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Modulatory influence of certain dietary supplements on the toxic effects of arsenite in rabbits

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ABSTRACT: The modulatory effects of three dietary supplements, honey, garlic (*Allium sativum* L.) and bitter kola (*Garcinia kola* seed) on sodium arsenite-induced toxicity were evaluated in rabbits. The experimental animals were fed 100 mg/kg body weight each of honey (Ho) and aqueous extracts of garlic (Ga) and bitter kola (Bi) either alone or in combination for five weeks. Sodium arsenite (2.5 mg/kg body weight) was fed at the beginning of the third week while feeding with the dietary supplements continued throughout the period of the experiment. Control rabbits were given distilled water.

The body weights of the animals were monitored while they were bled once a week for eight weeks. The packed cell volume (PCV) of the blood was determined and gamma glutamyltransferase (γ-GT) activity was assayed for in the serum. Serum albumin/globulin ratio (A:G) was also estimated. In general, the body weights of the animals did not show any appreciable difference during feeding of Ho, Ga, Bi and intoxication with sodium arsenite. Similar observations were made for the PCV of all the animals in the various groups. Conversely, serum γ-GT activity was elevated by about 51% in arsenite intoxicated rabbits. This activity was markedly reduced by the consumption of the dietary supplements before and after the intoxication. Although the toxic effects of arsenite decreased with time, the extent of disappearance of this toxic effect was more prominent in the second and third weeks following administration of arsenite. Whereas serum A:G ratio was appreciably decreased in rabbits intoxicated with arsenite, the consumption of the dietary supplements also caused a gradual reversal in this parameter till the eighth week of the experiment. The effect of the supplements is in the order Ho > Ga > Bi. Arising from these observations, it seems possible that dietary supplements may have long-term protective effects during and after exposure to sodium arsenite.

Key Words: Sodium arsenite; Arsenite toxicity; Honey; Garlic; Bitter kola.

Introduction

Arsenicals are well known for their beneficial and toxicological properties (1,2). For instance, of the organic arsenicals, the trivalent sodium arsenite has been shown to be the most cytotoxic and widespread of the group (3). Consequently, this toxicant has been used as a model arsenical for toxicity studies in animals. Several studies have shown that extensive exposure of humans to arsenic (As) results from mining, coal burning and the use of arsenicals in manufacturing industries, agriculture and in chemotherapy (4 – 6). More importantly, continuous exposure through long-term ingestion of drinking water containing high

concentration of As from wells has been widely reported (7, 8). Such exposure in areas such as West Bengal, India and Bangladesh (9) has been associated with the etiology of cancers of the bladder (10), lung and skin (11), diabetes (12), hypertension (13) and the Black-foot disease (14).

In order to check the incidence of diseases arising from ingestion of environmental contaminants, awareness has been created for the potential use of dietary inhibitors of mutagenesis and carcinogenesis in the prevention of human cancer (15). In this regard, dietary intervention has been shown to be effective in reducing the cytotoxic effects arising from the consumption of As contaminated water by humans. For instance, regular dietary administration of crude extracts of *Emblica officinalis* fruits (Indian goose berry) (16) or garlic bulbs (*Allium sativum* L.) to mice significantly reduced the cytotoxic and chromosome damaging effects of sodium arsenite in bone marrow cells (17). The list of dietary supplements (such as garlic) which are being used in traditional medicine is inexhaustible (18). Two of such supplements, bitter kola (*Garcinia kola* seeds) and honey are of interest in the present investigation. Honey is known for its decongesting, antiseptic and regenerating properties in wound dressing (19). Extracts of bitter kola have been used for the treatment of laryngitis and diverse liver diseases (20) and in the prevention of carbon tetrachloride-, amantia toxin- and thioacetamide-induced hepatoxicity in laboratory animals (22). An earlier investigation in our laboratory has shown that dietary supplementation with honey and aqueous extract of bitter kola offered some degree of protection against 2-acetylaminofluorene (2-AAF)-induced clastogenicity in mice *in vivo* (23).

The objective of the present study is to give a comparative assessment of the effectiveness of dietary supplementation with honey as well as aqueous extracts of bitter kola and garlic against the toxic effects of sodium arsenite in rabbits.

Materials and Methods

Chemicals and Reagents

Sodium arsenite ($NaAsO_2$) was obtained from Sigma Chemical Co., St. Louis Mo. The salt was dissolved in distilled water and the concentration was made equivalent to $1/10^{th}$ of the oral LD50, i.e. 2.5 mg sodium arsenite per kg body weight. This concentration has been shown to be highly toxic (24). Gamma glutamyltransferase diagnostic kit was obtained from Biolab SA, Fisme, France, while albumin and total protein kits were products of Randox Laboratories Ltd., Crumblin, N. Ireland. All other reagents and chemicals were of analytical grade and were obtained from Sigma Chemical Co., St. Louis, Mo, USA.

Experimental Animals

Three and a half month old male New Zealand rabbits of average weight of approximately 1.6 ± 0.15 kg were used. The rabbits were housed in the Experimental Animal House of the Department of Biochemistry, University of Ibadan, Nigeria. They were fed with rabbit pellets obtained from Ladokun Feeds Limited, Ibadan, Nigeria. The pellets contained at least 20% protein, 3.5% fat, 9.0% fibre, 1.2% calcium, 0.7% phosphorus, vitamin mix, mineral mix, antioxidant, antibiotics, carbohydrates, etc. The rabbits were also fed water *ad libitum*. The room temperature was 29 ± 2^{o}C with twelve hours light/dark cycle.

Dietary Supplementation

Garlic bulbs (*Allium sativum* L., single clove variety) and bitter kola (*Garcinia kola* seed) were purchased from Bodija Market, Ibadan, Nigeria. They were certified at the herbarium in the Department of Botany and Microbiology, University of Ibadan, Nigeria. Pure honey was purchased from the School of Forestry, Eleyele, Ibadan, Nigeria. The aqueous extract of garlic (Ga) was prepared from freshly sliced cloves that had been ground into paste and made up to 2.2% w/v stock suspension. A dose of 100 mg/kg body weight was fed to the rabbits based on a daily human intake of 60g garlic by a 60 kg individual. This dose also corresponds to the highest concentration of garlic extract that had been used beneficially against certain ailments (25). Bitter kola extract (Bi) was also prepared from freshly peeled seeds which were

blended with an electric blender and oven dried at 40°C. The preparation and administration were as described for garlic extract. Honey (Ho) was fed as purchased at 100 mg/kg body weigh of rabbits.

Experimental Protocol

The rabbits were distributed randomly into ten groups of three animals each. The rabbits in group 1 served as the negative control and were fed distilled water for the five weeks duration of the study. Those in group 2 were fed distilled water throughout the second, third, fourth, fifth and sixth week. Sodium arsenite (2.5 mg/kg) was fed once during the third week of the study. Animals in groups 3, 5 and 7 were separately fed with Ga, Ho and Bi throughout the second, third, fourth and sixth week respectively. Those in groups 4, 6 and 8 were treated in a similar manner to those in groups 3, 5 and 7, except that they were fed with sodium arsenite (2.5 mg/kg body weight) once during the third week. The rabbits in group 9 were fed simultaneously with Ga and Ho while those in group 10 were fed with Ga and Bi throughout the second, third, fourth, fifth and sixth weeks respectively. The animals in the two groups (i.e. groups 9 and 10) were also fed with sodium arsenite (2.5 mg/kg body weight) in the third week. All experimental animals had access to pellets and water *ad libitum* throughout the duration of the experiment. The body weights of the animals were measured weekly.

Preparation of Serum

The animals were bled via the external marginal ear vein once every week, starting from the first week to the eighth week. Blood samples were collected in two parts every week. One part was collected into anticoagulant tubes containing ethylene diamine tetraacetic acid (EDTA) for packed cell volume (PCV) determination and the other part into plain tubes fro serum γ-GT, total protein and albumin determinations. Serum was prepared by allowing the freshly drawn blood samples to stand at room temperature for 2 hours for clot formation and centrifugation at 3,500g for 10 minutes in a Beckman CS-15 centrifuge. The clear supernatant was immediately used for the estimation of γ-GT activity, total protein and albumin determinations.

Packed Cell Volume Determination

The Packed Cell Volume (PCV) was determined by the microhaematocrit method of Dacie and Lewis (26). Blood samples collected into EDTA treated bottles were mixed gently and then transferred into plain capillary tubes. The bases of the tubes were carefully sealed by heating with lighted Bunsen burner. The capillary tubes were then arranged in the space provided inside the microhaematocrit centrifuge and were centrifuged at 12,000g for 10 minutes. The PCV values (in percentage) were read using the haematocrit reader.

Serum Total Protein and Albumin

The serum total protein was determined by the Biuret method (27) with the aid of the total protein diagnostic kit. Serum albumin was also estimated using the albumin diagnostic kit (28). Serum globulin concentration was estimated on a weekly basis by calculating the difference between total protein and albumin. The albumin/globulin (A:G) ratio was also calculated.

Gamma Glutamyl Transferase Assay

Gamma-glutamyltransferase (γ-GT) was assayed by the method fo Szasz (29). The γ-GT diagnostic reagent kit was reconstituted by mixing the buffer (glycylglycine/Tris) with the substrate (L-glutamyl-p-nitro-anilide). The reconstituted enzyme is stable for 7 days at 2 – 8°C or for one day at 24 – 25°C. One ml of the reconstituted reagent was mixed at 30°C with 50µl of the serum sample and the mixture was incubated at 30°C. The initial absorbance was read after 30 seconds at 405 nm and subsequently at 1 min interval for 3 min. γ-GT activity was then calculated and expressed in International Units (IU) per litre as described by Szasz (29). The data were statistically analysed using Student's t-test.

Results

The effects of honey (Ho) and aqueous extracts of garlic (Ga) and bitter kola (Bi) on the body weights of rabbits fed with sodium arsenite are presented in Table 1. There is a gradual increase in body weight of the rabbits fed with distilled water only during the period of investigation. The observed increase is between 13% in the second week and 44% in the eighth week. The rabbits in group 5 fed Ho alone (second to sixth week) showed the same gradual increase in weight ranging from 13% in the second week to 50% in the eighth week. Although the animals in Group 6 fed with Ho for five weeks (second to sixth week) and sodium arsenite (third week) showed a gradual increase in their body weight. This increase (12% in the second week to 29% in the eighth week) is lower than that of Group 5 and the control animals in Group 1 for the same period.

Table 2 shows the weekly packed cell volume (PCV) of blood samples from the control and test rabbits. There is an increase of between 0.1% (second week) and 9.4% in the PCV of the control rabbits in Group 1. The PCV of the rabbits fed with Ho alone in Group 5 also increased between 0.1% (second week) and 12.5% sixth week with a slight reduction of 9.4% or an increase of 6.3% in the seventh and eighth week, respectively. It is also clear that the PCV of the rabbits Groups 2 – 4 and in Groups 7 – 10 decreased gradually from the second or third week to the sixth week, followed by a slight increase in the seventh and eighth week. The PCV of the animals fed with Ho (second to sixth week) and sodium arsenite (third week) increased (6.1%) in the third week and decreased again to the initial value before exposure to sodium arsenite (seventh to eighth week).

The effect of pre-treatment of the rabbits with Ho, Ga and Bi on sodium arsenite-induced serum γ-GT activity is presented in Table 3. From these data it is evident that administration of a single oral dose of sodium arsenite to rabbits in Group 2 in the third week significantly induced serum γ-GT activity by about 51% when compared to the serum γ-GT activity of the control animals in Group 1. Although the serum γ-GT activity of the Group 2 rabbits remained high (at least 33%) throughout the experimental period, it decreased gradually during the eighth week when about 20% increase in activity was observed. The serum γ-GT activity of Group 5 rabbits fed with Ho alone was similar to that of the control rabbits in Group 1. Pre-treatment of the rabbits from the second week to the sixth week with Ga or Bi alone (Groups 3 and 7) also resulted in a slight increase in serum γ-GT activity when compared with the controls.

Although the serum γ-GT activity observed in the rabbits pre-treated with Ga (Group 4) or Ho (Group 8) in combination with feeding with sodium arsenite in the third week was high, this activity was not as high as the activity observed in rabbits Group 2 which were treated with sodium arsenite alone. In this study, Ho (Group 6) was more effective in reducing the serum γ-GT activity, followed by Ga (Group 4) and Bi (Group 8) in rabbits intoxicated with sodium arsenite. In this regard, Ho, Ga and Bi reduced the γ-GT activity by 16%, 11% and 7% respectively when compared with animals intoxicated with sodium arsenite alone. Pre-treatment with a combination of Ga and Ho (Group 9) also reduced sodium arsenite-induced serum γ-GT activity to the level observed for Group 4 treated with Ga. Similar results were observed on pre-treatment with a combination of Ga and Bi where γ-GT activity was reduced to about the level of the protection offered by Bi alone (Group 8).

The data presented in Table 4 show the effect of orally administered Ho, Ga and Bi on sodium arsenite-induced changes in the serum albumin/globulin (A:G) ratio in the intoxicated rabbits. It is clear from this table that a single oral dose of sodium arsenite in the third week led to a decreased serum A:G ratio, compared to the control group fed distilled water only. This decrease was observed from the third to the sixth week and slight increases were observed in the seventh and eighth weeks. Similarly, the serum A:G ratio was slightly reduced in the groups of rabbits fed with the dietary supplements alone when compared with the animals in the control group. The degree of reduction is in the order Group 5 (Ho) > Group 3 (Ga) > Group 7 (Bi). Feeding of the dietary supplements between the second and sixth weeks plus the oral administration of sodium arsenite in the third week also caused a reduction in serum A:G ratio. However, this reduction was not as marked as what was observed with sodium arsenite alone. Ga, Ho and Bi could, therefore, decrease the ability of sodium arsenite to reduce the serum A:G ratio in the experimental animals. This effect is in the order Ga + Ho (Group 9) > Ho (Group 6) > Ga + Bi (Group 10) > Ga (Group 4) > Bi (Group 8).

Table 1: Effect of honey and aqueous extracts of garlic and bitter kola on the body weight of rabbits intoxicated with sodium arsenite

Group of rabbits	Treatment	Changes in Body Weight (kg)							
		1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
1	Distilled water H_2O	1.6±0.05	1.8±0.03	1.9±0.07	2.1±0.05	2.2. ±0.07	2.2±0.06	2..2±0.07	2.3±0.05
2	$NaAsO_2$ only	1.7±0.07	1.9±0.10	2.2±0.15	2.0±0.10	2.0±0.06	2.1±0.09	2.2±0.10	2.2±0.12
3	Ga only	1.6±0.03	1.7±0.05	1.7±0.09	1.8±0.05	1.7±0.03	1.7±0.07	1.8±0.03	1.9±0.05
4	Ga- $NaAsO_2$	1.6±0.06	1.8±0.11	1.7±0.05	1.7±0.11	1.6±0.10	1.6±0.05	1.7±0.06	1.8±0.10
5	Ho only	1.6±0.05	1.8±0.09	2.1±0.10	2.2±0.13	2.5±0.14	2.5±0.12	2.4±0.16	2.4±0.15
6	Ho- $NaAsO_2$	1.7±0.09	1.9±0.10	2.1±0.12	2.2±0.10	2.2±0.17	2.3±0.10	2.3±0.10	2.2±0.11
7	B_1 only	1.5±0.07	1.7±0.07	1.9±0.10	1.8±0.08	1.8±0.09	1.7±0.09	1.9±0.11	1.9±0.09
8	B_1- $NaAsO_2$	1.7±0.10	1.8±0.09	1.8±0.11	1.7±0.05	1.8±0.11	1.9±0.05	2.0±0.06	2.0±0.13
9	Ga-Ho+ $NaAsO_2$	1.5±0.05	1.7±0.10	1.8±0.09	1.8±0.07	1.7±0.08	1.7±0.10	1.8±0.05	2.0±0.10
10	Ga-B_1+ $NaAsO_2$	1.6±0.08	1.7±0.05	1.7±0.10	1.7±0.09	1.6±0.05	1.7±0.12	1.8±0.08	1.9±0.06

All animals were allowed to acclimatize for at least three weeks before the commencement of the experiment. Animals in groups 1 and 2 received distilled water only except that those in group 2 received 2.5 mg/kg sodium arsenite ($NaAsO_2$) in the third week only. Those in groups 3-10 were fed extract only as indicated above while the animals in groups 4,6,8,9&10 received 2.5 mg/kg ($NaAsO_2$) in the 3rd week only. Each value is a mean of at least ten determinations ± standard error. ($P<0.05$).

Table 2: Effect of honey and aqueous extracts of garlic and bitter kola on the packed cell volume of the rabbits intoxicated with sodium arsenite

Group of rabbits	Treatment	Changes in PCV							
		1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
1	Distilled water H_2O	32±0.50	32±0.45	33±0.33	34±0.25	33±0.51	35±0.46	34±0.35	35±0.55
2	$NaAsO_2$ only	33±0.10	33±0.25	31±0.42	28±0.35	26±0.20	29±0.35	30±0.20	32±0.41
3	Ga only	31±0.91	32±0.50	31±0.52	30±0.18	28±0.45	29±0.15	31±0.45	31±0.35
4	Ga+ $NaAsO_2$	31±0.80	31±0.23	29±0.27	27±0.40	28±0.31	29±0.25	29±0.63	30±0.20
5	Ho only	32±0.50	33±0.65	34±0.32	35±0.50	35±0.10	36±0.35	35±0.35	34±0.50
6	Ho+ $NaAsO_2$	33±0.30	33±0.18	35±0.40	33±0.25	32±0.50	33±0.41	34±0.15	34±0.40
7	B_1 only	32±0.50	32±0.43	30±0.70	29±0.55	27±0.25	29±0.55	29±0.25	31±0.30
8	B_1+ $NaAsO_2$	33±0.10	33±0.30	30±0.20	28±0.41	27±0.30	28±0.60	29±0.50	30±0.25
9	Ga+Ho+ $NaAsO_2$	32±0.42	33±0.51	32±0.30	31±0.32	30±0.31	30±0.28	30±0.20	31±0.40
10	Ga+B_1+ $NaAsO_2$	33±0.52	32±0.71	31±0.12	30±0.16	30±0.26	29±0.30	30±0.30	30±0.10

All animals were allowed to acclimatize for at least three weeks before the commencement of the experiment. Animals in groups 1 and 2 received distilled water only except that those in group 2 received 2.5 mg/kg sodium arsenite ($NaAsO_2$) in the third week only. Those in groups 3-10 were fed extract only as indicated above while the animals in groups 4,6,8,9&10 received 2.5 mg/kg ($NaAsO_2$) in the 3rd week only. Each value is a mean of at least ten determinations ± standard error. ($P<0.05$).

Table 3: Effect of honey and aqueous extracts of garlic and bitter kola on sodium arsenite-induced serum gamma glutamyl transferase (γGT) activity in rabbits.

Group of rabbits	Treatment	Changes in serum γGT activity							
		1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
1	Distilled water H_2O	9.1±0.50	8.9±0.40	9.1±0.35	9.0±0.25	9.0±0.40	9.1±0.30	9.0±0.50	9.1±0.55
2	$NaAsO_2$ only	9.0±0.25	9.1±0.32	13.6±0.65	13.0±0.48	12.4±0.26	12.0±0.38	11.1±0.51	10.8±0.60
3	Ga only	9.2±0.3	9.7±0.15	10.2±0.25	10.5±0.2	10.6±0.3	10.7±0.15	10.3±0.15	10.2±0.25
4	Ga+ $NaAsO_2$	8.8±0.6	9.4±0.5	12.1±0.48	11.7±0.11	11.3±0.50	11.4±0.25	10.6±0.32	10.3±0.45
5	Ho only	9.0±0.2	8.9±0.4	9.2±0.10	9.0±0.32	9.1±0.40	9.0±0.35	9.0±0.20	9.0±0.30
6	Ho+ $NaAsO_2$	9.1±0.32	9.0±0.2	11.4±0.32	10.9±0.26	10.4±0.12	9.8±0.40	9.4±0.25	9.2±0.50
7	B_1 only	8.9±0.4	9.5±0.3	9.9±0.50	10.1±0.40	10.2±0.25	10.3±0.50	9.8±0.50	9.6±0.15
8	B_1+ $NaAsO_2$	8.9±0.5	9.7±0.25	12.6±0.33	12.5±0.55	12.0±0.31	11.8±0.65	11.2±0.45	10.7±0.28
9	Ga+Ho+ $NaAsO_2$	9.0±0.25	9.4±0.3	11.8±0.12	11.5±0.70	11.3±0.45	11.2±0.26	11.0±0.31	10.8±0.33
10	Ga+B_1+ $NaAsO_2$	8.9±0.	9.8±0.45	12.3±0.35	11.9±0.45	11.8±0.52	11.5±0.18	11.3±0.50	10.7±0.25

All animals were allowed to acclimatize for at least three weeks before the commencement of the experiment. Animals in groups 1 and 2 received distilled water only except that those in group 2 received 2.5 mg/kg sodium arsenite ($NaAsO_2$) in the third week only. Those in groups 3-10 were fed extract only as indicated above while the animals in groups 4,6,8,9&10 received 2.5 mg/kg ($NaAsO_2$) in the 3rd week only. Each value is a mean of at least ten determinations ± standard error. ($P<0.05$).

Table 4: Effect of honey and aqueous extracts of garlic and bitter kola on sodium arsenite induced changes in serum albumin: globulin (A:G) ratio in rabbits.

Group of rabbits	Treatment	Changes in serum albumin : globulin ratio							
		1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
1	Distilled water H_2O	2.3±0.06	2.2±0.05	2.2±0.07	2.3±0.05	2.1±0.01	2.0±0.03	2.1±0.01	2.0±0.05
2	$NaAsO_2$ only	2.1±0.05	1.9±0.02	1.2±0.07	1.4±0.02	1.5±0.02	1.7±0.02	1.8±0.03	1.9±0.02
3	Ga only	2.3±0.01	2.2±0.06	2.0±0.05	1.9±0.03	1.7±0.03	1.7±0.01	1.8±0.01	1.8±0.03
4	Ga+ $NaAsO_2$	2.2±0.03	2.2±0.05	1.5±0.03	1.6±0.02	1.6±0.05	1.7±0.02	1.9±0.02	2.0±0.02
5	Ho only	2.3±0.07	2.3±0.03	2.2±0.03	2.0±0.04	2.0±0.02	2.1±0.04	2.0±0.05	2.1±0.04
6	Ho+ $NaAsO_2$	2.0±0.01	2.0±0.01	1.6±0.04	1.7±0.03	1.7±0.03	1.8±0.02	1.8±0.03	1.9±0.04
7	B_1 only	2.3±0.04	2.1±0.02	1.9±0.02	1.8±0.01	1.9±0.02	1.7±0.04	1.8±0.01	1.8±0.03
8	B_1+ $NaAsO_2$	2.1±0.02	1.9±0.05	1.5±0.05	1.6±0.04	1.7±0.05	1.8±0.05	1.9±0.04	2.0±0.01
9	Ga+Ho+ $NaAsO_2$	2.0±0.01	1.9±0.03	1.7±0.03	1.7±0.02	1.8±0.03	1.8±0.03	1.9±0.01	1.9±0.02
10	Ga+B_1+ $NaAsO_2$	2.1±0.05	2.0±0.04	1.6±0.04	1.7±0.01	1.8±0.02	1.8±0.05	1.9±0.02	1.9±0.03

All animals were allowed to acclimatize for at least three weeks before the commencement of the experiment. Animals in groups 1 and 2 received distilled water only except that those in group 2 received 2.5 mg/kg sodium arsenite ($NaAsO_2$) in the third week only. Those in groups 3-10 were fed extract only as indicated above while the animals in groups 4,6,8,9&10 received 2.5 mg/kg ($NaAsO_2$) in the 3rd week only. Each value is a mean of at least ten determinations ± standard error. ($P<0.05$).

Discussion

Despite intense research efforts and dietary intervention attempts, mortality resulting from arsenic poisoning in exposed individuals is still a cause for concern. The influence of dietary intervention with honey (Ho) and extracts of garlic (Ga) and bitter kola (Bi) in sodium arsenite-intoxicated rabbits, as observed in this study, show that sodium arsenite (2.5 mg/kg body weight; $1/10^{th}$ of the LD_{50}) markedly induced serum γ-GT activity (Table 3), compared with the negative control group (Group 1), following a single exposure in the third week of the experiment. It has been established that elevated serum γ-GT activity is an indication of liver damage resulting in an increase in the rate of synthesis of γ-GT and leakage of the enzyme into the serum from the liver (30, 31). Several studies have also shown that liver diseases are accompanied by a decrease in the serum albumin and an increase in total globulins and consequently a decrease in the A:G ratio (32). In the present study, the tested single dosage of sodium arsenite also decreases the serum A:G ratio (Table 4) when compared with the control (Group 1). Taken together, these results suggest sodium arsenite-induced liver damage in the rabbits. The results also confirm earlier reports on the toxicity of sodium arsenite in various organisms (23, 24, 33). Although various mechanisms have been proposed for arsenite-induced toxicity, it has been suggested that the strong cytotoxicity of arsenite might be mediated via the production of active oxygen species and protease activation (e.g. superoxide dismutase, catalase and glutathione) or by a peptide inhibitor of interleukin-1 beta-converting enzyme (34). It seems likely, therefore, that toxic effects of sodium arsenite may be due to arsenite-induced formation of reactive oxygen species in cells. This is because reactive oxygen species have been shown to be involved in arsenite-induced DNA damage (35), cell proliferation (36) and apoptosis (37).

The present study has shown that administration of the dietary supplements (Ho, Ga and Bi), alone for five weeks or in combination with a single oral dose of sodium arsenite in the third week, did not significantly affect the body weight of the experimental animals (Table 1). However, the degree of increase in body weight of the Group 5 animals treated with Ho alone, as compared with the remaining nine groups, may be due to the carbohydrate content of honey (38). The packed cell volume (PCV) is a measure of the relative mass of erythrocytes and is used in the diagnosis of anaemia. Anaemia is induced by a reduction in the production of erythrocytes as a result of defective or decreased erythropoiesis and/or haemorrhage. From the data presented in Table 2, it is clear that the PCV of all the groups of animals (Groups 2 – 4 and 6 – 10) with the exception of those in the negative control (Group 1) and those treated with Ho alone (Group 5) decreased in the five weeks of treatment. From the data presented here, this decrease was not marked as compared with the negative control Group 1, suggesting that under the present experimental conditions, the test substances may not necessarily induce anaemia, even in sodium arsenite treated rabbits.

The results of the effects of the oral administration of Ho, Ga and Bi on sodium arsenite-induced serum γ-GT activity and the decreased A:G ratio, as presented in Tables 3 and 4, show that the serum γ-GT activity and A:G ratio of the rabbits treated with Ho alone (Group 5) are similar to what was observed in the serum of negative control rabbits which received distilled water only. Although Ga or Bi, when administered alone (Groups 3 and 7), induced a certain degree of serum γ-GT activity when compared with the control (Group 1), the two dietary supplements also slightly decreased the A:G ratio. It is interesting that these dietary supplements have been shown in previous studies to have mild clastogenic and hepatotoxic effects in mice at a dosage of 100 mg/kg body weight (28, 29). Conversely, sodium arsenite-induced serum γ-GT activity was markedly reduced in the presence of Ho, Ga and Bi while there was a reversal of the effect on A:G ratio which was subsequently elevated during the course of the experiment (i.e. weeks 3 – 6; Table 4). Although the toxic effect of arsenite decreased with time, the extent of disappearance of this effect was more prominent in the fourth and fifth weeks of administering the dietary supplements. The degree of the effects of the supplements is in the order Ho > Ga > Bi. These effects may be attributed to the antioxidant and free radical scavenging properties of Ho, Ga (39) and Bi (40). Based on the fact that the cytotoxicity of arsenite might be mediated via the generation of active oxygen species and protease activation (34), it seems reasonable to propose that any compound exhibiting antioxidant properties such as Ga and Bi could be useful in alleviating arsenite-induced toxicities. It was observed here that Ga and Ho, when administered simultaneously before and after sodium arsenite intoxication (Group 9) reduced sodium arsenite-induced serum γ-GT activity and reversed the decreased A:G ratio to about the same level observed in animals that received Ga only (Group 3). This shows that Ho by itself has some inhibitory properties over both sodium arsenite and Ga-induced toxic effects.

It may, therefore, be concluded that dietary supplementation with Ho and Bi may, to a large extent, modify arsenite-induced toxicity as demonstrated for orally administered Ga, extracts of *Emblica officinalis* (16), mustard oil (41) and sodium selenite (42).

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References

1. Uthus EO. Arsenic essentiality and factors affecting its importance. In: Chappell WR, Abernathy CO, Cothern CR. Eds. Arsenic exposure. Science and Technology Letters 1994; 99: 199 – 208.
2. Hei TK, Liu SX, Waldren C. Mutagenicity of arsenic in mammalian cells: Role of reactive oxygen species. Proc. Nalt. Acad. Sci. (USA) 1998; 95: 8103 – 8107.
3. Abdulla FI, Rivera-Luna R, Garcia-Carranca A, Ayala-Fierro F, Espinosa-Aguirre JJ. Cytotoxic effect of three arsenic compounds in HeLa human tumour and bacteria cells. Mutat. Res. 2001; 493: 31 – 38.
4. Abernathy CO, Liu YP, Longfellow D *et al*. Arsenic: health effects, mechanisms of action and research issues. Environ. Health Persp. 1999; 107: 593 – 597.
5. Zhu J, Koken MH, Quignon F. *et al*. The arsenic-induced PML targeting onto nuclear bodies: Implications for the treatment of acute promyelocytic leukaemia. Proc. Natl. Acad. Sci. (USA) 1997; 98)8): 3979 – 3983.
6. Li YM, Broome JD. Arsenic targets tubulins to induce apoptosis in myeloid leukaemia cells. Cancer Res. 1999; 59(4): 776 – 780.
7. Xiao JG 96% well water is undrinkable. Asia Arsenic Network Newsletter. 1997; 2: 7 – 9.
8. National Research Council. Arsenic in drinking water. Washington DC. National Academy Press. 1999.
9. Chowdhury UK, Biswas BK, Chowdhury TR *et al*. Ground water arsenic contamination in Bangladesh and West Bengal, India. Environ. Health Persp. 2000; 108(5): 393 – 397.
10. Chow NH, Guo YL, Lin JS. *et al.* Clinicopathological features of bladder cancer associated with chronic exposure to arsenite. Br. J. Cancer. 1997; 75: 1708 – 1710.
11. Chen CJ, Chen CW, Wu MM, Kuo TL. Cancer potential in liver, lung, bladder and kidney due to ingested arsenic in drinking water. Br. J. Cancer 1992; 66: 888 – 892.
12. Lai MS, Hsueh YM, Chen CJ *et al*. Ingested inorganic arsenic and prevalence of diabetes mellitus. Am. J. Epidemiol. 1994; 139: 484 – 492.
13. Chen CJ; Hsueh YM, Lai MS *et al*. Increased prevalence of hypertension and long-term arsenic exposure. Hypertension 1995; 25: 53 – 60.
14. Tseng WP. Blackfoot disease in Taiwan – A 30-year follow-up study. Angiology 1989; 40: 547 – 558.
15. Hayatsu H, Arimoto S, Negishi T. Dietary inhibitors of mutagenesis and carcinogenesis. Mutat. Res. 1998; 202: 429 – 446.
16. Biswas S, Talukder G, Sharma A. Protection against cytotoxic effects of arsenic by dietary supplementation with crude extract of *Emblica officinalis.* Phytotherapy Research 1996; 13: 513 – 516.
17. Roychoudhury A, Das T, Sharma A, Taluker G. Dietary garlic extract in modifying clastogenic effects of inorganic arsenic in mice-two generation studies. Mutat. Res. 1996; 359: 165 – 170.
18. Kirtikar KR, Bau BD. In: Singh B, Singh MP eds. Indian Medicinal Plants 4. 2nd Edition. Dehra Dun, India. 1975.
19. Wilson RB, Crane E. Uses of honey. In: Honey: A comparative survey. Heinemann, London, 1975, pp. 378 – 391.
20. Iwu MM. Traditional Igbo Medicine. Institute of African Studies, University of Nigeria, Nsukka. 1982, 104pp.
21. Iwu MM, Igboko OA, Onwuchekwa U, Okunji CO. Evaluation of the antihepatotoxicity of the biflavonoids of *Garcinia kola* seeds. J. Ethnopharmacol. 1987; 21: 127 – 142.
22. Iwu MM, Igboko OA, Elekwa OK, Tempesta MS. Prevention of thioacetamide-induced hepatotoxicity by biflavonones of *Garcinia kola.* Phytotherapy Res. 1990; 4: 157 – 159.
23. Odunola OA, Adetutu A, Olorunnisola OS, Ola-Davis OE. Protection against 2-acetyl aminofluorene-induced toxicity in mice by garlic (*Allium sativum*), bitter kola (*Garcinia kola* seed) and honey. Afr. J. Med. Med. Sci. 2004; (in press)
24. Das T, Roychoudhury A, Sharma A, Talukder G. Modification of cytotoxic effects of inorganic arsenic by crude extracts of *Allium sativum* in mice. Int. J. Pharmacol. 1993; 31: 316 – 320.
25. Mansell P, Reckless JPD. Garlic effects on serum lipids, blood pressure, coagulation, platelet segregation and vasodilation. Brit. Med. J. 1991; 303: 379.
26. Dacie JU, Lewis SM. Practical Haematology. Sixth Edition. Edinburgh. Churchill Livingstone. 1984.
27. Weichselbaum TE. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am. J. Clin. Pathol. Tech. Bull. 1946; 10: 40.

28. Dumas BI, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chem. Acta 1971; 31; 87.
29. Szasz GA. A kinetic photometric method for serum glutamyl transpeptidase. Clin. Chem. 1976; 22: 2051 – 2055.
30. Keane PM, Garcia L, Gupta RN, Walker WHC. Serum gamma glutamyl transferase in liver disorders. Clin. Biochem. 1973; 6: 41 – 45.
31. Ivanor E, Kruster L, Adjarov D. *et al*. Studies on the mechanism of the changes in serum and liver gamma glutamyl peptidase activity II: Experimental hexachlorobenzene porphyria in rabbits. Enzyme 1976; 21: 8 – 20.
32. Grant GH, Kachman JF. The proteins of body fluids. In: Tietz N.ed. Fundamentals of Clinical Chemistry. W. B. Saunders, Philadelphia, 1976, pp. 298 – 400.
33. Ochi T. Arsenic compounds induced increases in glutathione levels in cultured Chinese hamster V79 cells and mechanisms associated with changes in gamma-glutamyl cysteine synthetase activity, cystine uptake and utilization of cycteine. Arch. Toxicol. 1997; 71: 730 – 740.
34. Sakurai T, Kaise T, Matsubara C. Inorganic and methylated arsenic compounds induce cell death in murine macrophages via different mechanisms. Chem. Res. Toxicol. 1998; 11: 273 – 283.
35. Wang TS, Hsu TY, Chung CH, Wang ASS, Bau DT, Jan KY. Arsenite induces oxidative DNA adducts and DNA-protein cross-links in mammalian cells. Free Rad. Biol. & Med. 2001; 31(3): 321 – 330.
36. Barchowsky A, Roussel RR, Klei LR *et al*. Low levels of arsenic trioxide stimulate proliferative signals in primary vascular cells without activating stress effector pathways. Toxicol. Appl. Pharmacol. 1999, 59: 65 – 75.
37. Wang TS, Kuo CF, Jan KY, Huang H. Arsenite induces apoptosis in Chinese hamster ovary cells by generation of reactive oxygen species. J. Cell. Physiol. 1996, 169: 256 – 268.
38. White JW (Jr.). Composition of honey. In: Crane E, ed. Honey: A comprehensive Survey. Heinemann, London. Pp. 157 – 206.
39. Reitz B, Belagyi J, Torok B, Jacob R. The radical scavenging ability of garlic examined in various models. Bull. Chim. Farm (Italy). 1995; 134: 69 – 76.
40. Farombi EO, Tahnteng JG, Agboola AO, Nwankwo JO, Emerole GO. Chemoprevention of 2-acetyl aminofluorene induced hepatotoxicity and lipid peroxidation in rats by kolaviron, a *Garcinia kola* seed extract. Food Chem. Toxicol. 2000; 38: 535 – 541.
41. Choudhury AR, Das T, Sharma A. Mustard oil and garlic extract as inhibitors of sodium arsenite-induced chromosomal breaks *in vivo*. Cancer Letts. 1997; 121: 45 – 52.
42. Biswas S, Talukder G, Sharma A. Prevention of cytotoxic effects of arsenic by short-term dietary supplementation with selenium in mice *in vivo*. Mutat. Res. 1999; 441: 155 – 160.