EFFECT OF SOYBEAN EXTRACT ON NITROSAMINES FORMATION. IN VITRO STUDY

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Soybean (SB) extracts markedly inhibit formation of diphenylnitrosamine (DPNA) from the precursors diphenylamine (DPA) and nitrite (NO_2^-). SB extracts interact with nitrite, as shown by the decrease in the nitrite level. The rate of interaction between SB extracts and nitrite was lower than the rate of inhibition of DPNA formation. Thus the reduction of DPNA yield by SB extracts is not caused mainly through their competition for the available nitrite, but it might be due to the adsorption of the DPNA formed to the SB proteins, internitrosation between SB constituents and DPNA formed, or through the breakdown of the DPNA.

Nitrosamines (NAs) are potent carcinogens in many animal species. Nitrosamines, as well as their precursors are widely present in human environment. Evidence has been obtained for their occurrence in foodstuffs[1,2].

During the past decades considerable attention has been focused upon dietary items which might inhibit the carcinogenic processes. Wattenberg[3] divided the inhibitors of carcinogenesis into 3 categories. The first consists of compounds that prevent the formation of carcinogens from their precursors. The second one, called blocking agents, inhibits carcinogenesis by preventing the carcinogenic compounds to reach or react with critical target sites in the tissues. The third category is known as supressing agents which supress the expression of neoplasia in cells previously exposed to carcinogenic agents.

Soybean which is widely used today in some foodstuffs, such as minced meat, beef and animal feeds, was found to counteract the effect of the hepatocarcinogen dimethylaminoazobenzene (DAB) when administered to rats[4]. This finding was confirmed by Radwan[5] using hepatocarcinogens ethionine or DAB. It was found to have a protective action against the effect of NAs precursors when orally administered to mice[6].

The present study is an in vitro study, conducted in order to ascertain the effect of SB extracts on NAs and their formation from precursors. It represents also a trial

to clarify the main role of SB in counteracting the action of NAs, i.e. whether it acts through prevention of the formation of NAs from precursors or through the breakdown of the NAs already formed.

EXPERIMENTAL

Soybean (SB) *extracts*: Soybean grains cultivated in Egypt and dried for about 1 year have been in either of the following forms. (a) Powder suspension: SB grains were ground in an electric grinder. The powder was suspended in 0·1M acetate buffer, pH 3. (b) Cold extract: the powder was vigorously shaked in cold 0·1M acetate buffer, pH 3. (c) Boiled extract: The powder was boiled for 15 min in distilled water, cooled and vigorously shaked. Sodium acetate was added to the concentration 0·1 mol l^{-1} and the pH was adjusted to 3.

The SB extracts were prepared fresh for every experiment.

Effect of SB *extracts on nitrite concentration*: 1%, 5% and 10% SB extracts were added to 20 ppm nitrite at room temperature. Unreacted nitrite was determined immediately after SB addition, and after 30 min until 24 h. The nitrite was determined according to Montgomery and Dymock[7] using Griess reagent. The pink colour produced was measured spectrophotometrically at 535 nm.

Effect of SB *on* DPNA *formation*: 50 ppm DPA was added to 100 ml of 5% SB powder suspension; 2 500 ppm NO_2 was immediately added to the mixture, and left for 4 h in the dark. The DPNA formed was then determined by comparing its yield with that formed in the absence of SB.

Effect of SB *on the already formed* DPNA: DPNA was prepared according to the method of Walter et al.[8] by adding 1 000 ppm DPA to 4 000 ppm nitrite in acetate buffer, pH 3. Then, 12 ppm DPNA was added to 5% SB extracts. The remaining DPNA was redetermined in the mixture after 4 h.

Determination of total NAs: NAs were extracted three times with dichloromethane (1 : 3, v/v). Samples were dehydrated using anhydrous sodium sulphate, then concentrated to 5 ml at 40°C. Total NAs were determined using Griess reagent after decomposing it into amine and nitrite with 3% hydrobromic acid in glacial acetic acid according to the method of Eisenbrand and Preussmann[9].

RESULTS

Effect of SB *extracts on nitrite concentration*: Table I shows that SB extracts at the concentration of 1% did not show any immediate effect on the nitrite concentration. The interaction occured when SB concentration increased from 1 to 5 or 10%. After 24 h a considerable decrease in the nitrite concentration was observed.

Effect of SB *on* DPNA *formed from its precursors*: Table II shows the effect of SB on the DPNA formation after the addition of nitrite, amine and SB. It is obvious that SB reduced the yield of DPNA, especially if SB powder suspension was used.

Effect of SB on the already formed DPNA: It is obvious from Table III that the already formed DPNA is eliminated from the reaction mixture after the addition of SB.

DISCUSSION

An important way of eliminating cancer in man is to prevent or counteract the formation of chemical carcinogens. Among the most important compounds which inhibit N-nitrosation of secondary amines are those which can be nitrosated themselves, by competing with amines for the nitrosating agents or by internitrosation between NAs and the compound.

Application of SB with various carcinogens was found to counteract the effect of these carcinogens[10-12]. A fat emulsion for infusion therapy (Intralipid) composed of soybean oil and yolk lecithin, subsequently inhibited NAs formation[13]. Mokhtar[6] reported that application of soybean in addition to nitrosamine precursors, namely dibutylamine and nitrite, resulted in a decrease in the biological changes caused by the nitrosamine precursors.

The present findings agree with the previous findings concerning the effect of SB on chemical carcinogens, namely DPNA. Diphenylamine was chosen in the present work as a precursor for NAs, since the rate of its reaction with nitrite to form DPNA is very high[14]. All the reactions were carried out at pH 3 to simulate the pH of the stomach which is considered as the main site for NAs formation, and as it is the optimum pH for nitrosamine formation[15].

In the present study, it was found that SB reduced the formation of NAs when nitrite and amines were added together.

TABLE I

Transformation of nitrite by soybean extracts. Results are expressed as percents of the unreacted nitrite, the values in absence of SB being taken as 100%

Type of SB used	Conc. of SB %	Time, h						
		0	0·5	1	2	3	4	24
None[a]		100 (20)	100 (18·4)	100 (17·5)	100 (16·8)	100 (16·4)	100 (16)	100 (12·4)
Powder susp.	5%	94·0	91·3	83·0	72·0	92·0	67·5	40·3
Cold ext.	5%	94·0	91·3	86·4	77·4	78·0	76·3	85·5
Boiled ext.	5%	87·0	83·7	81·8	78·6	79·3	78·8	74·2
Powder susp.	10%	86·5	89·1	75·0	69·0	67·1	63·8	40·4
Cold ext.	10%	90·0	89·7	81·8	75·0	74·4	73·8	74·2
Boiled ext.	10%	70·0	72·8	72·7	73·8	73·2	72·5	61·3

[a] In parentheses are given absolute values of the remaining nitrite in ppm.

It is possible that NAs ingested in diet together with SB powder are adsorbed to the SB particles. Proteins, amino acids or other constituents of SB might also bind the carcinogen or intearct with it, thus counteracting its carcinogenic activity. In this respect, it is important to mention that Kanisaki and Hayashi[16] found that amino acids, such as cysteine, glutathione and methionine, inhibit nitrosamine formation. Zeibarth and Scheuing[17] reported similar effect of proteins.

The nitrite–SB interaction was proved to be time and dose dependent (Table I). The rate of nitrite consumption by SB was not equivalent neither to the rate by which DPNA formation was inhibited by SB (Table II), nor to the rate of DPNA disappearence from the mixture containing SB (Table III). Thus, the effect of SB on DPNA formation or stability is not completely due to its reaction with nitrite, but other means might be responsible also. This is obvious from Table III, which shows that the effect of SB on the already formed DPNA may be through its breakdown, trans-

TABLE II

The effect of SB on the formation of NAs. Reaction mixture: 50 ppm diphenylamine + 2 500 ppm nitrite + 5% SB powder or extract in 100 ml 0·1M acetate buffer, pH 3, incubated for 4 h at 25°C

Type of SB	Percents of NAs formed
None	100 (23·5)[a]
SB powder suspension	1·0
Cold extract	9·3
Boiled extract	32·4

[a] In parentheses is the absolute value in ppm.

TABLE III

The effect of SB on NAs. Reaction mixture: 12 ppm DPNA + 5% SB powder suspension or extract in 100 ml 0·1M acetate buffer, pH 3, incubated for 4 h at 25°C

Type of SB	Percents of NAs remaining in the reaction media
None	100 (11·9)[a]
SB powder suspension	5·1
Cold extract	18·4
Boiled extract	18·4

[a] In parentheses is the absolute value in ppm.

nitrosation between SB components and DPNA, or through adsorption of DPNA to SB proteins. It is worth mentioning that Kurechi et al.[18] reported that the soya products effectively reduced nitrite concentration, in vitro, under mild acidic conditions. They also inhibited the formation of N-nitrosodimethylamine from its precursors at gastric pH. They reported that the unsaturated fatty acids present in soybean products might be responsible for the reduction in nitrite concentration and the inhibition of nitrosamine formation. Sosulski[19] reported that this inhibitory effect might be due to phenolic compounds in soybean.

On the other hand, Tahira et al.[20] found that soya sauce treated with nitrite was found to be mutagenic to *Escherichia coli*. The mutagenicity of soya sauce treated with nitrite was affected by the concentration of soya sauce in the mixture and a concentration of 5% at pH 3 resulted in the highest specific activity.

Still, the consumption of nitrite from the reaction mixture after 24 h represents an important finding. Howe et al.[21] found an increased risk of bladder cancer in males who have no access to tap water and consume unpurified water containing high levels of nitrates and nitrites. El-Aaser et al.[22] found a positive correlation between the presence of nitrite in urine and the cytological findings in the exfoliated cells from the bladder mucosa, such as atypia in the form of dysplasia.

It can be concluded, that the present findings concurrent with the previous studies concerning the protective effect of soybean against the carcinogenic action of NAs and its presursors. It also clarifies to a certain extent the mode of action of SB on NAs and their formation from precursors.

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