



Journal of Medical Sciences

ISSN 1682-4474

science
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J. Med. Sci., 5 (3): 208-211
July-September, 2005

Prevention of 2, 4-dinitrophenylhydrazine-induced Tissue Damage in Rabbits by Orally Administered Decoction of Dried Flower of *Hibiscus sabdariffa* L.

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The effects of orally administered decoction of the dried flower of *Hibiscus sabdariffa* on 2, 4-dinitrophenylhydrazine-induced lipid peroxidation and reduced glucose concentration in blood, brain and liver have been examined in rabbits. Following oral administration of the decoction (200 mg kg⁻¹ body wt.) once/day for 3 days, 2, 4-dinitrophenylhydrazine (2, 4-DNPH) was administered in saline (28 mg kg⁻¹ body wt.) intraperitoneally. Three hours after 2, 4-DNPH administration the rabbits were sacrificed. The results show that treatment with 2, 4-DNPH alone significantly ($p < 0.05$) reduced brain glucose when compared with the control (control 0.23±0.02 mM; 2, 4-DNPH treated: 0.12±0.03 mM). Treatment with the decoction before 2, 4-DNPH caused significant ($p < 0.05$) increase in brain glucose relative to that of the group treated with 2, 4-DNPH alone (DNPH alone-0.12±0.03 mM; Decoction + DNPH-0.18±0.01 mM). Exposure of rabbits to 2, 4-DNPH produced significant ($p < 0.05$) increase in lipoperoxidation in blood (28.20±4.19 units/mL x 10⁻⁷) brain (21.30±4.28 units/mL x 10⁻⁷) and liver (36.97±2.72 units/mL x 10⁻⁷) as indicated by malondialdehyde level when compared to the control (blood: 1.54±0.22; brain: 5.25±0.56; liver: 7.50±2.67 units/mL x 10⁻⁷). In rabbits treated with the decoction prior to DNPH the level of malondialdehyde was significantly ($p < 0.05$) decreased in the blood (6.73±0.55 units/mL x 10⁻⁷) brain (6.93±0.20 units/mL x 10⁻⁷) and liver (9.37±2.14 units/mL x 10⁻⁷) relative to the group treated with DNPH alone. The data obtained in this study show that the decoction of the dried flower of *H. sabdariffa* was able to prevent not only 2, 4-DNPH-induced decrease in brain glucose but also its lipoperoxidative effect in the blood, brain and liver in the rabbit.

Key words: *H. sabdariffa*, 2, 4-dinitrophenylhydrazine, blood, brain, liver, glucose, Malondialdehyde

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INTRODUCTION

Among the more than 300 species of *Hibiscus* is *Hibiscus sabdariffa* L., which has many medicinal uses^[1,2]. The dried calyces contain the flavonoids-gossypetin, sabdaretine, hibiscetine and anthocyanins^[3]. Flavonoids are phenolic substances. They act in plants as antioxidants. It is thought that in humans absorbed flavonoids and their metabolites may display an *in vivo* antioxidant activity. This is evidenced experimentally by the increase in plasma antioxidant status, the sparing effect on vitamin E of erythrocyte membranes and Low Density Lipoproteins (LDL) and preservation of erythrocyte membrane polyunsaturated fatty acids^[3,4]. Antioxidant vitamins such as vitamin C and vitamin E along with flavonoids have been shown to be effective in reducing atherosclerosis^[5,6].

Phenylhydrazine is a long established haemolytic agent^[7]. The lytic effect has been attributed to its autoxidation and subsequent oxidation of enzymes, membrane proteins, haemoglobin and its ability to initiate lipid peroxidation in membrane phospholipids^[8]. There are indications that the red extracts of the dried flower of *Hibiscus sabdariffa* is protective against oxidative stress in rat primary hepatocytes^[9]. Since the underlying basis of the biological effects of phenylhydrazine is oxidation its effects in experimental animals provides a model for testing the claim that *H. sabdariffa* flower extract possesses antioxidant property. So the purpose of this investigation was to evaluate this claim. We have done this by giving the decoction of the dried flower to experimental rabbits prior to phenylhydrazine exposure and compared the effect with that in rabbits exposed to phenylhydrazine alone.

MATERIALS AND METHODS

Preparation of decoction: One hundred grams of the dried flower minus the seeds were put in a pot containing one litre of clean tap water and allowed to boil for 15 min. After the period of boiling the mixture was left to cool. Thereafter it was filtered through 4 layers of cheese-cloth and a clear, red coloured decoction was obtained. One millilitre of the decoction was evaporated to dryness in a pre-weighed watch glass to determine the concentration of its solute content. This was found to be 80 mg mL⁻¹.

Animals and treatment: Pathogen-free rabbits (*Oryctolagus cuniculus*; initial mean weight = 1.02 kg) were obtained from a Breeder in Benin City. The animals were housed in galvanized rabbit cages and acclimatized on guinea growers mash (product of Bendel Feed and Flour Mills, BFFM Ltd, Ewu, Nigeria). They were subsequently assigned to 4 groups (3 per group).

Members of group 1 (control) were given 0.5 mL H₂O kg⁻¹ body wt. by gavage once a day for 3 days. Rabbits in groups 2 and 4 were given the equivalent of 200 mg of the decoction kg⁻¹ body wt. once a day for 3 days by gavage. Following each daily treatment all groups were fed *ad libitum* on mash and allowed free access to clean drinking water, but were fasted overnight on the third night. Following the overnight fast rabbits in group 3 (2, 4-DNPH only) and group 4 (Decoction + 2, 4-DNPH) were given a single dose of 28 mg 2, 4-DNPH kg⁻¹ body wt. in saline (i.p.) Three hours after 2, 4-DNPH administration the rabbits were anaesthetised using diethylether. While under anaesthesia the abdominal and thoracic regions were opened to expose the heart and liver. Blood was obtained by heart puncture by means of a 10 mL heparinized syringe and needle and placed in ice-cold heparinized bottles. From these plasma samples were obtained by centrifugation, 2000xg for 10 min (SM 9026 B bench Centrifuge, Surgifriend Medicals, England). From each rabbit liver was excised. One gram was homogenized in ice-cold saline (1:4 wt./v) and subsequently centrifuged, 2000xg for 10 min to obtain a clear supernatant. The brain of each rabbit was also excised and 1 g homogenized and centrifuged as described for the liver.

Biochemical assays: Glucose in blood plasma and in brain and liver homogenate supernatants was determined by the glucose oxidase method. The procedure adopted is as described in Randox Glucose Oxidase Kit Leaflet (Randox Laboratories Ltd, UK). Fifty microlitres of the plasma, brain and liver homogenate supernatants were used, respectively with 5 mL of the glucose oxidase/oxidase reagent. After 15 min incubation at 37°C a red-violet coloured complex that absorbs at 500 nm was obtained. The absorbance was determined using 210 Milton Ray Unicam SP1800 uv/visible spectrophotometer. Malondialdehyde (MDA) was estimated colorimetrically based on its reaction with 2-thiobarbiturate to yield a pink-coloured complex that absorbs strongly at 532 nm^[10]. The MDA concentration of each sample was calculated using the extinction coefficient of 1.56x10⁵ M⁻¹ cm⁻¹ indicated by Buege and Aust^[10].

Statistics: The data are presented as means±SEM. The mean values of the various treatment groups were compared using ANOVA and the least square difference test^[11]. The 0.05 level of probability was used as the criterion of significance.

RESULTS

In group 3 rabbits brain glucose was significantly (p<0.05) decreased (Table 1). In the same group MDA

Table 1: Effect of *Hibiscus sabdariffa* dried flower decoction on 2, 4-DNPH-induced changes in blood, brain and liver glucose concentration

Groups	Treatment	Glucose concentration (Mean±SEM(n))		
		Blood (plasma)(mM)	Brain (mM)	Liver (mM)
1	Water control (0.5 mL kg ⁻¹ body wt.)	5.12±0.92(3) ^a	0.23±0.02(3) ^a	5.53±1.56(3) ^a
2	Decoction (200 mg kg ⁻¹ body wt.)	4.74±0.35(2) ^a	0.21±0.01(2) ^a	5.55±0.19(2) ^a
3	DNPH (28 mg kg ⁻¹ body wt.)	2.42±0.94(4) ^a	0.12±0.03(3) ^b	3.39±0.59(3) ^a
4	Decoction (200 mg kg ⁻¹ body wt.) DNPH (28 mg kg ⁻¹ body wt.)	3.49±0.06(3) ^a	0.18±0.01(3) ^a	5.18±0.58(3) ^a

^a Values with same superscript within a column are not statistically significantly different from each other (p>0.05)

^b Value significantly lower than the others within the same column (p<0.05)

Table 2: Effect of *Hibiscus sabdariffa* dried flower decoction on 2, 4-DNPH-induced changes in blood, brain and liver malondialdehyde concentration

Groups	Treatments	Malondialdehyde concentration (Mean±SEM(n))		
		Blood (plasma)(Units/mL) 10 ⁻⁷	Brain (Units/mL homogenate) 10 ⁻⁷	Liver (Units/mL homogenate) 10 ⁻⁷
1	Water control (0.5 mL kg ⁻¹ body wt.)	1.54±0.22(3) ^a	5.25±0.56(3) ^a	7.50±2.67(3) ^a
2	Decoction (200 mg kg ⁻¹ body wt.)	1.25±0.10(2) ^a	4.90±0.48(2) ^a	6.44±1.06(2) ^a
3	DNPH (28 mg kg ⁻¹ body wt.)	28.20±4.19(3) ^b	21.30±4.28(3) ^b	36.97±2.72(3) ^b
4	Decoction (200 mg kg ⁻¹ body wt.) DNPH (28 mg kg ⁻¹ body wt.)	6.73±0.55(3) ^a	6.93±0.20(3) ^a	9.37±2.14(3) ^a

^a Values with same superscript within a column are not statistically significantly different from each other (p>0.05).

^b Value significantly greater than the others within the same column (p<0.05)

was significantly (p<0.05) increased (Table 2) in the plasma and in brain and liver homogenate supernatants due to 2, 4-DNPH treatment when compared with controls. In group 4 rabbits exposed to the decoction before 2, 4-DNPH treatment, there was significant (p<0.05) increase in brain glucose level (Table 1) but a significant (p<0.05) decrease in MDA levels (Table 2) in the blood and brain and liver homogenate supernatants when compared to their corresponding values in group 4 rabbits exposed to 2, 4-DNPH alone.

DISCUSSION

In this study, glucose and malondialdehyde levels in blood plasma, brain and liver were used as indices of DNPH-induced tissue damage and protection by *H. sabdariffa* dried flower decoction against the same effect in rabbits.

Table 1 show that DNPH caused significant depletion in brain glucose. This finding is in agreement with the report of previous workers^[12]. Table 2 show that DNPH causes membrane lipid peroxidation in the blood, brain and liver. DNPH-induced lipid peroxidation has been reported by others as well^[13].

Increase in blood and tissue levels of thiobarbituric acid reactive substances, malondialdehyde in particular, are reliable indices of oxidative stress and lipoperoxidative tissue damage^[15]. So the profile of MDA in the blood,

brain and liver of 2, 4-DNPH-treated rabbits (Table 2) is a clear indication that 2, 4-DNPH provokes oxidative stress in these tissues/organs. *In vivo*, this toxicant is believed to undergo autoxidation and so becomes a strong oxidant with the ability to initiate lipid peroxidation in membrane phospholipids^[8] once the antioxidant defense system of the cell, tissue or organ has been overwhelmed.

The hypothesis on which this investigation was based is that if the decoction of *H. sabdariffa* dried flower possesses antioxidant principles it would prevent lipid peroxidation and other metabolic side effects of DNPH caused by its oxidant action. Present data demonstrates reasonably well that treatment of rabbits with the decoction prior to 2, 4-DNPH intoxication significantly impaired the latter's ability to deplete brain glucose and induce lipoperoxidation in the blood, brain and liver (Table 1 and 2). This finding supports our hypothesis and lends weight to the report of Tseng *et al.*^[9] that extract obtained from the dried flower of this plant possesses antioxidant activity.

Presently, though, the constituent(s) of this decoction that possesses the antioxidant property is yet to be specifically identified but there is evidence that the flower of this plant contains gossypetine, sabdaretine, hibiscetine and anthocyanin which are flavonoids. Since flavonoids are known to exhibit antioxidant activities^[3,4,7] any or a combination of these flavonoids may be the agent(s) that impaired the oxidant action of 2, 4-DNPH in the rabbit.

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