

EFFECT OF BETEL NUT CONSTITUENTS ON SULFHYDRYL METABOLISM

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Abstract. In order to study the effect of different constituents of betel nut on sulfhydryl metabolism. betel nut aqueous extract, polyphenol fraction and arecoline, an important alkaloid of betel nut were injected i.p. to Swiss mice. Glutathione, protein-SH and protein-SH+S-S contents were measured in liver, lung, kidney and muscle tissues. Tannic acid was injected as a standard polyphenol for comparison. Betel nut aqueous extract, polyphenol fraction and tannic acid increased glutathione content while arecoline decreased the same in almost all the tissues. All the test substances decreased the protein-SH content in majority of the tissues studied.

Key Phrases. Arecoline Polyphenol Tannic acid Sulfhydryl groups

Introduction

Biological studies on the possible carcinogenic properties of betel nut aqueous extract and its constituents are in progress in this laboratory. It has been reported that s.c. injections of hot and cold aqueous extracts of dried Mangalore betel nut produced 60% fibrosarcoma in Swiss strain mice (Ranadive et al., 1976) and polyphenol fraction of betel nut produced 100% fibrosarcoma in Swiss strain mice (Gothoskar et al., 1977). Further, it was observed that oral feeding of arecoline, pharmacologically the most important alkaloid of betel nut, produced 41% liver and lung tumours in Swiss mice (unpublished data).

Arecoline was found to be excreted in rats as mercapturic acid, like so many other carcinogens (Boylard, 1968) and the same was reviewed by them as a possible carcinogen from betel nut. Like betel nut polyphenol fraction, polyphenol fractions from so many other plant sources have been found to possess tumour producing properties in laboratory ani-

mals (Pradhan et al., 1974; Kirby, 1960). Many polyphenolics have been shown to form complexes with sulfhydryl group compounds i.e. enzymes etc. (Lacombe, Mitjavila and Carrera 1976; Luciani, 1969; Rodney et al., 1950).

In continuation of the search of specific changes brought about by different fractions of betel nut which have been found to produce tumours in Swiss mice, it seemed worthwhile to study sulfhydryl group metabolism in different tissues of Swiss mice treated with different constituents of betel nut. The present paper reports the effect of betel nut constituents on glutathione level (soluble-SH) and protein-SH and protein-SH +S-S (fixed-SH) in different tissues of Swiss mice.

Materials and methods

Male Swiss strain mice were obtained from the Animal Colony of the Cancer Research Institute, Bombay.

Arecoline HCl was obtained from Sigma Chemical Co., U.S.A. and

tannic acid from B.D.H., England. Rest of the chemicals used were of analytical grade. Betel nut aqueous extract and betel nut polyphenol fraction were prepared as described by Shivapurkar, Bhide and Ranadive (1978). Both the extracts were diluted ten times for treatment. The amount of arecoline and polyphenol was measured by the methods described by Sharp (1932), and Swan and Hills (1959) respectively. 0.1 ml of the aqueous extract was found to contain 1.5 mg of arecoline and 1.9 mg of polyphenol (in terms of mg of tannic acid). 0.1 ml of the polyphenol fraction was found to contain 1.9 mg of polyphenol (in terms of mg of tannic acid). The amount of arecoline, betel nut aqueous extract polyphenol fraction and tannic acid to be injected was determined as described earlier (Shivapurkar et al., 1978). In the present experiment 0.06 mg/g body weight of arecoline, 0.1 ml of betel nut aqueous extract/animal, 0.1 ml of polyphenol fraction/animal and 1.9 mg tannic acid/animal were injected i.p to Swiss mice (6-8 weeks old) and the animals were sacrificed 24 h after injection.

Liver, lung, kidney and muscle were dissected out and kept frozen at -20°C . All the tissues were immediately weighed and used for estimation of glutathione, protein-SH and protein-SH+S-S group content.

For glutathione estimation, tissues were homogenized in 4% cold sulfosalicylic acid in Potter Elvehgen homogenizer to precipitate proteins, and filtrate was used for estimation

of glutathione by titrimetric method described by Patterson & Lazarow (1955).

For sulfhydryl group estimation, tissues were homogenized in 0.2 M phosphate buffer pH 7 (ice cold) in Potter Elvehgen homogenizer. Proteins were precipitated from the homogenate by addition of equal volume of 10% TCA (ice cold) and the precipitate was dissolved in 0.2 M phosphate buffer pH 7.0 and protein sulfhydryl group content was estimated by spectroscopic method described by Sela, White and Anfinsen (1957).

For protein-SH+S-S estimation suitable aliquot containing about 6 to 7 mg of protein was treated with 0.37 ml of 0.8% thioglycolic acid and 0.48 g urea. pH was adjusted to 8.5 with 10% aq. trimethylamine and volume was made up to 1 ml. The mixture was incubated at room temp. for 4 1/2 h. The protein was precipitated with 10 volumes of acetone +HCl mixture (39:1 ml) and allowed to stand for 15 min in ice-salt mixture. The precipitate was centrifuged and washed with 3:10 ml portions of acetone+HCl mixture and then with ether (peroxide free). The dried precipitate was dissolved in a known quantity of 0.5 M phosphate buffer pH 7.0 and sulfhydryl content was estimated by spectroscopic method described by Sela et al. (1957). Unless and otherwise stated, all the steps were carried out at 0°C .

R e s u l t s

Table 1 summarizes the effect of

Table 1
Glutathione content in different tissues of swiss mice treated with different ingredients of betel nut

Group	Glutathione content (mg/g dry weight) of different tissues (Mean ± S.E.)			
	Liver	Lung	Kidney	Muscle
Untreated	2.60 ± 0.10	12.60 ± 1.50	5.30 ± 0.40	2.00 ± 0.70
Betel nut aqueous extract containing 1.5 mg arecoline and 1.9 mg polyphenol/0.1 ml extract	14.40 ± 1.90*	16.80 ± 2.70	17.00 ± 4.40*	11.40 ± 1.70*
Polyphenol fraction containing 1.9 mg polyphenol/0.1 ml fraction	10.85 ± 0.81*	23.12 ± 0.90*	11.95 ± 0.31*	12.68 ± 0.36*
Tannic acid (1.9 mg/0.1 ml soln.)	13.10 ± 0.53*	10.88 ± 0.70	20.02 ± 0.33*	7.60 ± 0.52*
Arecoline (1.5 mg/0.1 ml soln.)	1.24 ± 0.03*	5.02 ± 0.20*	1.80 ± 0.02*	1.49 ± 0.12*

0.1 ml of each compound was injected i.p.
 Values represent mean of 4 mice
 • P<0.05

Table 2
 Protein-SH group content in different tissues of swiss mice treated with different ingredients of betel nut

Group	Protein-SH content ($\mu\text{M}/\text{mg}$ dry weight) of different tissues (Mean \pm S.E.)			
	Liver	Lung	Kidney	Muscle
Untreated	0.14 \pm 0.05	0.25 \pm 0.02	0.12 \pm 0.01	0.14 \pm 0.01
Betel nut aqueous extract containing 1.5 mg arecoline and 1.9 mg polyphenol/0.1 ml extract	0.03 \pm 0.01*		0.09 \pm 0.003*	0.03 \pm 0.007*
Polyphenol fraction containing 1.9 mg polyphenol/0.1 ml fraction	0.069 \pm 0.006*	0.14 \pm 0.03*	0.07 \pm 0.012*	0.076 \pm 0.002*
Tannic acid (1.9 mg/0.1 ml soln.)	0.076 \pm 0.017*	0.25 \pm 0.01	0.08 \pm 0.010*	0.050 \pm 0.004*
Arecoline (1.5 mg/0.1 ml soln.)	0.060 \pm 0.010*	0.20 \pm 0.01*	0.17 \pm 0.010*	0.090 \pm 0.008*

0.1 ml of each compound was injected i.p.

Values represent mean of 4 mice

* P<0.05

Table 3
Protein-SH+S-S group content in different tissues of miss mice treated with different ingredients of betel nut

Group	Protein-SH+S-S content ($\mu\text{M}/\text{mg}$ dry weight) of different tissues (Mean \pm S.E.)			
	Liver	Lung	Kidney	Muscle
Untreated	0.180 \pm 0.020	0.27 \pm 0.009	0.29 \pm 0.050	0.180 \pm 0.000
Betel nut aqueous extract containing 1.5 mg arecoline and 1.9 mg polyphenol/0.1 ml extract	0.040 \pm 0.004*	—	0.14 \pm 0.080*	0.050 \pm 0.000*
Polyphenol fraction containing 1.9 mg polyphenol/0.1 ml fraction	0.100+0.018*	0.16 \pm 0.014*	0.16 \pm 0.017*	0.140 \pm 0.010*
Tannic acid (1.9 mg/0.1 ml soln.)	0.084 \pm 0.020*	0.30 \pm 0.010	0.21 \pm 0.010	0.100 \pm 0.008*
Arecoline (1.5 mg/0.1 ml soln.)	0.085 \pm 0*000	0.22 \pm 0.010*	0.18 \pm 0.010*	0.088 \pm 0.020*

0.1 ml of each compound was injected i.p.

Values represent mean of 4 mice

* P<0.05

different betel nut constituents on the glutathione content of various tissues of Swiss mice. It is evident that aqueous extract, polyphenol fraction and tannic acid increase glutathione content in all the tissues whereas arecoline decreases the same in all the tissues.

Table 2 summarizes the effect of different betel nut constituents on the protein-SH content of various tissues of Swiss mice. It may be observed that the aqueous extract and polyphenol fraction decrease protein-SH levels in all the tissues whereas tannic acid decreases the same in liver, kidney and muscle tissues. Arecoline decreases protein-SH contents in all the tissues under investigation.

Table 3 summarizes the effect of different betel nut constituents on the protein-SH+S-S content of various tissues of Swiss mice. Aqueous extract and polyphenol fraction as well as arecoline decrease the protein-SH+S-S levels in all the tissues while tannic acid treatment decreases the same in liver and muscle tissues.

Discussion

The results clearly show that treatment with betel nut aqueous extract, polyphenol fraction and tannic acid (used as a typical polyphenol to compare effects due to polyphenol fraction) increased glutathione content while protein-SH and protein-SH+S-S content were decreased in different tissues. Arecoline decreases glutathione as well as protein-SH and protein-SH+S-S content in

different tissues of Swiss mice. Increased or decreased glutathione level or decrease in protein-SH and protein-SH+S-S levels are notable in the light of reported tumorigenicity data of the above mentioned betel nut constituents. Sulfhydryl groups are suspected to play a key role in cell division (Alexander and Boyer, 1971) differentiation (Brachet, 1962) and carcinogenesis (Egyud and Szent-Gyorgyi, 1966).

It is also reported that various carcinogens are detoxicated by conjugation with SH containing compounds (i.e. glutathione) and thus cause a decrease in SH levels. It is further known that this decrease is then followed by an increase in GSH level after an interval of time ranging from few weeks to few months (Dijkstra, 1964; Dijkstra and Pepler, 1964).

In the present studies, the observed decrease in glutathione level in different tissues due to the treatment with arecoline might probably be the first stage in the exposure of the organism to carcinogenic principle from betel nut and it will be worthwhile to pursue the pattern of SH levels during the malignant transformation and ultimately in the tumour.

Many carcinogens have been found to inhibit -SH enzymes as a part of their carcinogenic process (Burk and Winzler, 1944; Potter, 1942). Further, alkylation of nucleoprotein by some sulfhydryl reacting carcinogens has also been postulated as the principal mode of action of some carcinogens (Wheeler, 1962). It is inte-

resting to see whether the observed decrease in protein-SH and protein-SH+S-S content seems to have some implication in tumour production. Further studies may help us to locate the precise mode of action.

It is rather difficult to interpret the observed increase in glutathione levels of aqueous extract and polyphenol fraction treated mice. Glutathione plays a crucial role in various metabolic reactions (Beeley, 1971; Eggleston and Krebs, 1974; Sutter and Moldave, 1966; Morris, 1967) and it is important to ascertain if this increase is due to *de novo* synthesis of glutathione or due to decreased rate of oxidation of reduced glutathione. Work on these lines is in progress and will be reported later.

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