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Antiteratogenic Effects of Leaf Extract of *Mentha Cordifolia* Opiz. (Mint), *Allium Sativum* L. (Garlic), and *Pterocarpus Indicus* Willid. (Narra) on *Mus Musculus* (White Mice)

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ABSTRACT

The study was conducted to compare the antiteratogenic effects of leaf extracts from mint, garlic and narra. Specifically, this aimed to determine the fertility, implantation and gestation indices, percentage dead implants, and percentage female resorptions; examine and describe the gross anatomy of the fetuses; and compare the sperm morphology of all treatments. Twenty-five (25) sexually mature male and fifty (50) sexually mature female mice were used in this study. These were randomly allocated to five groups labeled as T0+ (distilled water), T0- (tetracycline), T_1 (tetracycline + mint extract), T_2 (tetracycline + garlic extract), and T_3 (tetracycline + narra extract) with five replicates. Antiteratogenic activities were monitored using dominant Lethal Test. Treatments were administered daily in male mice subcutaneously for six days. Treated mice were allowed to mate with two female mice for 24 hours. Sperm morphology assay was done to all male mice after mating. After 18 days mated female mice were sacrificed and pups were examined for any malformations. Positive control showed 100% fertility, 0% resorption, and fetal lethality while negative control revealed 0% fertility. At 0.3 ml of pure extracts per 20 g mice, narra treated mice significantly increased the frequency of fertile matings to 40%, mint to 30% and garlic to 20%; significantly increased implantation to 83% (mint), 85% (garlic), and 82% (narra); significantly decreased the number of females with resorptions to 25%, 33.3% and 50% (mint, narra and garlic, respectively); and significantly decreased the number of dead implantations to 17.6% (garlic), 15.2% (narra), and 4% (mint). Gross morphological examination of pups showed no signs of congenital malformations. Sperm morphology assay revealed significant decrease in the frequency of abnormal sperm cells in mice administered with leaf extracts of mint, garlic and narra.

Keywords: Antiteratogenic effects, *Mentha cordifolia* Opiz. (mint), *Allium sativum* L. (garlic), *Pterocarpus indicus* Willid. (narra), *Mus musculus* (white), sperm morphology, antifertility

INTRODUCTION

A number of Philippine plants, indigenous or not, are used for treatment of a variety of ailments. In fact, this is the practice of our "herbularios" who have been using plants in the cure of some common ailments particularly in places where modern medicine has yet to be fully accepted. As modern technology progresses mutation becomes a more prevalent phenomenon, which may be brought about

by environmental chemicals such as pesticides, food additives, synthetic drugs, atmospheric, and water pollutants (Sylianco, 1995). These chemicals produce genetic changes and decrease the body resistance to disease (Villaseñor *et al*., 1997).

Genotoxicity to somatic cells can induce cancer while genotoxicity to germ cells can lead to genetic disorders or teratogenesis that can be transmitted from one generation to the next (Sylianco and Wu, 1990). The manifestations are embryo lethality, birth defects, growth retardation, functional alterations of the nervous, immune and endocrine systems, and childhood cancer (Villaseñor *et al*., 1997). Congenital defects have been a major health problem even in ancient time. Today, despite the great advancement in medicine, very little progress has been made in the prevention of congenital defects.

A number of extracts from Philippine plants have been reported to reduce the genotoxicity of mutacarcinogens (Sylianco *et al*., 1986 and 1988). The search therefore for substances that can reduce or remove the genotoxicity of environmental chemicals is a global concern. Three medicinal plants namely: mint *(Mentha cordifolia* Opiz.), garlic *(Allium sativum* L.), and narra *(Pterocarpus indicus* Willid.) may have the potential to counteract the effects of teratogens. Quisumbing (1978) labeled these plants as "cure-all medicine". Sylianco *et al.* (1986 and 1988) reported that mint and garlic extracts are antigenotoxic while Takasaki *et al.* (1995) reported that narra leaf extract exhibits anti-cancer effect in mice.

Since these medicinal plants have been proven to be antigenotoxic, these are likely potential antiteratogens. Thus, it is assumed that extracts from their leaves would reduce or minimize teratogenesis. It is in this aspect that this study was conceived.

This study attempted to compare the antiteratogenic effects of leaf extracts from mint, garlic and narra. Specifically, this aimed to determine the fertility, implantation and gestation indices, percentage dead implants, and percentage female resorptions; to describe the gross anatomy of the fetuses; and to compare the sperm morphology of all treatments.

Mint, garlic and narra are widely distributed in the countryside and are used to cure diseases including cancer (Quisumbing, 1978). Antigenotoxic potential of these plants has already been elucidated but their antiteratogenic activities have not been studied. The importance of this study lies in the counteraction of the mutagenic activity of environmental chemicals, such as pesticides, food additives, synthetic drugs, atmospheric and water pollutants, and in the probable antiteratogenic activities of these antimutagenic plants.

RESEARCH DESIGN AND ANALYTICAL FRAMEWORK

Leaf extracts of mint, narra, and garlic bulbs were prepared and LD_{50} was established at 0.4 ml per 20 g body weight of mouse for all treatment using the procedure of Sylianco *et al.* (1988).

Twenty-five (25) male and fifty (50) female Swiss albino mice were used in the experiment. Twenty-five (25) rectangular plastic cages with screen covers were prepared with a size of 8x6x8 cubic inches for each treatment. There were five replicates for each treatment and each housed 3 mice, 1 male and 2 female mice. Each cage group was marked To+, To-, T1, T2 and T3. The treatments were allocated following the procedures of Villaseñor *et al*. (1997) as follows:

To+ = positive control - distilled water was given to male mice. This determined the fertility of mice.
To- = negative control- 0.2 ml tetracycline per 20 g mouse was administered subcutaneously using 1ml hypodermic needle. This determined the teratogenic activity of tetracycline.
T_1 = 0.2 ml tetracycline per 20 g mouse was administered subcutaneously followed by intraperitoneal injection of 0.3 ml mint leaf.
T_2 = 0.2 ml tetracycline per 20 g mouse was administered subcutaneously followed by intraperitoneal
injection of 0.3 ml garlic leaf extract.
T_3 = 0.2 ml tetracycline per 20 g mouse was administered subcutaneously followed by intraperitoneal
injection of 0.3 ml narra leaf extract.

The mice were acclimatized for 3 days prior to treatment. Treatments were administered for six (6) days. Commercial pellet feed and water were given to each cage in *ad libitum.*

The Dominant Lethal Test (Searle, 1974) was used for bioassay of antiteratogenic potential. Fertility, implantation, and gestation indices as well as % dead implants and % female with resorption were computed as follows:

Fertility Index	=	number of pregnant females / number of mated females	X	100
Implantation Index	=	total implantation / number of pregnant female	X	100
Gestation Index	=	no. of live implants / no. of implantation	X	100
% Dead Implants	=	100 - Gestation Index		
% Female with Resorption	=	number of females with resorption / number of females pregnant	X	100

Note: Resorption refers to early death.

Sperm morphology assay was undertaken using the procedure of Pagulayan *et al.,* (1994). Sperms were checked of any abnormality. Criteria of sperm morphology of Coffin (1953), Pagulayan *et al.*,(1994) and Subhan *et al.* (1997) were utilized. Specimens were observed under light microscope using the high power objective. Photographs were taken on suitable specimens.

Completely Randomized Design was used in this experiment with positive and negative controls, 3 treatments and 5 replications. Statistical analysis was undertaken to determine the significance of the results using One-Way Analysis of Variance (ANOVA). Least Significant Difference (LSD) Test was used as post hoc test to determine the difference among treatments at 5% and 1% level of significance.

RESULTS AND DISCUSSION

Three herbal plants were used in this study as test samples namely: *Mentha cordifolia* Opiz. (mint), *Allium sativum* L. (garlic), and *Pterocarpus indicus* L. (narra). Table 1 reveals the antiteratogenic potentials of these herbal plants to tetracycline-induced teratogenesis. The table shows that at 0.3 ml /20 g mouse, the pure extracts significantly increased the fertility index to 20% (garlic), 30% (mint) and 40% (narra) compared to the negative control. Implantation index also significantly increased to 83% in mint, 85% in garlic and 82.5% in narra treated mice compared to the negative control but slightly lower that the positive control (92.0%). Gestation index significantly increased from 0% (negative control) to 82.4%, 84.8% and 96% for garlic, narra and mint extracts, respectively. These suggest that there were more pregnant females and more live implants in a litter compared to the negative control. Lethality index was significantly low (4% mint, 15.5% narra and 17.6% garlic). A significant decrease in the number of females with resorption was noted from 100% (positive control) to 50%, 33.3%, and 25% (garlic, mint and narra, respectively). This means that a significantly higher ratio of pregnant females and live implants existed compared to the negative control.

These observations imply that the pure leaf extracts of mint, garlic and narra were able to counteract the teratogenic effects of tetracycline as there were significant increase in the frequency of fertile matings and implantation, significant decrease in percentage of female with resorptions, and significant decrease in the number of early and late fetal deaths.

Table 1. Antiteratogenic effects of pure extracts of mint, garlic, and narra leaves

TREATMENT	FERTILITY INDEX	IMPLANTATION INDEX	GESTATION INDEX	% DEAD IMPLANTS	% FEMALE WITH RESORPTION
Positive Control	100[a]	92[a]	100[a]	0[e]	0[e]
Negative Control	0[e]	0[c]	0[d]	100[a]	100[a]
Mint Extract	30[c]	83[b]	96[b]	4[d]	33.3[c]
Garlic Extract	20[d]	85[b]	82.4[c]	17.6[b]	50[b]
Narra Extract	40[b]	82.5[b]	84.8[c]	15.2[c]	25[d]

Means with the same letters are not significantly difference at 0.05 level.

Of the three extracts, narra showed the highest significant increase of fertile matings and significant decrease in the number of females with resorption (Table 1). Several bioactive components are present in narra that could be responsible for these antiteratogenic potentials. According to Santos *et al*. (1981), narra leaves contain narrin, bitter principle, angolensin, formononetin, eromopterocarpin, b-eudesmol, pterocarpol, and alkaloid. List and Horhammer (1979) reported the presence of prunetin and isoliquiritigenin in the leaves of narra. Isoliquiritigenin inhibits the induction of aberrant crypt foci thereby stabilizing DNA molecules (Takasaki *et al*., 1995). Prunetin, a flavonoid, had been shown to inhibit the catalytic activity of DNA topoisomerase by stabilizing the Topo II DNA complex preventing DNA damage induced by genotoxins (Constatinou *et al*., 1995). Earlier report of Endo and Miyasaki (1972) showed that growth of carcinoma cells were significantly inhibited by narra leaf extract. These revealed the antimutagenic activity of narra leaf extract.

Meanwhile, mint extract showed the highest significant increase of pregnancy and significant decrease of incidence of fetal death (Table 1). Study of Villaseñor *et al*. (1997) revealed that chloroform extract of mint at 2.5 mg/ 20 g mouse increased fertile matings to 100%, decreased to 0% the number of female with resorption, and decreased to 3.4% the number of dead implantation. This shows that chloroform extract of mint has higher antiteratogenic activity than pure extract because bioactive components of mint are fat-soluble (Ode, 1993). Santos *et al*. (1981) reported the presence of carvone, methyl acetate, piperitone oxide, transcarveol, and 1-carvone in the leaves of mint. An extensive search of literature showed that the structures of these bioactive constituents are not yet elucidated and their antigenotoxic mechanism ascertained.

On the other hand, garlic also showed antiteratogenic effects. The frequency of fertile matings was significantly increased, the number of female with resorption and the number of early and late deaths were significantly decreased (Table 1). These antiteratogenic potentials may be due to the presence of several bioactive constituents. Garlic bulbs contain alliin, allicin, inulin, choline, allyl disulfide, myrosinase, fructosan, scoldinine A and B, alliinase, biotin, s-allyl-L-cysteine, glutamyl peptides, amino acids, glycosides, and pantathine (Santos *et al*., 1981). Garlic contains high amounts of trace elements sodium, potassium, calcium, phosphorous, iron, magnesium manganese, fluorine, and iodine (Janjua, 1990). These mineral ions were shown to be antimutagenic as to reduce the genotoxicity of tetracycline (Sylianco and Daya, 1984). Furthermore, s-allyl cysteine, a sulfur-containing amino acid found in garlic, shows antigenotoxic effect by reducing the chromosome breaking effects of tetracycline (Sylianco and Wu, 1990).

Examination of embryos revealed normal morphology with two eye slits, two ears, lower jaw slightly shorter than the snout, four legs that point in the proper direction, four digits on each front paws, five digits on hind paws, with an anal and genital openings, a tail, an umbilicus, and a smooth epidermis with no protrusions. According to EPA (1999) paternally-induced teratogenesis ends mostly in embryo loss rather than a live birth with malformation.

The significant decrease in the number of fertile mating was supported by sperm morphology assay. An important part of any breeding soundness exam is an evaluation of sperm morphology and in the most fundamental case the size and shape of the head, midpiece and tail are examined (EPA, 1999).

The results revealed that positive control has the lowest percentage of abnormal

sperm (15.88%). The negative control has the highest occurrence of abnormal sperm percentage of 38.72%. Among treatments, garlic extract reduced the sperm abnormalities to 25.52%, mint extract to 21.4%, and narra extract to 20.88%.

Compared with the controls, significant decrease in the number of abnormal sperm cells was observed in treated mice (Table 2). Analysis of variance (ANOVA) of abnormal sperm cells among treatments revealed a significant F-value of 18.233 indicating that at least one treatment group had a significant mean difference with another group. Because of a significant F-value, the least significant difference (LSD) was used to determine which groups were significantly different (Table 2).

Table 2. Mean percentage of sperm abnormalities between treatments

TREATMENTS	MEAN PERCENTAGE OF ABNORMAL SPERMS
Positive Control	15.88[c]
Negative Control	38.72[a]
Mint Extract	21.4[bc]
Garlic Extract	25.52[b]
Narra Extract	20.88 [bc]

Means with same letters are not significantly different at 0.05 level

Table 2 shows that the mean percentage of abnormal sperm cells in the positive control was significantly lower compared to negative control. Furthermore, mean percentage of abnormal cells in mice treated with mint extract, garlic extract, and narra extract were significantly lower than that of the negative control. Moreover, garlic extract showed significantly higher percentage of abnormal cells than with positive control. On the other hand, no significant differences in mean percentage of abnormal cells were observed between mint, garlic, and narra extracts.

There were twenty-one (21) types of sperm abnormalities observed on both control and test mice. These types were as follows in decreasing incidence: bent midpiece, banana, head agglutination, beak, tailless, coiled tail, headless, hookless, corrugated tail, periform, balloon, rhomboid, calyx, distal droplet, sunflower, triangular, funnel, amorphous, double headed, double tailed, and proximal droplet types (Table 3). Each type of abnormality occurred in varied frequencies in each treatment.

Among the various types of abnormalities, the bent midpiece, with mean frequency of 133.4, was the most common (Table 3). Furthermore, double headed, double tailed, and proximal droplets occurred the least with mean frequency of 3.8, 3.2, and 2.2, respectively.

Table 3 reveals that in the positive control, bent midpiece was the most common abnormality followed by tailless, headless, and banana types. Furthermore, triangular, funnel, and double tailed occurred the least. Moreover, sperm cells with proximal droplet and double-heads were not observed in positive control mice. In the negative control group, bent midpiece was also observed to be the most frequent abnormal type followed by beak, banana, and coiled tail types. Double tailed and proximal drop types were rare. All identified types of sperm abnormalities revealed in this study were observed in the negative control group.

Among treatments, the numerous abnormal type was the bent midpiece. Head agglutination and coiled tail types followed in mint treated mice; beak, banana, and head agglutination types in garlic treated mice; banana and tailless types in narra treatment mice. Rarely observed abnormalities were, the double headed and proximal droplet types in mint treated mice; double headed and distal droplet types in garlic treated mice; calyx, triangular, sunflower, funnel, and double tail types in narra treated mice.

Although most sperm abnormalities were observed in all treatments, double-headed sperms were not observed in positive control. While proximal droplet was found in negative control and mint treated mice, this was not observed in positive control, garlic and narra extract treated mice.

This variation in the frequency of abnormalities (mean percentage) is significantly different (Table 2). The mint, garlic, and narra extracts decreased significantly the number of abnormal sperms compared to negative control. On the other hand, mint and narra extracts showed insignificant frequency difference with the positive control. This means that mint and narra extracts were more effective in decreasing the frequency of sperm abnormalities compared to garlic extract.

Table 3. Frequency of sperm abnormalities among treatments

TYPES OF SPERM ABNORMALITIES	POSITIVE CONTROL	NEGATIVE CONTROL	MINT EXTRACT	GARLIC EXTRACT	NARRA EXTRACT	SUM
Bent midpiece	23.6	36	17.6	30.4	25.8	133.4
Banana	6	20	6.2	13.4	18.2	63.8
Head agglutination	3.6	15.8	14.4	12.4	10	56.2
Beak	2.6	21	7	17.2	5.2	53
Tailless	9	14.2	5.4	5.2	12.8	46.6
Coiled tail	4.6	16.4	13.8	4	3	41.8

Headless	6.8	10.2	6	7.2	6.2	36.4
Hookless	2	5.8	6.2	6.2	4.8	25
Corrugated tail	4.8	2.4	6.2	5.6	5.6	24.6
Periform	3	7	3.2	6.6	2.6	22.4
Balloon	5	6.6	4.2	3	2	20.8
Rhomboid	2.4	6.4	1.2	4	1.6	15.6
Calyx	2	5.2	3	3	0.8	14
Distal droplet	0.8	8	1.6	0.2	2.2	12.8
Sunflower	1	3.2	3.6	3.8	0.8	12.4
Triangular	0.2	3.8	1.8	2.2	0.8	12.4
Funnel	0.4	4.8	1.4	1	0.8	8.4
Amorphous	1.2	2.6	1.8	1.2	0	6.8
Double headed	0	1.8	0.6	0.4	1	3.8
Double tailed	0.40	0.8	1.2	0.6	0.2	3.2
Proximal droplet	0	1.6	0.6	0	0	2.2
Total	79.4^{c}	193.6^{a}	107^{bc}	127.6^{b}	104.4^{bc}	612

Means with same letters are not significantly different at 0.05 level

The unique hook shape of mouse's sperms easily reveals any variations in normal morphology (Wyrobeck *et al*., 1983). The penetrating capability of the sperm, according to Pagulayan *et al.,* (1994), is related to the head shape acrosome and tail of sperms. Abnormalities in the sperm could affect their fertilizing ability by lessening the chances of successfully penetrating the egg. Bent midpiece, the

most frequently observed abnormality, seemed to be critical in term of motility because of its curved shape. Coiled tail and corrugated tail types also lessen motility. Calyx, beak, rhomboid, and triangular types appeared to be less motile because of their distorted heads compared to normal sperms. However, balloon, periform, sunflower, funnel, and hookless types appear to be critical conformations among different abnormalities since they lack the hook and considering the fact that the hook is a vital part in penetration. Chance of reaching the egg is decreased in amorphous type due to its enlarged head that lessens motility. According to Wyrobeck *et al* (1983), significant reduction of sperm motility is associated with infertility even when percentage of motile sperm is not affected. Furthermore, Coffin (1953) stated that fertility is also dependent on the degree on which sperms are normal. Unable to penetrate the egg were the head agglutination and double-headed types because of their clumped heads. Proximal and distal droplet types also appeared less motile due to enlarged middle piece. On the other hand, banana and double-tailed types do not seem to be critical in terms of motility and penetrating capability owing to their slender shape and presence of hook in the former and presence of extra tail in the latter.

Meanwhile, the headless sperm without its haploid nucleus cannot penetrate the egg nor the tailless sperm reached the egg. Wyrobeck *et al.* (1983) pointed out that these abnormal sperms might not reach the oviduct or participate in fertilization. It is implied that the higher the number of ejaculated abnormal sperm, the higher the probability of reduced fertility (Parastie, 2000).

Tetracycline, a known mutagen and teratogen, induces sperm abnormalities and teratogenesis in mice (Sylianco and Blanco, 1984). This antibiotic is not degraded in the body and excreted in the urine in the same chemical form as absorbed in both humans and farm animals (Bowman and Rand, 1980).

The mechanism by which sperm abnormalities are induced by mutagens is poorly understood or unknown (Parastie, 2000; EPA, 1999; Sylianco, 1995; Pagulayan *et al*., 1994). The abnormal morphology is said to be transmitted for at least two generations, and the increase in the frequency of abnormalities are thought to be consequence of either point mutation or small deletion (De Marini, 1983).

The characteristic shape of the head is carried on the autosomes (Topham, 1980). Some studies suggested that the Y chromosomes has a role in determining the sperm head shape such that the absence of this chromosome may lead to a significant increase in the frequency of deformed heads (Matthew *et al*., 1992).
The increased value of fertile matings may be attributed to the significant decrease in the frequency and occurrence of defective sperms that might have been able to fertilize the eggs of females. *In vivo* conditions of conception, according to EPA (1999), have demonstrated that significantly higher pregnancy rates were observed in cases of better sperm than damaged ones. However, pregnancy rates in *in vivo* conditions are influenced by multiplicity of parameters (WHO, 1992).

CONCLUSION AND RECOMMENDATION

Based on the objectives and findings of the study, the following conclusions and recommendations were drawn: a) leaf extracts of mint, garlic and narra decreased teratogenesis, b) embryos of treated mice showed no physical malformations, c) mint, garlic, and narra extracts were effective in decreasing sperm abnormalities in white mice.

From the results, the following are recommended: a) an identical study be conducted on organic solvent-extracted leaf extract and other plant organs using other known teratogens for comparison, b) a study be done on the anatomy of the embryos, c) a similar study on sperm motility be conducted, and d) a chemical study on identification, purification, and structure elucidation of the bioactive constituents of these medicinal plants..

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