary antioxidants of free radicals.

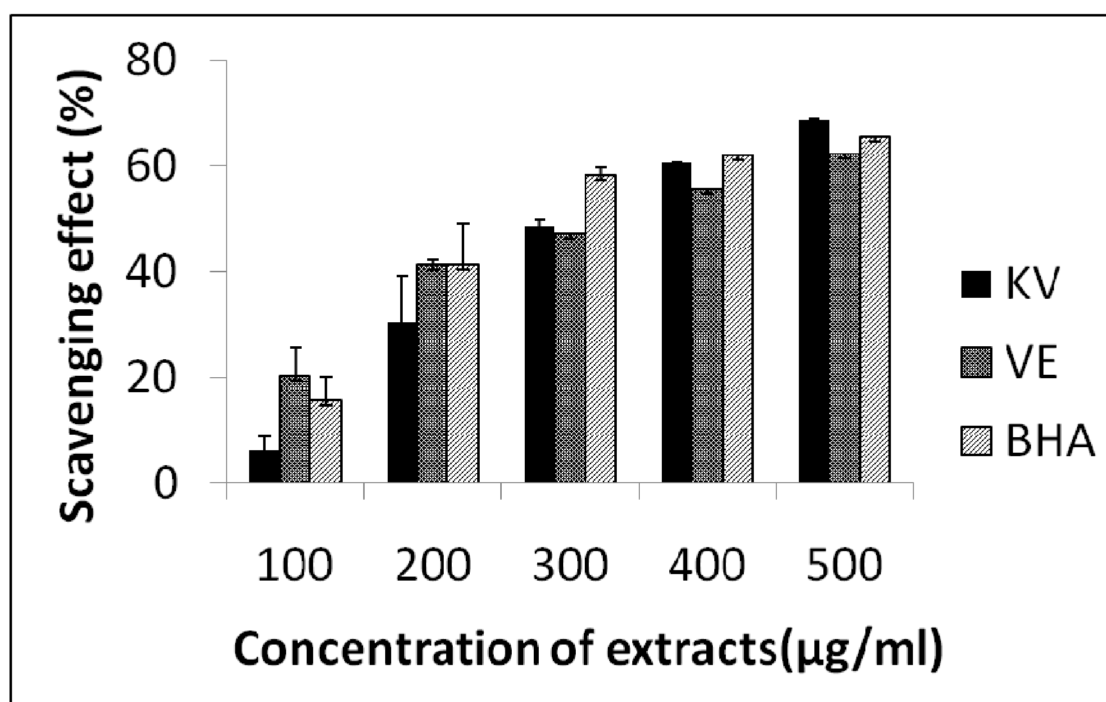

Figure 2: The comparative scavenging effect of *Garcinia Kola* Seed extract (KV) and VE on superoxide anions radical *in vitro.* Values are means± S.D of five determinants.

Our data suggest that GKSE and VE scavenged both hydrogen peroxide and superoxide anion radicals effectively. This observation is in consonance with the findings of Farombi et al [7], who reported marked scavenging effect of the *Garcinia Kola* extract on hydrogen peroxide and superoxide anion radicals *in vitro*. The scavenging effect of GKSE was more than 45% at concentrations of 400 and 500 µg/ml for both reactive species.

Inhibitory effect of GKSE and VE on rat kidney microsomal lipid peroxidation

It has been reported that hydrogen peroxide has only a weak activity to induce lipid peroxidation, but its activity as an active oxygen species comes from its potential to produce the highly reactive hydroxyl radical through the Fenton reaction [1].Therefore the scavenging effect of GKSE is attributable to its inhibition of lipid peroxidation *in vitro* at the initiation stage. In the present work, the scavenging effect of GKSE was comparable with VE.

Our study also demonstrated the *in vivo* protective effect of Garcinia kola extract and VE on rat kidney microsomal lipid peroxidation induced by potassium bromate. **Table 1** shows the inhibitory effect of GKSE and VE on potassium- bromate induced peroxidation of rat kidney microsomes. There was a significant (P<0.05) increase in lipid peroxidation product in potassium bromate- treated rat kidney when compared with the control. VE reduced $KBrO_3$ induced MDA formation in the kidney by 87% while GKSE gave an 82% reduction.

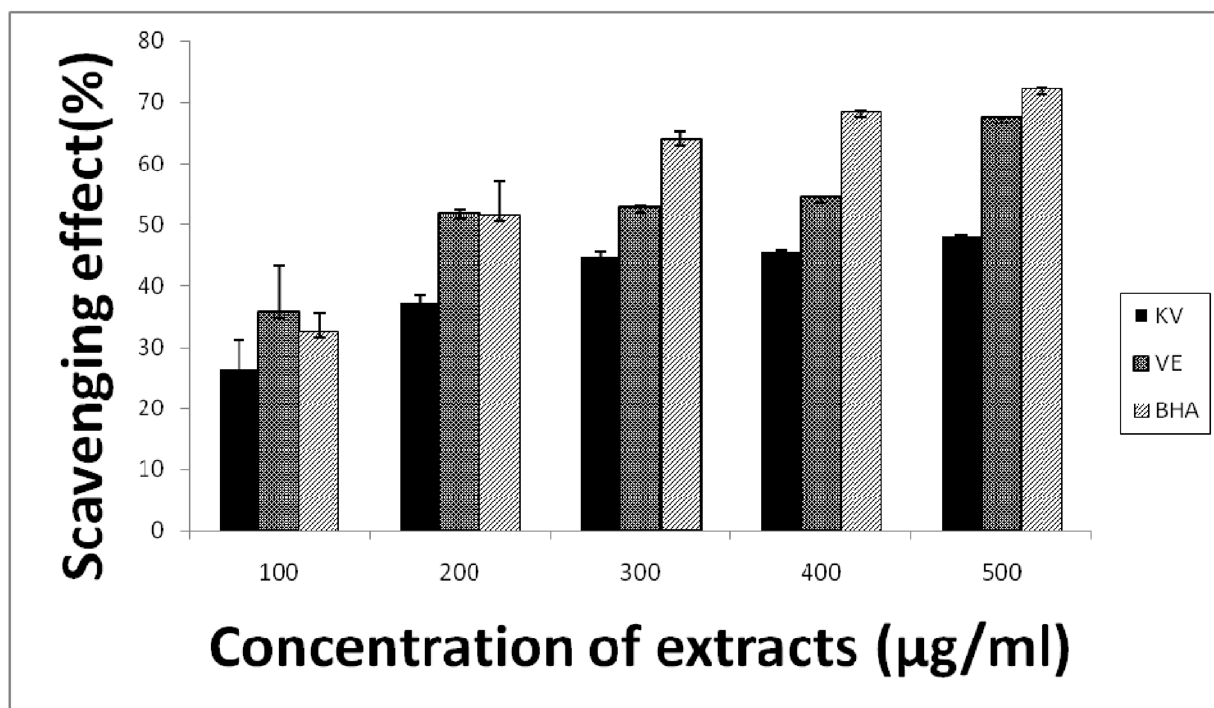

Figure 3: The comparative scavenging effect of *Garcinia Kola* Seed extract (KV) and VE on DPPH radical in vitro. Values are means± S.D of five determinants.

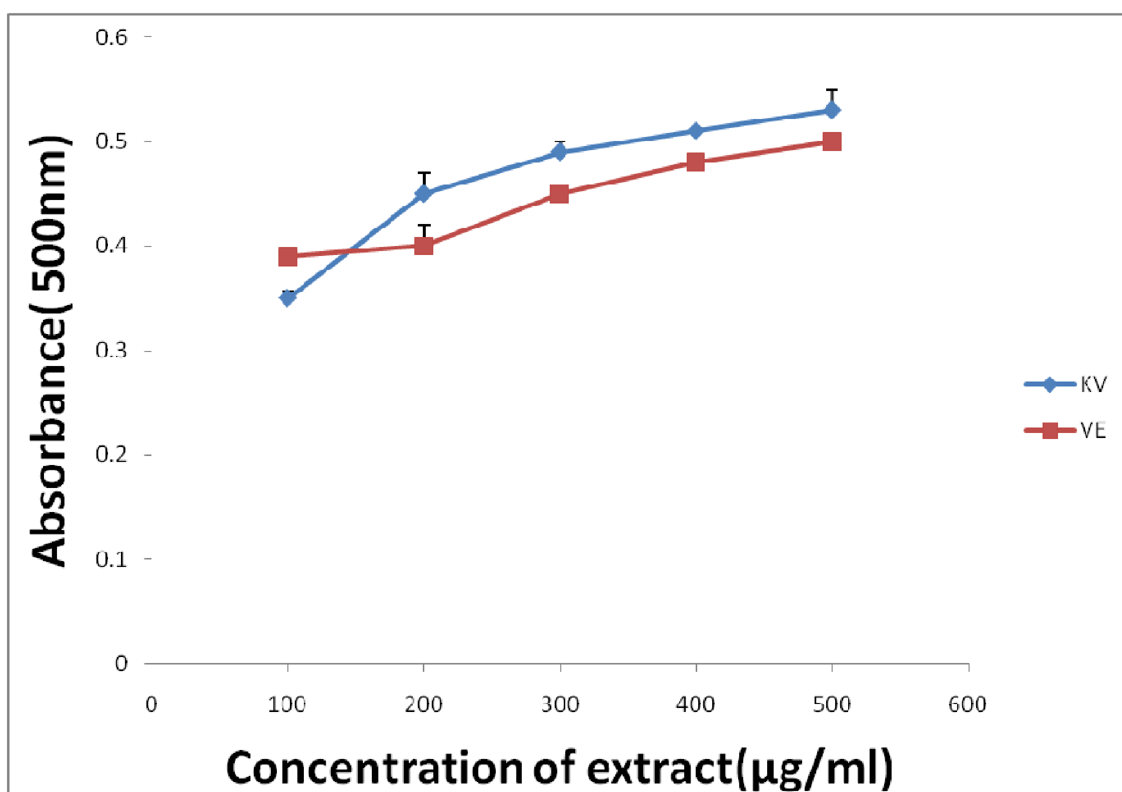

Figure 4: The comparative scavenging effect of Garcinia Kola Seed extract (KV) and VE on lipid hydroperoxide formation. Values are means± S.D of five determinants. Comparative KV and VE activities were not significantly different at p>0.05.

Various authors had earlier posited that potassium bromate induced the formation of free radicals which react with some cellular components such as membrane lipids and produce lipid peroxidation products [30, 31]. MDA is one of the major oxidation products of peroxidized polyunsaturated fatty acids, and increased MDA content is an important indicator of lipid peroxidation [32]. The observed significantly high levels of kidney MDA concentrations in rats treated with $KBrO_3$ may be a consequence of higher levels of superoxide radicals, which are produced in significant amounts in response to potassium bromate exposure. In the kidney of the $KBrO_3$-treated rats, MDA levels were slightly elevated by 19% increase when compared with the control. This is consistent with the reports by Shivarajashankara et al [33] that rats treated with fluoride afforded a 27% increase in the red blood cells when compared with the control group. GKSE and VE significantly decreased the $KBrO_3$-mediated lipid peroxidation in rats. This had also been reported by Scientists who suggested that the anti-lipoperoxidative effect of GKSE might be linked to their free radical scavenging ability. Also, an earlier study had reported that treatment of mice with kolaviron, a biflavonoid extract of *Garcina kola,* at 100 and 200 mg/kg and vitamin E at 100 mg /kg significantly decreased the CCl_4-mediated increase in renal lipid peroxidation [34].

Table 1: Effect of GKSE (100mg/kg) and VE (100mg/kg) on kidney lipid peroxidation following potassium bromate pre-treatment (125mg/kg body wt)

Treatment	MDA(µmol/mg protein)
Control	**38.1±0.15**
GKSE	**2.2±.002**
VE	**4.0±.005**
$KBrO_3$	**45.3±0.37***
GKSE +$KBrO_3$	**8.10±0.04***
VE +$KBrO_3$	**4.70±0.02***

*Values are means ± S.D. of five determinants. *$P<0.05$ significantly different from control.*

Similarly, several researches have been conducted highlighting the antioxidative effects of α-tocopherol, Vitamin C and ß-carotene. They have been reported to reduce oxidative stress and the cellular dysfunction that results from oxidative damage to cellular constituents. In particular α-tocopherol is generally accepted to act as a potent antioxidant in the protection of biological membranes against free radical damage [35].The ability of VE to reduce MDA formation induced by potassium bromate is in consonance with its antioxidative properties.

CONCLUSION

A number of conclusions are evident from this work. The first is that GKSE and Vitamin E exert significant protection against $KBrO_3$-induced toxicity by their ability to ameliorate the lipid peroxidation through their radical scavenging abilities. This study also showed that GKSE was as effective anti-lipoperoxidative agent as Vitamin E. This suggests that the protective effect of this extract on tissue damage may be similar to that of Vitamin E in mechanistic terms and could act effectively in preventing oxidative damage to lipids.

Also in view of the recent quest for natural antioxidants to replace the synthetic ones, *Garcinia Kola* extract, as a potential natural antioxidant, may find relevance in chemoprevention of free radical-mediated diseases.

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