Innovations in Pharmaceuticals and Pharmacotherapy

www.innpharmacotherapy.com

IPP

eISSN: 2321–323X

Original Article

Prevention of Fe^{2+} induced lipid peroxidation by aqueous extract of Garcina Kola leaf in some rat tissues

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Abstract

Cell injury in aerobic organism subjected to oxidative stress has been caused by lipid peroxidation. The ability of aqueous extract of Garcina kola leaf (3.3-33.3µg/ml) to prevent 60µM Fe^{2+} induced lipid peroxidation in rat liver and brain were assessed respectively using TBARS (Thiobarbituric acid reactive species. Fe^{2+}- chelating ability of the extract was also determined. The result of the study revealed that incubating the liver and brain in the presence of iron exhibited high percentage inhibition against thiobarbituric acid reactive species (TBARS) induced by iron (ii) sulphate (60µM) with IC_{50} value of 72.58±29.16µg/ml and 89.36µg/ml respectively, while the extract shows strong iron chelating ability of 79.93% at concentration (2.3µg/ml) with an EC value of 62.0µg/ml. The inhibitory effect of aqueous extract of Garcinia kola shown in TBARS and Iron chelation assays were concentration dependent. The results however suggest that Garcinia kola is beneficial in the treatment of various cellular damages due to its ability to reduce lipid peroxidation.

Keywords: Lipid peroxidation Garcina kola Thiobarbituric acid reactive species.

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1. Introduction

Plants have evolved the ability to synthesize chemical compound that help them defend against attack from a wide variety of predators such as fungi, insect and herbivorous mammals. By chance, some of these compounds whilst being toxic to plant predators turn out to have beneficial effects when used to treat human diseases [1]. All plants produce chemical compounds as part of their normal metabolic activities. The use of herbs to treat disease is almost universal among non-industrialized society. Herbs may be harmful if taken for the wrong conditions, used in excess amounts, combined with prescription drugs or alcohol [2]. *Garcinia kola*, an angiospermae, belonging to the family Guttiferae, is known in commerce as bitter kola. On Bitter cola is a highly valued ingredient in African ethno medicine because of its varied and numerous uses which are social and medicinal; thus making the plant an essential ingredient in folk medicine.Medicinal

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plants such as *Garcinia kola* are believed to be an important source of new chemical substances with potential therapeutic benefits [3].

Garcinia kola is regarded as a wonder plant because every part of the plant (bark, leaves, root and wood) have been found to be of medicinal importance. The medicinal importance of bitter kola is based mainly on the phytochemical components of the plant. From its roots to its leaves, the plant is known to contain several phytochemicals noted for their medicinal importance [4]. *Garcinia kola* seed is believed to contain a wide spectrum of organic compounds such as flavonoids which confer on it some antimicrobial and antifungal actions against gram negative and gram positive micro organisms.

Oxidative stress represents an imbalance between the production and manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA [5].

Antioxidants are widely used as ingredients in dietary supplements and have been investigated for prevention of diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplement may be harmful [6]. The objective of this study is to test for the antioxidant potential of cold water extract of Garcinia kola leaf using Iron (ll) Sulphate as prooxidant.

2. Materials and Methods

Reagents

Thiobarbituric acid (TBA), 1, 10-Phenenthroline, (w/v) Sodium Dodecyl Sulphate (SDS), Tris HCl, Iron II Sulphate ($FeSO_4$). Other reagents used include Ascorbic acid, Sodium Chloride (Saline).

Preparation of plant extract

The leaves of *Garcinia kola* were collected in June, 2011 from Egun Farm settlement in Ifaki Ekiti, Nigeria and authenticated in the department of plant science by a botanist (Mr. F.O. Omotayo) at the Ekiti State University, Ado-Ekiti, Nigeria. The leaves were sliced into small pieces and dried with air at room temperature for about four (4) weeks. Voucher specimens were deposited in the herbarium of the department of plant science, Ekiti state university state university, Ado-Ekiti. The dried samples were powdered with a blender and one (1) gram of the powdered sample was dissolved in 100 milliliters of distilled water. The mixture was allowed to stand for 24 hours and filtered using a filter paper, the filtrate contains 1 gram per 100 milliliters of the plant extract.

Test animals

All animals' procedures conform strictly to the NIH Guide for the care and use of laboratory animals. Two (2) to three (3) months old wistar-albino rats weighing between 200g to 250g were used for the *invitro* studies.

Production of Thiobarbituric reactive species

Production of Thiobarbituric acid reactive species (TBARS) was determined using a modified method of [7] as described by [8]. The rats were sacrificed by cervical dislocation method. The liver and brain were removed and placed on ice. 1 gram of the tissues were homogenized in cold 0.1M tris buffer at pH 7.4 (1:10 w/v) with about ten (10) up and down strokes in a Teflon-glass homogenizer. The homogenates were centrifuged at 12,000 revolutions per minute for 5 minutes to yield a supernatant which was used for the assay and pellet that was discarded.

The supernatant (100µl) with or without 50µl of the freshly prepared prooxidant (Iron ii Sulphate), different concentration of the plant extract and an approximate volume of distilled water which gives a total volume of 300µl were incubated at 37^{o}c for 1 hour. The colour reaction was carried out by adding 200µl, 500µl and 500µl of each of 8.1% Sodium Dodecyl Sulphate (SDS), 1.33M Acetic Acid (pH 3.4) and 0.6% Thiobarbituric Acid (TBA) respectively.

The reaction mixture was incubated at 97^{o}c for 1 hour and the absorbance was read after cooling the tubes at a wavelength of 532nm in a spectrophotometer.

Iron chelation assay

The ability of the cold water extract to chelate Iron (ii) Sulphate was determined using a modified method of Puntel et al., (2005). Briefly, 20µl of freshly prepared 1mM Iron (ii) Sulphate was added to a reaction mixture containing 168µl of 0.1mM Tris HCl (pH 7.4), 218µl Saline (0.9% NaCl) and the cold water extract of the plant.

The reaction mixture was left to stand for 5 minutes before the addition of 13µl of 0.25% 1, 10-Phenanthroline (w/v). The absorbance was subsequently read at 510 nm in the spectrophotometer.

Statistical analysis

All the values were expressed as mean ± standard error of mean (mean ± SEM). The significance of the results was evaluated using one way analysis of variance (ANOVA), followed by Duncan's multiple range test when appropriate. The correlation coefficient (r^2) between the parameter tested was established by regressive analysis.

3. Result and Discussion

Oxidative stress, an imbalance between the production and manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage, is implicated in many chronic diseases including cancer, heart disease and aging [6,9]. Oxidative stress is now recognized to be associated with more than 100 diseases as well as normal aging process [10]. Disturbances in the normal redox state of tissues can cause toxic effects through the production of free radicals that damage all components of the cell, including Protein, Lipids and DNA.

There is a strong correlation between thiobarbituric acid reactive species (TBARS) as a marker of lipid peroxidation and products that reflects oxidative damage to DNA. To counteract oxidative stress, the body produces an armoury of antioxidants to defend itself. Thus, it is the job of antioxidants to neutralize or inhibit free radicals production that cause extensive damage to tissues and biomolecules leading to various disease conditions of lytic and degenerative nature.

The effects of cold water extract of *Garcinia kola* leaf as an antioxidant was determined in an attempt to test the ability of the extract to scavenge free radicals. Iron (ll) sulphate was used to induce lipid peroxidation in brain and liver homogenates of rat so as to generate free radical.

Subsequently, the antioxidants potential of the extract was evaluated. Tables 1 and 2 shows the inhibitory effects of the plant extract to free radical generation in the iron (ll) sulphate induced lipid peroxidation in rat brain and liver respectively. The inhibitory activity of the plant extract on lipid peroxidation was compared on both tissues. When compared with liver, the extract exhibited more potency on inhibition against Iron (ll) sulphate induced lipid peroxidation in brain. As revealed, the extract at concentration 333.33µg/ml showed maximum inhibition of 97.99% and 87.69% in the brain and liver respectively.

Garcinia kola is a potent antioxidant. This is manifested in its ability to reduce the free radical

Table 1: The inhibitory effect of the cold water extract of *Garcinia Kola* leaf on Fe_2SO_4 induced lipid peroxidation in a rat liver homogenate.

Concentration (µg/ml)	Absorbance (at 532nm)	% Inhibition
Basal	0.017±0.003[a]	NA
Control	0.773±0.123[b]	NA
3.33	0.130±0.021	83.19±2.72
7.67	0.104±0.004	86.61±0.45
20.33	0.090±0.009	88.36±1.65
45.33	0.060±0.009	92.24±1.17
121.33	0.043±0.004	94.50±0.45
333.33	0.016±0.006	87.69±0.64
Results are expressed as means of three experiments in duplicate ± SE		
Log of equation	y=3.155ln(x)±79.60	R^2=0.990
IC_{50}-9.36±52.89		

Table 2: The inhibitory effect of the cold water extract of *Garcinia Kola* leaf on Fe_2SO_4 induced lipid peroxidation in a rat liver homogenate.

Concentration (µg/ml)	Absorbance (at 532nm)	% Inhibition
Basal	0.092±0.000[a]	NA
Control	1.002±0.015[b]	NA
3.33	0.205±0.005	57.69±10.06
7.67	0.366±0.087	87.69±0.64
20.33	0.190±0.011	78.09±1.22
45.33	0.107±0.056	81.39±1.16
121.33	0.111±0.090	87.17±1.04
333.33	0.161±0.010	76.36±0.52
Results are expressed as means of three experiments in duplicate ± SE		
Log of equation	y=5.719ln(x)±58.35	
IC_{50}-72.58±29.16	R^2=0.789	

production as shown in the table 1 where it reduces the values of the extract-treated samples as compared with the high absorbance value of the brain and liver obtained 0.773±0.123 and 0.865±0.137 respectively. The concentration that gave the lowest inhibition of 83.19±2.72 (Brain) and 57.69±10.06 (Liver) was found out to be 3.33µg/ml. thus, the study revealed that the extract is more effective in the Brain than in the Liver however, it is an effective antioxidant.

In accordaance with IC_{50} which represents the concentration of a drug required for 50% inhibition invitro, both the brain and the liver expresssed a high IC_{50} value (Brain: 89.36±52.89µg/ml; Liver: 72.58±29.16µg/ml). As shown above, the IC50 value is higher in the brain than in the Liver and hence, indicating a greater effect of the plant extract in the brain.

Table 3: The Iron Chelating ability of cold water extract of Garcinia Kola leaf

Concentration (µg/ml)	Absorbance (at 532nm)	% Inhibition
0	0.069	74.82
2.3	0.055	79.93
6.1	0.067	75.54
13.6	0.091	66.79
36.4	0.09	67.15
Log of equation	y=2.39ln(x)±78.57	
IC50-53.20+30.92	R^2=0.808	

Table 3 shows the result obtained from iron chelation assay which revealed that the cold water extract of *Garcinia kola* leaf has maximum chelating activity at a concentration of 2.3µg/ml. this chelating ability of the extract on Fe^{2+} results in the inhibitory effect on Fe^{2+} lipid peroxidation. The protective ability of the cold water extract extract of Garcinia kola leaf can be attributed to the presence of antioxidant compounds especially tocopherol (vitamin E), phenols and phytochemicals e.g. flavonoids. The protection offered by this antioxidant compounds demonstrated high electron donating capacity which initiates chain termination in the lipid peroxidation mechanism, and transforming reactive free-radical species into more stable non reactive products.

Conclusion

In conclusion, the results obtained from this study clearly indicate that the administration of leaf extract of *Garcinia kola* produced antioxidant effect and hence can induce protective response against the destructive effects of free radicals on both brain and liver. There are bioactive constituents basically flavonoids and phenols present in the extract as reported and both are responsible for confering the antioxidant activity to the plant extract.

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