Bergenin, A Nigerian Alcoholic Beverage Additive From Sacoglottis Gabonensis As An Antioxidant Protector Of Mammalian Cells Against 2,4—Dinitrophenyl Hydrazine-Induced Lipid Peroxidation

H Maduka, Z Okoye

Citation

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Abstract

The effect of bergenin isolated from Sacoglottis gabonensis bark extract, on membrane lipid peroxidation and tissue ascorbic acid level was studied using rat as the experimental animal and 2,4-dinitrophenyl as the experimental oxidant. Pretreatment with bergenin significantly reduced but did not completely abolish DNPH-induced lipid peroxidation in the liver,brain and red blood cell as evidenced from the levels of the two intermediates, lipid hydroperoxide and aldehydes, measured. Bergenin also protected against DNPH-induced depletion of tissue ascorbic acid and had a sparing effect in untreated animals. The results suggest that bergenin may be responsible for the earlier observed anti-lipid peroxidizing effect of the bark extract.

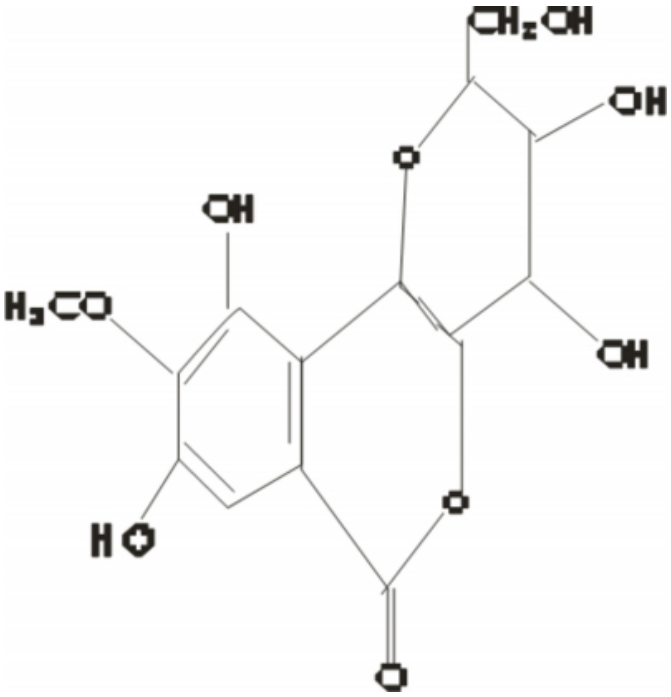
INTRODUCTION

Sacoglottis gabonensis stem bark is commonly used as palm wine additive in some rainforest communities of Southern Nigeria. Laboratory studies have shown that the extract bergenin (Fig 1) may possess antioxidant properties (Ekong and Ejike 1974, Madusolumuo, 1993; Okechukwu 1998). Ekong and Ejike reported that bergenin isolated from the bark extract had antioxidant properties towards yeast alcohol metabolism. A similar observation was made with the crude extract by Okechukwu (1998) who demonstrated that it was a non-competitive inhibitor of yeast alcohol and aldehyde dehydrogenases. Madusolumuo (1993) studied the effect of the bark extract in mammalian system and found that pretreatment with the additive protected the rat liver from acetaminophen- induced cytotoxicity.

We have recently examined the antioxidant activity of the crude extract in the mammalian system in detail using 2,4-dinitrophenyl hydrazine as the experimental peroxidant. The crude extract was found to effectively inhibit experimental lipid peroxidation in the liver, brain and red blood cells as determined by the tissue concentrations of three stable intermediates of the lipid peroxidation pathway, lipid hydroperoxide, lipid hydroperoxide carbonyl and lipid aldehyde (Maduka and Okoye 2002a). The crude extract was subsequently shown to exert a sparing effect on tissue antioxidant vitamins, ascorbic acid and vitamin E, protecting them against the 2,4-DNPH-induced depletion in the liver, brain and red blood cell, and to remove the inhibitory effect of 2,4-DNPH on the activity of the antioxidant enzyme, superoxide dismutase (Maduka and Okoye 2002b).

Figure 1

Figure 1: Structure of bergenin (2,1-benzopyrone derivative of isocoumarin, molecular weight C H O).



This paper reports on a study on the effect of bergenin isolated from S. gabonensis on 2,4-DNPH experimental lipid peroxidation and tissue ascorbic acid, primarily for the purpose of ascertaining whether or not it contributes to the observed antioxidant effect of the crude extract on lipid peroxidation.

MATERIALS AND METHODS

ISOLATION OF BERGENIN

Bergenin was isolated from the 4% aqueous ethanol extract of freshly harvested Sacoglottis gabonensis stem bark essentially as described by Madusolumuo (1993). The procedure has been routinely used in this laboratory. The isolate which was recrystallized from absolute ethanol as a white crystalline powder exhibited the characteristic melting point (238°C), UV and IR spectra earlier reported for bergenin from S gabonensis bark (Ogan, 1971, Faparusi and Bassir 1972). The purity of the bergenin used in the study was well over 99% based on its physico-chemical properties.

ANIMAL TREATMENT AND TISSUE SAMPLE COLLECTION

Weanling male wistar strain rats (body weight 90-120g) used in the study were obtained from the animal house of the Nigerian Institute of Trypanosomiasis Research (NITR), Vom, and left for 3 days to acclimatize to their new environment. During the period, they were fed ECWA laboratory chow and drinking water only. At the end of the period, the rats were distributed randomly and evenly into five standard metal/plastic rat cages, four rats per cage, such that the mean weights of the groups were about equal. The groups were labeled 1-5 respectively. Rats in groups 4 and 5 were given (1:10w/v) aqueous solution of bergenin ad libitum as drinking water for 3 days while the other groups received, also ad libitum drinking water as drinking water. All groups were fed ad libitun on ECWA laboratory chow. At the end of the 3 days pretreatment period, each rat in groups 3 and 5 was given a single intraperitoneal dose (2.8mg/kg body weight) of 2,4-dinitrophenyl hydrazine dissolved in 0.5ml normal saline to induce peroxidation (Clemens et al 1984) while those in groups 2 and 4 received also i.p., the equivalent volume of the solvent (saline) only. Rats in group 1 being the overall control, received neither 2,4-DNPH nor the solvent. At the end of the 3hr period following the administration of 2,4-DNPH or normal saline, as the case may be, rats in each group were sacrificed by cervical dislocation and tissue samples were quickly collected, starting with blood sample. Blood samples were colleted by cardiac puncture into sodium citrate bottles and samples from each group were pooled. Red blood cells were subsequently obtained by centrifugation (1000 x g for 10 min. at 4°C), washed three times with 10 times its volume of saline and stored in the refrigerator (4°C) pending analysis. The liver of each rat was quickly excised and removed to an ice bath. Samples from each group were pooled after washing with ice-cold KCl-Tris buffer pH 7.4. The group pooled samples were quickly mopped with filter paper and weighed in a beaker on a top loading balance. Each pool was subsequently homogenized in ice-cold KCl-Tris buffer pH 7.4 in the proportion of 1g liver tissue to 5ml homogenizing medium. The supernatant was obtained from the homogenate by centrifugation (1000 x g and 4°C for 10min) and stored in refrigerator pending analysis. The brain was also quickly excised and appropriate aliquots of the group pooled samples were homogenized in 0.9% NaCl solution in the ratio of 1g tissue to 5ml medium. The supernatant obtained by centrifugation at 1000 x g and 4°C for 10min was stored at 2°C pending analysis.

BIOCHEMICAL ANALYSES OF SAMPLES

Lipid hydroperoxide content was determined as malondialdehde by the method of Hunter et al (1963) as modified by Kirkova et al (1995). Fatty acid aldehyde content was determined as malonaldehyde by the

thiobarbituric acid method of Gutteridge (1984). Ascorbic acid determination was by the method of Roe and Kuether (1943) as modified by Tietz (1970). The data were analysed statistically by the student's t-test and confirmed by Mann-Whithney 2-Sample t-test.

RESULTS

The results are summarized on Tables 1-3. As can be seen from Tables 1 and 2, administration of DNPH expectedly induced lipid peroxidation in the three tissues, the values of the two intermediates determined (lipid hydroperoxide carbonyl and lipid aldehydes) being statistically higher in DNPH-treated rats (Group 3) than in the corresponding saline controls (Group 2). In both cases, pretreatment with bergenin significantly reduced the level of DNPH-induced peroxidation; the level of each intermediate was significantly lower in rats given DNPH after pretreatment with bergenin (Group5) than in non-pretreated rats given DNPH (Group 3). However, pretreatment with bergenin did not completely prevent oxidative damage by DNPH. As can be seen from Table 2, the concentration of the peroxidation intermediate, lipid aldehyde in each tissue was significantly higher in bergenin-pretreated rats given DNPH (Group 5) than in bergenin controls (Group 4). For lipid hydroperoxide carbonyl (Table 1), the difference was significant only in the case of the liver.

Figure 2

Table 1: Effect of bergenin on the level of lipid peroxidation product, lipid hydroperoxide (as determined by malondialdehyde) in the rat liver, red blood cells and brain during 2,4-DNPH-induced peroxidation.

Treatment group	Level of lipid hydroperoxide carbonyl (mg malondialdehyde/g tissue)		
	Liver	Red blood cells	Brain
(group 1) (Water)	0.46 ± 0.06	1.18 ± 0.21	4.55 ± 0.87
(group 2) (Water + Saline i.p)	0.51 ± 0.06[b]	3.13 ± 0.06[b]	4.20 ± 0.79[b]
(group 3) (Water + DNPH in Saline)	1.05 ± 0.18[acd]	5.06 ± 0.18[acd]	15.72 ± 1.69[acd]
(group 4) (Aq. Bergenin)	0.50 ± 0.11[b]	2.80 ± 0.56[b]	3.34 ± 0.05[b]
(groups 5) (Aq. Bergenin + DNPH, i.p.)	0.80 ± 0.07[bcd]	3.51 ± 0.09	4.59 ± 0.87

Tabulated values are means ± S.D. of triplicate determinations. Statistical analysis was by Mann-Whithney 2-Sample t-test.
[a]Significantly higher than saline control (p<0.05)
[b]Significantly lower than DNPH-treated (p<0.05)
[c]Significantly higher than bergenin- treated control (p<0.05)
[d]Significantly higher than water control (DNPH-solvent) control (p<0.05)

Figure 3

Table 2: Effect of bergenin on the level of lipid peroxidation product, fatty acid aldehydes in rat tissues after 2, 4-DNPH-induced perxidation

Treatment	Lipid aldehyde concentration (mg malonaldehyde/g tissue)		
	Liver	Red blood cells	Brain
(group 1) (Water)	1.59 ± 0.08	38.49 ± 1.41	3.51 ± 0.52
(group 2) (Water + Saline i.p)	1.73 ± 0.13	42.32 ± 1.97	4.61 ± 0.25
(group 3) (Water + DNPH in Saline)	4.71 ± 0.53[abd]	69.90 ± 6.17[abd]	7.85 ± 0.81[abd]
(group 4) (Aq. Bergenin)	2.76 ± 0.25[abc]	32.74 ± 2.14[cef]	2.63 ± 0.52[cef]
(groups 5) (Aq. Bergenin + DNPH, i.p.)	4.02 ± 0.18[bcd]	48.28 ± 0.39[bcd]	4.28 ± 0.39[acd]

Tabulated values are means ± S.D. of triplicate determinations.
[a]Significantly higher than water control p<0.05)
[b]Significantly higher than saline control (p<0.05)
[c]Significantly lower than DNPH- treated control (p<0.05)
[d]Significantly higher than bergenin-treated control (p<0.05)
[e]Significantly lower than water control (p<0.05)
[f]Significantly lower than saline control (p<0.05)

Tissue ascorbic acid content (Table 3) was generally lower in DNPH -treated rats (Group 3) than in the corresponding saline controls (Group 2) but the difference was significant only in the case of the red blood cell, suggesting marked DNPH-induced depletion. Ascorbic acid content of each of the three tissues was significantly higher in rats given DNPH after pretreatment with bergenin (Group 5) than in non-pretreated rats given DNPH (Group 3), an indication that bergenin protected against DNPH-induced depletion of liver, brain and red blood cell ascorbic acid. Also in each case, tissue ascorbic acid content of rats given only bergenin (Group 4) was significantly higher than in water controls (Group 1), suggesting that bergenin also protects against endogenous oxidants.

Figure 4

Table 3: Effect of bergenin on tissue ascorbic acid level during experimental peroxidation.

Treatment group	Ascorbic acid content (mg/ml tissue homogenate)		
	Liver	Red blood cells	Brain
(group 1) (Water)	0.014 ± 0.002	0.057 ± 0.01	0.014 ± 0.002
(group 2) (Water + Saline i.p)	0.014 ± 0.004	0.060 ± 0.01	0.020 ± 0.005
(group 3) (Water + DNPH in Saline)	0.012 ± 0.002	0.047 ± 0.009[d,e]	0.015 ± 0.002
(group 4) (Aq. Bergenin)	0.024 ± 0.003[abc]	0.095 ± 0.03[ab]	0.036 ± 0.007[abc]
(groups 5) (Aq. Bergenin + DNPH, i.p.)	0.022 ± 0.006[bs]	0.055 ± 0.003[b]	0.021 ± 0.001[ab]

Tabulated values are means ± S.D. of triplicate determinations.
[a]Significantly higher than water control (p<0.05)
[b]Significantly higher than DNPH- treated (p<0.05)
[c]Significantly higher than saline control (p<0.05)
[d]Significantly lower than water control (p<0.05)
[e]Significantly lower than saline control (p<0.05)

DISCUSSION

Ekong and Ejike (1974) first reported that bergenin isolated

from S.gabonensis bark exhibited antioxidant properties in palm wine metabolism but did not state the site or mode of action. Okechukwu (1998) subsequently demonstrated that the crude extract of the bark inhibited palm wine yeast alcohol dehydrogenase and aldehyde dehydnegenase. The results obtained in this study suggest that bergenin also exhibits antioxidant properties towards experimental peroxidation in the mammalian system. For the observed reduction in the level of experimental peroxidation and in the rate of depletion of tissue ascorbic acid when rats were pretreated with bergenin, are evidences of antioxidant effect.

The initiation stage of lipid peroxidation is a free radical reaction with lipid hydroperoxides-products of the reaction between lipid peroxyl radical and oxygen-being the first stable intermediate (Wills 1987). Lipid hydroperoxides subsequently give rise to lipid aldehydes. The fact that pretreatment with bergenin reduced the levels of these two stable intermediates in experimental lipid peroxidation is an indication that the photochemical exerts its antioxidant action prior to the formation of the first stable intermediate. More work is required to understand the exact site and mode of action as well as the part of the bergenin molecule responsible for the antioxidant activity in a peroxidation reaction. However, in the case of the later, indications from reports on another antioxidant photochemical point to attachment of both polar and lipophilic substituent groups to the coumarin nucleus. For example, Sugiyama et al (1993) has shown that the presence of both polar and lipophilic substituent groups on the nucleus of purpurogallin molecule was responsible for its antioxidant properties against peroxyl radicals in human erythrocytes.

Theoretical mechanistic biochemistry concept has used the theoretical bioactivation of phenyl hydrazine-induced lipid peroxidation as well as the structure of bergenin as models (Maduka,2005a and Maduka 2005b) to justify the observed antioxidant properties of Sacoglottis gabonensis(Maduka and Okoye,2002,Maduka et.al,2003).Theoretical mechanistic biochemistry relies on the structures of oxidants to predict their reactivities in tissues.From thje structure of bergenin,positions 1,2,3 and 4 are positions of antioxidant properties with positions 1 and 2 being greater than positions 3 and 4.Bergenin could give off any of the protons to react with the free radicals generated during the theoretical bioactivation scheme of phenyl hydrazine.

Ascorbic acid is a reducing agent which plays a general antioxidant role in the body. The observed reduction of its tissue level in experimental peroxidation is therefore, an indication that it participates in the body's antioxidant defences against xenobiotics like DNPH. Furthermore, the fact that the ascorbic acid sparing effect of bergenin was observed even in the absence of the experimental oxidant, DNPH is an indication that bergenin exerts antioxidant action on endogenous oxidizing agents as well.

Since similar antioxidant effect on lipid peroxidation and tissue ascorbic acid has been observed with the crude extract of S. gabonensis stem bark (Maduka 2000, Maduka and Okoye 2002a, 2002b, Maduka et al., 2003) the present data would confirm that bergenin is a majoy component of Sacoglottis gabonensis bark extract responsible for the antiperoxidising properties of the extract. Bergenin appears to act partly by sparing tissue ascorbic acid,a preferred antioxidant in the brain.As already reported,bergenin is a major isolate of Sacoglottis gabonensis(Ogan,1971)

Which is an alcoholic beverage additive in Nigeria.The case being reported in this presentation is a typical case of manipulation of alcohol effects in the tissues described including the brain.

CORRESPONDENCE TO

H.C.C. Maduka Department Of Biological Sciences, The University Of Hull, HU6 7RX, UK. E-mail: chmaduka@yahoo.com

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Author Information

H. C. C. Maduka
Department Of Biochemistry, Faculty Of Medical Sciences, University Of Jos

Z. S. C. Okoye
Department Of Biochemistry, Faculty Of Medical Sciences, University Of Jos