Toxicity and Mutagenicity Testing of Selected Herbal Drugs

Gloria D. Reyes and Asuncion K. Raymundo
National Institutes of Biotechnology and
Applied Microbiology (BIOTECH) and
Institute of Biological Sciences (IBS)
University of the Philippines Los Baños
College, Laguna,

ABSTRACT

Three of the 10 herbal drugs tested showed toxicity based on chicken embryo assay of water and oil extracts. These herbal drugs (golden seal root, black walnut and herbal tea) taken to improve memory, exhibited fatal effects on the chicken embryo four days past inoculation. This finding does not support the superfluous therapeutic claims that go with these drugs.

Mutagenicity test using the Ames-Salmonella bacterial test system employing rat liver homogenate (S9) as mammalian metabolic activator revealed that eight of the herbal drugs were not mutagenic. The possibility, however, of mutagenicity with higher doses cannot be ruled out. The mutagenicity potential of golden seal root and black walnut was not revealed at 20% water extract concentration used due to the direct toxic effect on the tester strain. Such determination was shown to be possible at 2% water extract concentration for black walnut. Nevertheless, this shows the validity of the method when used in conjunction with S9 which could be prepared locally from Sprague-Dawley rats. This further confirms the necessity of using S9 in assessing mutagenicity potential.

INTRODUCTION

Herbal drugs are processed dosage forms usually from plants used traditionally in the treatment of diseases. These products have active ingredients that may be acting synergistically or antagonistically in producing the activity necessary in the treatment of diseases (**8**). In preparing drugs from plants, primary consideration is no doubt given to effectiveness in treating or curing health problems. However, as with any pharmaceutical production , evaluation of toxicity and hazards should always be linked to the efficacy that enables consumers to weigh the benefits versus risks. Although these constitute the standard operating procedure in developing a plant into a drug, such information is not available despite the fact that herbal medicine is now gaining wide acceptance.

Toxicological testing aims to establish the safety of a drug sample (i.e., lack of toxic and mutagenic components). Chemical contaminants may find their way into the drug preparations through contamination of raw materials during processing or throught infection by a variety of pests. When taken in small doses over a period of time with the drug, they may become toxic (**15**). It is also of interest to note that toxic compounds can be formed during processing. Residues from compounds used in growing the plants, such as pesticides and fertilizers, could also be considered as contaminants. Fungal contamination may also produce toxic effects due to production of mycotoxin. Moreover, plants have been known to contain specific substances that are considered toxic or may be mutagenic (**7**). In fact, studies have already shown that plant extracts contain inactive substances which, when metabolized by specific enzymes, are activated and converted into mutagens. There has also been considerable evidence that most mutagens are carcinogens and vice versa. Hence, the products proven to be effective as drugs for certain illness may still pose potential hazards.

In the face of growing concern for safety and lack of safety information on herbal drugs now locally being sold in the local market, it would be of interest to evaluate these products by conducting toxicity and mutagenicity tests. Hence, this study was conducted at the Antibiotics Laboratory of the National Institutes of Biotechnology and Applied Microbiology (BIOTECH), UPLB, College, Laguna from October 1989 to July 1990.

MATERIALS AND METHODS

Herbal Drug Samples

Herbal drugs for internal use in humans were evaluated in this study (Figs. 1-3). They were selected and obtained from the different drug outlets (Table 1). Prescribed uses and actual costs were also indicated. For each sample, approximately 100 g was placed inside a plastic bag, sealed and stored at 4°C until tested.

Toxicity Test

The chicken embryo assay method (**5, 9**) was used to determine the presence or absence of toxicity in the drug samples. Both the water and oil extracts were tested. A 20% water extract was prepared by homogenizing the mixture of drug and distilled water in Waring blendor and collecting the aqueous extract by filtration and centrifugation at 14,000 rpm at 4°C for 15 minutes. The oil extract was prepared according to standard procedure (**5**) using soya oil.

The experiment was conducted at the Bureau of Animal Industry (BAI) Poultry Station in Alabang, Muntinlupa, Metro Manila. One- day-old fertile eggs were incubated at 37-38°C at 65% relative humidity for five days. After incubation, the eggs were candled to select only those with live embryo and to outline the location of the air sac. After surface sterilization of selected eggs with 95% ethanol, they were aseptically and slowly bored with a flamed-sterilized dissecting needle within the air sac area. The test material was aseptically injected into each egg through the hole using 1.0 ml tubercullin syringe. The hole was immediately sealed with sterile melted paraffin. Immediately, the eggs were incubated at 37-38°C (65% RH) for four days. After incubation, the inoculated eggs were candled and examined for viability of embryo. The number of surviving embryo was determined for each treatment. After 21 days incubation, percent hatchability was also determined. The Chi square test of goodness of fit was done to determine if the number of surviving embryo was equally distributed among the treatments. Differences between treatments were assessed using the Z-test for proportions against the positive control. Two trials were conducted and the results were combined as average of the two after analysis of the repeated experiment showed no significant differences between the two trials.

Mutagenicity Test

The Ames-Salmonella test (**16**) was used as a measure of the mutagenicity of the herbal drug samples. This was conducted both in the absence and presence of a mammalian metabolic activation system using rat liver homogenate (S9).

Bacterial strain and media used. *Salmonella typhimurium* TA98 BIOTECH 1326 was used and was maintained on a plate of Minimal Glucose Agar (MGA) containing histidine, biotin and ampicillin. For the assay, the organism was inoculated into L-broth and was shaken overnight until highly turbid growth occurred.

The following media and reagents were prepared before the experiment: 0.5mM histidine and biotin solutions, top agar (0.6% agar) and Minimal Glucose Agar which served as the base agar. S9 Mix was prepared just prior to use and was maintained on ice. The S9 Mix is a NADPH-generating system produced by the addition of NADP and glucose 6-phosphate to the S9 fraction (**2**). This was done to simulate NADPH-dependent mammalian metabolism.

Preparation of rat liver homogenate (S9). Two Sprague-Dawley male rats weighing approximately 200 g each were used. Five days before sacrifice, the rats were induced to activate microsomal system by 0.1% sodium phenobarbital given through their drinking water. The rats were fed ad libitum until the 5th day. On the 5th day of induction, they were killed by cervical dislocation.

To remove the liver from a rat (for liver homogenates preparation), all procedures were carried out under aseptic conditions using sterile glassware and surgical tools. The animal was placed on its back on a styrofoam board, its feet secured with pins, its abdominal fur removed with scissors and its skin thoroughly swabbed with 95% ethanol. A cut was made through the skin using a sterile scalpel. The skin flaps were folded back and pinned onto the board. The muscle layer was cut through carefully with a fresh pair of sterile scissors and the liver was excised.

To prepare the liver S9 fraction, the procedure of Garner et al. (**12**) was followed. All steps were carried out at -4°C using cold, sterile solutions and glasswares. The freshly excised livers were placed in a preweighed beaker containing approxi-

mately 1 ml of chilled 0.15M KCl/g of wet liver. The livers were washed in fresh chilled KCl to ensure a sterile preparation and to remove hemoglobin which could be inhibitory to the activity of Cytochrome P-450 enzymes. The washed livers were transferred to a beaker with 3 ml 0.15M KCl/g wet liver, minced with sterile scissors and homogenized in a Waring blendor. The homogenate was centrifuged for 10 min at 9000 xg and the supernatant was decanted and saved. The preparation was distributed in 1 ml portions in Eppendori tubes, frozen quickly on a bed of crushed ice and stored immediately at -70°C. The sterility of the preparation was determined prior to use by a small test streak on a nutrient agar plate.

Plate incorporation test. Water extracts prepared as previously described were used as test material. The experiment employed a completely randomized design (CRD) with 14 treatments (10 drug samples + 4 controls) and 3 replicates/treatment. The experimental unit was an MGA plate. The four controls were for sterility (no organism added), for spontaneous reversion (solvent added in place of test material), for positive response (a standard mutagen added in place of test material) and for positive response using a locally available medicinal plant previously reported as mutagenic.

The test was done by combining 0.1 ml of the bacterial tester strain, 0.1 ml of the test material and 0.5 ml of the phosphate buffer in a top agar. This mixture was poured on to an MGA plate. To treatments requiring metabolic activation, 0.5 ml of the S9 Mix was added in place of the phosphate buffer before pouring the top agar. The poured plate was quickly tilted, rotated and allowed to harden on a level surface to achieve uniform distribution of the top agar. Within an hour, the plates were inverted and placed in a 37°C incubator for 48 h. Following incubation, revertant colonies against a background lawn were counted and recorded. A sample was considered mutagenic if the average number of revertants/plate was greater than two times the spontaneous level (**13, 22**). Colonies appearing on a plate without background lawn were not revertants and were not counted. Two trials were conducted to determine replicability of results. Results reported are the averages of the two trials.

RESULTS AND DISCUSSION

Toxicity Potentials of the Herbal Drug Samples

Effects of Water Extract on the Chicken Embryo

The possible toxic effects of water extracts were revealed by viability and hatchability of chicken embryo (Table 2). Tests for significance of differences in percentage of surviving embryo four days after inoculation showed that seven of the samples elicited the same effect on the chicken embryo as in the controls. This showed that these samples did not contain water soluble toxic material constituents and were not contaminated with any toxic chemical.

The other three samples (herbal tea for memory, golden seal root and black walnut) each resulted in 62.5% live embryo which were significantly lower than the control. Hence, water extracts of these samples contained toxic constituents which might be inherent in the preparation or present as a result of chemical contamination or biodegradation attributable to presence of microbial contaminants.

Percentage of eggs hatched after 21 days of incubation showed the same statistical significance. However, the value for the uninoculated control was lower compared to that of the uninoculated control of the live embryo. Nevertheless, this was greater than the normal value of 80% by commercial hatching eggs (**6**). Based on the percentage of eggs hatched, herbal tea for improved memory, golden seal root and black walnut again exhibited the same toxic effects as revealed by 25% to 30% eggs hatched. This was significantly lower than the percentage of eggs hatched in the controls. This confirmed the presence of water soluble toxic components in these samples.

Effects of Oil Extract on the Chicken Embryo

The percentage of live embryo four days after inoculation with oil extracts (Table 3) showed a relatively lower survival rate than those inoculated with water extracts. It appeared that oil effected the survival of the embryo. This was shown in the significantly lower percentages of live embryo elicited by benostrum, benergen, lagundi syrup, herbal tea for longevity, turgor bran and alfalfa tablet. This showed that the effects on the

embryo by the samples were due to presence of oil and not due to presence of toxic material inherent or present in the sample. The other samples (balaya, herbal tea for memory and golden seal root) elicited 7.5% to 15.0% live embryo only, which was significantly lower than for both controls. None of the 20 embryos inoculated with black walnut survived four days after. This shows the presence of toxicity in its oil extract.

The percentage of eggs hatched 21 days after inoculation with oil extracts (Table 3) was lower than the percentage of live embryo. All the samples (including sterile oil) exhibited significantly lower percentage of eggs hatched than the uninoculated control. However, the percentages of eggs hatched by balaya, benostrum, benergen, lagundi syrup, herbal tea for longevity, turgor bran and alfalfa tablet were comparable with that by sterile oil. Hence, the low hatchability by these samples was due to the effect of oil on the development of the embryo and not due to the presence of toxic metabolites in the sample oil extract. Balaya oil extract was shown to be toxic on the embryo but its effect on the percentage of eggs hatched was not significant. Thus, it was not considered toxic. A component of balaya oil extract might have affected the embryo significantly but that effect was not significant when determined by the percentage of eggs hatched. The very low hatchability, however, of 5.0% by herbal tea for memory and golden seal root, and the absence of eggs hatched by black walnut, implies a clear presence of toxic metabolite in these samples.

Toxicity Assessment

Based on the results of the chicken embryo assay of water and oil extracts of herbal drug samples, herbal tea for memory, golden seal root and black walnut were toxic using 20% and 10% (w/v) water and oil extracts, respectively. These smaples had exhibited fatal effects against the embryo, resulting in lower embryo survival and eggs hatched. The toxicity of herbal tea for memory does not support the claim of the manufacturer that it improves the inellectual capacity of a person (Table 1). Likewise, the toxicity of golden seal root and black walnut does not support the claim that they can cure almost all diseases and correct bodily disorders (Fig. 3).

Toxicity of herbal preparations has been previously reported (**3, 10, 18, 19, 21**). Majority of these reports point to herbal tea as a health hazard and not as the herbal remedy to various diseases and disorders claimed by numerous users. Consumers should be wary about the therapeutic claims of these products. For instance, Paraguay tea is very popular in Brazil and in the United States, but it was implicated in a case of veno-occlusive disease of the liver in Britain (**18**). Another herbal tea of proven toxicity is the camomile tea which has been well documented as a cause of contact dermatitis (**4**). In the Philippines, many kinds of herbal teas are currently available. Among the popular ones, the Taheebo herbal tea, which claims to be approved by the FDA and recognized by the governments of Brazil, Canada and the United States (**24**), has been promoted extensively through brochures and leaflets which extol the healing properties of this product. Many Filipinos, however, have now expressed questions or doubts regarding the curative properties and safeness of this product.

No herbal drug is registered with the Bureau of Foods and Drugs (**1**). Aside from the herbal products endorsed by the Department of Health (DOH) for use in government hospitals and drugstores, no other herbal products have been submitted for registration with the BFAD. Moreover, herbal drugs are far from being approved by the regulatory body if manufacturers could not satisfy requirements, among which are the identification and isolation of the active constituent responsible for the claimed therapeutic effect. Toxicity studies, therefore, of products generally regarded as safe (GRAS) should be one of the priorities of institutions engaged in health related researches.

Mutagenicity Potentials of the Herbal Drug Samples

Mutagenicity was measured in terms of the number of histidine revertants/plate induced by the test material (**2**). This is based on the premise that mutagenic material will induce the reversion to the prototrophic state of histidine auxotrophic strains deficient in its repair system mechanisms. To test the validity of the bacterial test system, several controls were included in the assay. Control treatments using solvent only in place of test material, assessed the spontaneous reversion property of *Salmonella typhimurium* TA 98. Using dis-

tilled water, the solvent used for extraction of herbal samples, the strain elicited an average of 38.0 revertants/plate in the absence of S9 (Table 4). This was well within the acceptable range of 30-50 revertants/plate in the absence of S9 (**16**). Using dimethyl sulfoxide (DMSO), the solvent for 2-aminofluorene, the value obtained was 32.0 revertants/plate in the absence of S9. A value lower than that obtained from distilled water was similarly reported by Shahin (**22**) and Shibuya et al. (**23**).

Another equally necessary control treatment is a positive control employing a diagnostic mutagen to confirm the reversion property of the strain in the presence of the mutagen. The arylamine and a potent hepatocarcinogen (**20**), 2-aminofluorene (2-AF) was used as a positive control. Its selection facilitated the validation since 2-AF requires metabolic activation for the expression of its genotoxic potential (**17**). Hence, the effectivity of using S9 could also be determined. Dissolved in DMSO and used at a concentration of 20 ug/plate, 2-aminofluorene elicited an average of 44.0 and 126.0 revertants/plate in the absence and presence of S9, respectively (Fig. 4). The value was within the acceptable range, both in the absence and presence of S9. Thus, 2-AF was considered mutagenic only in the presence of S9 based on the accepted basis of mutagenic activity which is greater than two-fold increase of revertants over the spontaneous level. Hence, the S9 Mix prepared from liver homogenate of phenobarbital-induced Sprague-Dawley rats was effective as a mammalian metabolic activation system.

A further check on the test system was the inclusion of a medicinal plant reported to be mutagenic. Raw garlic bulb water extract at a concentration of 20% (w/v) was used. In the absence of S9, garlic aqueous extract elicited an average of 84.0 revertants/plate; whereas in the presence of S9, a lower average count of 64.0 revertants/plate was obtained. Previous mutagenicity screening tests on this medicinal plant revealed that raw garlic bulb was mutagenic in the Ames-Salmonella test without S9 (**14**). However, the mutagenicity was lost upon metabolic activation when measured by the host-mediated assay. Host-mediated assay as an alternative to using S9 as a metabolic activation system has a major weakness. The conversion to active mutagenic forms may have taken place in the mouse upon injection of the test ma-

terial. However, the concentration of these active forms reacting with the test strain present in the peritonium of the mouse could be lower (possibly too low) to produce a significant number of mutations (**11**). The lower concentration stems from the fact that not all of the active forms reach the peritonium where mutagenicity reaction takes place. Nevertheless, the use of the Ames-Salmonella test with and without S9 confirmed the elimination of the mutagenic effect of raw garlic bulb upon metabolic activation. This was shown in the greater than twofold increase in the number of revertants in the absence of S9 and the lower count obtained in the presence of S9. This result, therefore, agreed with the reported nonmutagenicity of this medicinal plant upon metabolic activation.

Among the samples, only lagundi syrup and herbal tea for memory elicited revertant counts comparable with the value in spontaneous reversion count. These two samples, therefore, could be considered nonmutagenic as well. The rest of the samples, balaya, benostrum, benergen, herbal tea for longevity, turgor bran and alfalfa tablet, elicited counts higher than the spontaneous reversion count. However, these counts failed to reach the greater than twofold increase over the spontaneous level criterion in assessing mutagenicity. Thus, the samples could still be considered nonmutagenic. It seemed that the herbal drug samples did not contain water-soluble components that could be activated to mutagenicity. High reversion rates, however, may imply that the samples might be mutagenic at a higher dose, but not too high as to exert toxic effect to the tester strain. Furthermore, other extracting solvents and strains prescribed in the Ames-Salmonella test should be tried with S9 to determine if components extractable with a solvent could be activated to mutagenicity. Hence, the use of S9 as a metabolic activation system is indispensable in assessing mutagenicity employing the Ames-Salmonella test.

Examination of mutagenicity plates of golden seal root and black walnut showed no background lawn. Therefore, no counts were done on these plates. Apparently, the concentration of 20% water extract was toxic to the tester strain. Hence, no growth, and no background lawn occurred. Few small colonies appeared on the plates, having survived on the trace of histidine present on the medium. This condition has

been repeatedly observed by Ames et al. (**2**) and Maron and Ames (**16**) on highly toxic compounds. To prevent this, the originators have prescribed that the concentration or dose of samples to be assayed should be taken from the linear portion of a dose-response curve. If no toxicity determination is done, a 2.5-fold increase over the spontaneous level will also be considered an indication of mutagenicity if found in repeated experiments (**22**). A 2% concentration was tried for black walnut and the normal background lawn appeared with revertant colonies. Therefore, at this concentration, black walnut lent itself to the detection of mutagenicity potential but at 20% water extract concentration, such determination was not possible due to the direct toxic effect on the tester strain.

Table 1. **Source, manufacturer, prescribed uses and actual cost of herbal drugs studied toxicologically**

SAMPLE	SOURCE AND MANUFACTURER	PRESCRIBED USES[a]	COST (₱)
Balaya	Ben Cruz Herbal Clinic, Taft Avenue, Manila and Ben Cruz Herbal Clinic, Taft Avenue, Manila	As a remedy for high blood pressure intestinal and respiratory tract infections	0.30/ tablet
Benostrum	Ben Cruz Herbal Clinic, Taft Avenue, Manila and Ben Cruz Herbal Clinic, Taft Avenue, Manila	Medicine for diarrhea (LBM), food poisoning, cough, allergy and colds.	0.30/ tablet
Benergen	Ben Cruz Herbal Clinic, Taft Avenue, Manila and Ben Cruz Herbal Clinic, Taft Avenue, Manila	For improvement of digestion and metabolism and prevention of premature graying of hair; also as energy food	30.00/ 250 mL

continued next page

Table 1. continued . . .

SAMPLE	SOURCE AND MANUFACTURER	PRESCRIBED USES[a]	COST (₱)
Lagundi Syrup	Medicinal Plant Section, Ethnobotany Lab. IBS, UPLB College, Laguna and Medicinal Plant Section, Ethnobotany Lab. IBS, UPLB Coolege, Laguna	As analgesic, antipyretic, bronchodilator and expectorant	9.00/ 60 mL
Herbal tea for longevity (Fo-ti-tieng)	Sto. Niño Botanical Center Sta. Cruz, Manila and Unknown	Rejuvenates internal organs; revitalizes glands; adds years to life; gives vigor and virility, also sharpens memory.	50.00/ 25 g
Herbal tea for memory	Sto. Niño Botanical Center Sta. Cruz, Manila and Unknown	Helps maintain and improve memory as antidote to mental fatigue and forgetfulness; as remedy for bringing agility to intellect	50.00/ 25 g
Turgor bran	Ben Cruz Herbal Clinic, Taft Avenue, Manila and Ben Cruz Herbal Clinic, Taft Avenue, Manila	As brain and nerve food; for diabetes, constipation hemorroid; anti-anemia, vacular; respiratory, height and weight problems; effective for pimples, high blood pressure, varicose veins; prevents fat tumors.	10.00/ 500 g

continued next page

Table 1. continued . . .

SAMPLE	SOURCE AND MANUFACTURER	PRESCRIBED USES[a]	COST (₱)
Alfalfa tablet	Bio-Synergy Inc. Agrix Supermarket Los Baños, Laguna and International Vitamin Corp. USA	As multi-vitamin supplement rich in chloropyll, calcium and phosphorus; scrubs out cholesterol deposit in the arteries	1.00/ tablet
Golden seal root	Herbix Enterprises, Los Baños, Laguna and Wachter's Organic Sea Products Corp. California, USA	As remedy for a wide range of diseases and disorders (Fig. 3)	8.00/ tablet
Black walnut	Herbix Enterprises, Los Baños, Laguna and Wachter's Organic Sea Products Corp. California, USA	As remedy for a wide range of diseases and disorders (Fig. 3)	5.00/ tablet

[a] Taken from product label and/or brochure obtained from the drug outlet

Table 2. Effects of water extract of herbal drug samples on viability and hatchability of chicken embryo[a]

TREATMENTS	LIVE EMBRYO %	EGGS HATCHED %
Control: Uninoculated	95.0[a]	85.0[a]
Control: Sterile water	90.0[a]	62.5[a]
Balaya	85.0[a]	62.5[a]
Benostrum	95.0[a]	80.0[a]
Benergen	95.0[a]	60.0[a]
Lagundi Syrup	80.0[a]	70.0[a]
Herbal tea for longevity	85.0[a]	62.5[a]
Herbal tea for memory	62.5[b]	30.0[b]
Turgor bran	90.0[a]	65.0[a]
Alfalfa tablet	85.0[a]	60.0[a]
Golden seal root	62.5[b]	30.0[b]
Black walnut	62.5[b]	25.0[b]

[a] Based on two trials each using 20 eggs/treatment. Chi-square test and Z test for proportion against the positive control were conducted to determine significance of the differences in percentages between treatments. Percentages followed by same letter are not significantly different.

Table 3. Effects of oil extract of herbal drug samples on viability and hatchability of chicken embryo[a]

TREATMENTS	LIVE EMBRYO %	EGGS HATCHED %
Control: Uninoculated	90.0[a]	90.0[a]
Control: Sterile oil	47.5[b]	37.5[b]
Balaya	15.0[c]	15.0[b]
Benostrum	72.5[ab]	60.0[b]
Benergen	52.5[ab]	50.0[b]
Lagundi Syrup	72.5[ab]	62.5[b]
Herbal tea for longevity	70.0[ab]	60.0[b]
Herbal tea for memory	7.5[c]	5.0[c]
Turgor bran	65.0[ab]	62.5[b]
Alfalfa tablet	52.5[b]	47.5[b]
Golden seal root	7.5[c]	5.0[c]
Black walnut	0[c]	0[c]

[a] Based on two trials each using 20 eggs/treatment. Chi-square test and Z test for proportion against the positive control were conducted to determine significance of the differences in percentages between treatments. Percentages followed by same letter are not significantly different.

Table 4. Number of histidine revertants/plate induced by water extracts of herbal drug samples on *Salmonella typhimurium* TA98 with and without S9 mix

SAMPLES	REVERTANTS/PLATE[a]	
	-S9 Mix	+S9 Mix[b]
Control, distilled water	38.0	61.0
Control, Dimethyl sulfoxide (DMSO); 100 u1	32.0	55.0
Control, 2-Aminofluorene; 20 ug/plate	44.0	126.0
Control, garlic bulb aqueous extract	84.0	64 0
Samples:		
Balaya	73.0	82.0
Benostrum	62.0	68.0
Benergen	50.0	54.0
Lagundi Syrup	32.0	32.0
Herbal tea for longevity	53.0	53.0
Herbal tea for memory	34.0	42.0
Turgor bran	48.0	55.0
Alfalfa tablet	44.0	48.0
Golden seal root	0[c]	0
Black walnut	0	0

[a] Average of two trials. The number of spontaneous revertants has not been subtracted from the totals.

[b] 0.5 mL per plate used contained 0.02 ml S9 or 4% S9.

[c] No background lawn observed. This might be due to toxicity of the extract to the tester strain. Hence, no counts were done.

A

B

Figure 1. Formulation and appearance of packed herbal drug samples balaya and benostrum (A) and benergen and lagundi syrup (B).

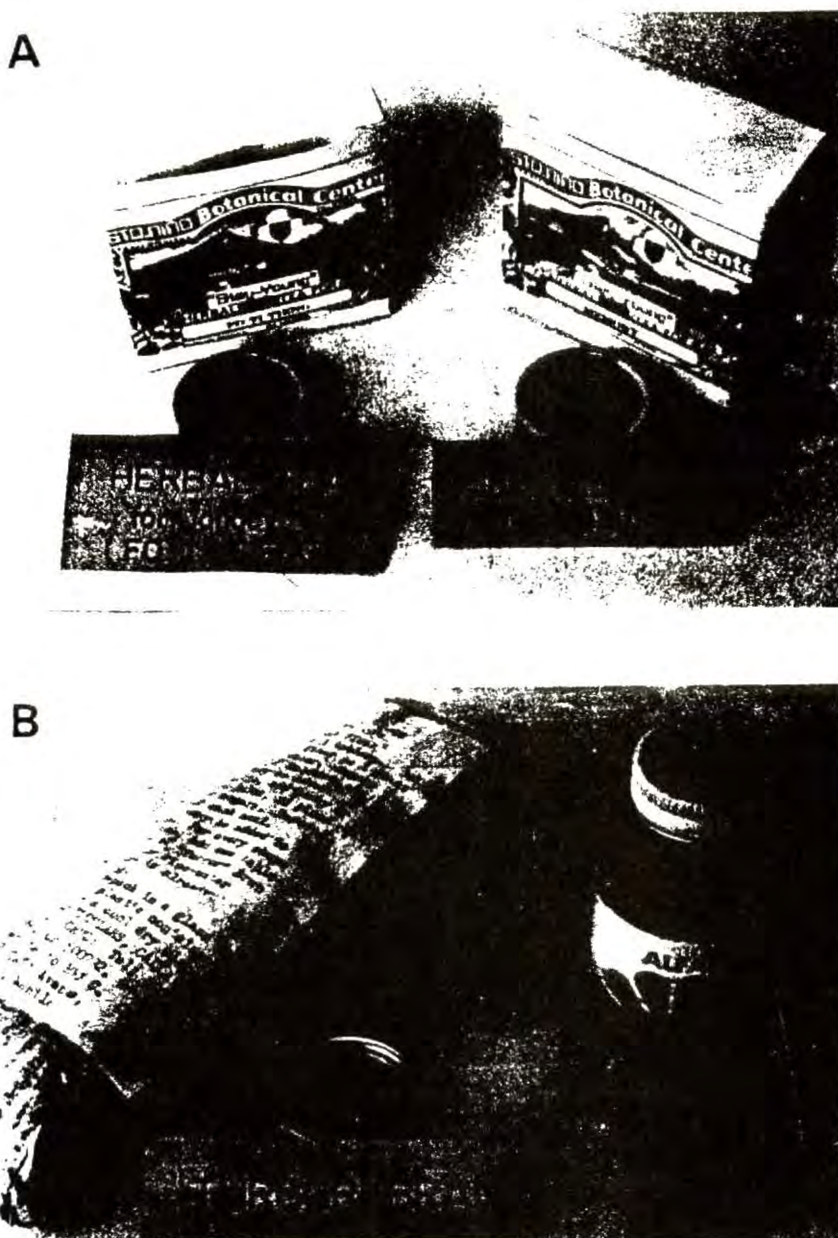

Figure 2. Formulation and appearance of packed herbal drug samples herbal teas for longevity and memory (A) and turgor bran and alfalfa tablet (B)

Has been used for the following:
Allergies, antibiotic, appetite, asthma, bladder infections, bleeding bowel, bronchitis, burns, cankers, catarrh, chicken pox, circulation, colds, colitis, constipation, coughs, diabetes, digestion, earache, eyewash, eczema, flu, gall bladder, gonorrhea, sore gums, hay fever, heart, hemor rhages, hemorrhoids, infections, inflammation, kidney, liver, measles, menstruation, morning sickness, mouth sores, nasal passages, nausea, nerves, pancreas, prostate gland, psorlasis, respiratory, ringworm, skin, skin cancer, small pox, syphillis, sore throat, ulcers, urethra, weight loss, wounds.

Has been used for the following:
Antiseptic purposes, athlete's foot, boils, cancer, colitis, dandruff diarrhea, eczema, electrocution, antidote, hair, hemorrhoids, hoarseness, infections, inflammations, impetigo, leucorrhea, malaria parasite, mouth sores, nails, ringworm, skin rash, ulcerated sores, syphillis, tape worm, sore throat, thyroid, tooth enamel, tuberculosis, tumors, ulcers, prolapsed uterus, vaginal discharge, varicose veins, expulsion of worms and parasites

Figure 3. **Formulation and appearance of packed product and the prescribed uses of herbal drug samples golden seal root (A) and black walnut (B)**

Figure 4. Histidine revertants on Minimal Glucose Agar (MGA) of standard mutagen 2- aminofluorene in the absence (-S9) and presence (+S9) of rat liver homogenate (S9) mix

LITERATURE CITED

Alcala, J.C. 1990. Interview, Aug. 14, 1990. Bureau of Food and Drugs (BFAD), Alabang, Muntinlupa, Metro Manila, Philippines.

Ames, B.N., W.E. Burston, E. Yamasaki and F.D. Lee. 1973. Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proceedings of the National Academy of Sciences (PNAS) 70: 2281- 2284.

Anderson, L.A. and J.D. Phillipson. 1982. Mistletoe - the magic herb. Journal of Pharmacy 229. 437-439.

Anon. 1988. Many herbal teas are toxic. American Pharmacy NS28 4.18-19

Association of Official Analytical Chemists (ADAC). 1980. Official methods of Analysis of the Association of Official Analytical Chemists. 13th ed. W. Horowitz (Ed.). Washington, D.C., Association of Official Analytical Chemists. 426-427.

Aves, Y. 1986. Production and utilization of crude tylosin as feed additives for broilers. UPLB Unpublished MS Thesis, UPLB, College, Laguna.

Clark, A.M. 1982. Endogenous mutagens in green plants Environmental mutagenesis, carcinogenesis and plant biology E.J. Klekowski (Ed.) Praeger Publishers 99-132. vol. 1, New York.

De Padua, L.S., G.C. Lugod and J.V. Pancho. 1981. Handbook on Philippine Medicinal Plants Vol. 3. UPLB Hortorium. Research Reports. College, Laguna p. 1.

Dey, B.P. 1976. Production, nutritional and toxicological evaluation of Cellulomonas for a protein source. PhD. Thesis. Univ. of Missouri, Columbia 75-82.

Dickstein, E.S. and F.W. Kunkel. 1980. Foxglove tea poisoning. American Journal of Medicine. pp. 69. 167-169.

Flamm, W.G. 1979. Strengths and weaknesses of tests for mutagenesis, in Banbury report 1, Assessing chemical mutagens: The risk to humans. V.K. McElheny and S. Abrahamson (Eds.) (Cold Spring Harbor Laboratory). p. 367.

Garner, R.C., E.C. Miller and J.A. Miller. 1972. Liver microsomal metabolism of aflatoxin B1 to a reactive derivative toxic to *Salmonella typhimurium* TA1530. Cancer Research 32. 2058-2066.

Hannan, M.A., A.A. Al-Dakan, H.Y. Aboul-Enein and A.A. Al-Othaimeen. 1989. Mutagenic and antimutagenic factors extracted from a desert mushroom using different solvents. Mutagenesis 4(2). 111-114.

Lim-Sylianco, C.Y. 1989. Mutagenic and antimutagenic activities of Philippine medicinal plants in Proceedings of UNESCO symposium on the development of drugs from plants. E.C. Quinto (Ed.) (UST Research Center for the Natural Sciences), 101-112.

Lucas, J. 1975. Our polluted food: A survey of the risks. London: Charles Knight and Co. Ltd. 37-38.

Maron, D.M. and B.N. Ames. 1984. Revised methods for the Salmonella mutagenecity test. Mutation Research 113. 173-215.

McCan, J., N.E. Spingarn, J. Kobori and B.N. Ames. 1975. Detection of Carcinogens as mutagens: Bacterial tester strains with R factor plasmids. Proceedings of the National Academy of Sciences 72. 979-983.

McGee. J.R.S. Patrick, C.B. Wood, L.H. Blumgart. 1976. A case of veno-occlusive disease of the liver in Britain associated with herbal tea consumption. Journal of Clinical Pathology. 29. 788- 794.

Penn, R.G. 1988. Adverse reaction to herbal medicines. Australian Prescriber 11(1). 16-18.

Rees, R.W., A.J. Brice, J.B. Carlton, P. J. Gilbert and I. de G. Mitchell. 1989. Optimization of metabolic activation for four mutagens in a bacterial, fungal and two mammalian cell mutagenesis assays. Mutagenesis 4(5). 335-342.

Ridker, P.M. 1989. Health hazards of unusual herbal teas. American Family Physician 39(5). 153-156.

Shahin, M.M. 1989. Evaluation of the mutagenicity of azo dyes in *Salmonella typhimuriun:* A study of structure-activity relationships. Mutagenesis 4(2). 115-125.

Shibuya, T., N. Tanaka, M. Katoh, Y.T. Matsuda and K. Morita. 1985. Mutagenecity testing of ST-film with the Ames test, chromosome test *in vitro* and micronucleus test in female mice. Journal of Toxicological Sciences 10(2). 135-141.

True II, G.N. 1989. Health frontiers: Herbal teas. Philippine Daily Inquirer Sept. 14, 1989. 3.