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Modulating effects of *Piper nigrum* Fruit extract Against Adriamycin Induced Genotoxicity in Swiss Albino Mice

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ABSTRACT

In the present research, studies were carried out to examine the modulating effects of crude *Piper nigrum* fruit extract against Adriamycin induced genotoxicity in germ cells of Swiss Albino mice using Sperm Morphology Assay. Two experiments were conducted using three different doses 200, 300, 400 mg/kg body weight of the crude *Piper nigrum* fruit extract and 16mg/kg body weight Adriamycin in germ cells of mice. In first test the antigenotoxic effects of *Piper nigrum* extract was studied and in the second test one group of animals were treated with Adriamycin alone and other groups of animals were primed with crude *Piper nigrum* fruit extract prior to adriamycin treatment . Control animals were fed only with physiological saline. All the exposed and control animals were sacrificed on last day of fifth week and cauda epididymis was dissected out, smeared, stained and screened for the presence of sperm abnormalities in all control and treated animal groups. The results were found to be insignificant in crude *Piper nigrum* fruit extract treated animal groups. A significant increase in the percentage of abnormal sperms were noted when treated with Adriamycin alone and the cells showed inhibition in the percentage of abnormal sperms when animals were primed with crude *Piper nigrum* fruit extract prior to adriamycin treatment. The present study reveals that the crude *Piper nigrum* fruit extract nonmutagenic nature. Hence crude Piper nigrum fruit extract supplementation is safer in chemotherapeutic strategy.

Keywords: *Piper nigrum* Fruit Extract, Adriamycin, Genotoxicity, Germcells.

I. INTRODUCTION

Toxicity is the level to which a chemical substance or a particular mixture of substances can harm an organism.Toxicity can refer to the effect on a whole organism, such as an animal, bacterium, or plant, as well as the effect on a substructure of the organism, such as a cell (cytotoxicity) or an organ such as the liver (hepatotoxicity). Antineoplastic drugs are medicines that are used to treat some forms of cancer.

Genotoxic drugs are chemotherapy agents that affect nucleic acids and alter their function. These drugs may directly bind to DNA or they may indirectly lead to DNA damage by affecting enzymes involved in DNA replication[1]. The mode of action also explains many of the side effects of treatment with these drugs. Rapidly dividing cells, such as those that line the intestine or the stem cells in bone marrow, are often killed along with the cancer cells [1.] In addition to being cytotoxic (cell poisons), these drugs are also mutagenic (cause mutations) and carcinogenic (cause cancer). Treatment with these drugs carries with it the risk of secondary cancers, such as leukemia. These drugs are used to treat a variety of solid cancers and cancers of blood cells, often in combination with other drugs[1]. Some examples of chemotherapy drugs are :Carboplatin, Cisplatin, Cyclophosphamide, Daunorubicin, Doxorubicin(Adriamycin), Epirubicin, Idarubicin, Mitomycin C., etc,.

Adriamycin is an anti-cancer ("antineoplastic" or "cytotoxic") chemotherapy drug and derived by chemical semisynthesis from a bacterial species [2]. It is an anthracycline antibiotic closely related to the natural product daunomycin. This medication is classified as an "anthracycline antiobiotic." Its generic name is Doxorubicin and other brand name is Rubex. The drug is administered intravenously, as the hydrochloride salt.

Used in combination with cyclophosphamide, vinca alkaloids and other agents, it is an important ingredient for the successful treatment of lymphomas. It is valuable component of various regimens of chemotherapy for adjuvant and metastatic carcinoma of the breast and small cell carcinoma of the lung. The drug also is particularly beneficial in a wide range of pediatric and adult sarcomas including osteogenic, Ewing and in soft tissue sarcomas. Doxorubicin (Adriamycin) is an antineoplastic drug which is cell cycle specific for the S-phase of cell division [3].

Natural remedies have been investigated for centuries for a wide variety of ailments. The use of plants in medicine goes as far back as thousands of years and still continues today [4.] Many plants are used for the treatment of different diseases. Several dietary constituents modulate the process of carcinogenesis and prevent genotoxicity. As far as diet is concerned, the value of fruit and vegetable consumption was promoted; with researchers claiming that plant based diets prevent 20%-50% of cases of cancer. The positive effects of consuming fruits and vegetables have been attributed to the antioxidant properties of certain substances contained in them. It is well known that antioxidants are almost universal antimutagenic agents. [5,6].

Dietary spices have been traditionally used to increase the flavor of food and as medicinal tools for the treatment of disease [7,8].. In literature, several studies have established there is inverse relationship between diet high in plant foods (spices) and risk for disease[9]. Epidemiological data supported the health benefits associated with intake of dietary spices are naturally rich source of phytochemicals including low molecular weight polyphenolics, flavonoids, carotenoids, anthocyanins. Many spices contain high molecular weight polysaccharides which may have positive effect on human health [10,11].

Black pepper (*Piper nigrum*) is a flowering vine in the family Piperaceae, cultivated for its fruit, which is usually dried and used as a spice and seasoning. Peppers are an important source of nutrients in the human diet and an excellent source of Vitamins A and C, neutral and acidic phenolic compounds, which are important antioxidants for a variety of plant defense responses[12]. Medicinal plants continue to play an important role in the healthcare system of a large number of the world population [13]. Natural antioxidants were known to exhibit a wide range of biological effects including antibacterial, antiviral, anti-inflammatory and vasodilatory activities[14].Secondary metabolites mainly phenolics provide antioxidants, antimicrobial, antitumour, antiviral, enzyme inhibiting and radical scavenging properties[15]. Hence in the present investigation an effort has been made to test the efficiency of *Piper nigrum* fruit extract against adriamycin induced genotoxic damage in germ cells of mice using Sperm Morphology Assay.

II. METHODS AND MATERIAL

2.1 PROCUREMENT AND IDENTIFICATION OF PLANT MATERIAL:

The plant material that is fresh dried fruits of *Piper nigrum* were procured from wholesale spice and herbs market, Hyderabad and were identified in Department of Botany, Osmania University, Hyderabad. Fresh dried fruits of Piper nigrum were cleaned and washed with deionised water, sliced and dried in the sun for one week and again dried at 50°C

in a hot air oven for 6 hours. Dried fruits were powdered by electronic mill.

2.2 DRUGS AND CHEMICALS :

Adriamycin of Pfizer company was bought from Apollo Pharmacy, Hyderabad and Eosin stain (Himedia). The chemicals and glassware used in the study are purchased from Rahul Scientifics , Hyderabad, Telangana.

2.3 PREPARATION OF EXTRACT:

Piper nigrum (Black pepper)fruits were collected from the local Super market. Dry spices (100 gm) were crushed and sieved through mesh cloth to get the fine powder. Powdered spices were soaked in 200ml of distilled water and were kept at room temperature for 24 hours, then were filtered using Whattman no. 1 filter paper. The filtrate was heated at 40-50°C using water bath, until thick paste is formed. The thick paste was considered as 100% concentration of extract. These extracts were stored at 4°C in refrigerator [16].

2.4 EXPERIMENTAL ANIMALS :

Eight to ten weeks old male mice (*Mus musculus*) weighing about 25gm, procured from National Institute of Nutrition, Hyderabad, were used in this study. The mice were housed in polypropylene cages in a well ventilated room and were provided with standard pellet diet (M/S Lipton India limited) and water ad libitum. They were maintained under controlled conditions of temperature and light.

For Sperm Morphology Assay, in the first test three doses of *Piper nigrum* fruits extract (PFE) i.e. 200, 300 and 400 mg/kg body weight were administered for seven days and control group of animals were fed only with physiological saline. In the second test animals were divided into groups and group I ,group II, group III, group IV and were given 16 mg/kg body wt adriamycin (ADR), 200(PFE)+16(ADR) mg/kg body wt, 300(PFE)+16(ADR) mg/kg body wt,400(PFE)+16g(ADR) mg/kg body wt respectively. *Piper nigrum* fruits extract was dissolved in double distilled water and administered as a single dose in 2 ml per mouse for 7days prior to adriamycin administration. The drug was supplied by Apollo Pharmacy, Hyderabad. For each dose group three animals were used and for each animal 500 sperms were screened. The animals were given adriamycin intravenously in a single dose within 24 h interval. The control group of mice received physiological saline simultaneously.

2.5 SPERM MORPHOLOGY ASSAY:

All the exposed and control animals were sacrificed on last day of fifth week by cervical dislocation after exposure to adriamycin. Spermatogonial stage which is exposed to drug would reach to cauda epididymis after undergoing a series of changes during the process of maturation to give rise to sperm. Animals were sacrificed and dissected out for both testis and cauda epididymis, which were removed and placed in petridish containing 0.9% NaCl (hypotonic physiological saline) solution. The cauda epididymis were teased thoroughly to release the sperm and stained with 1% aqueos eosin for about 20-30 minutes. A drop of sperm suspension was smeared on a clean slide. Five sperms per animal were scored for each group for the presence of sperm shape abnormalities[17].

2.6 STATISTICAL ANALYSIS:

For statistical evaluation of the experimental data Chi-Square test was performed. To determine the frequency of various sperm head abnormalities about 500 sperms were scored for each animal for the presence of amorphous , banana shaped, hammer headed, pin headed sperms etc,. All the data was analysed for the significance of experimental versus control data using the Chi-square test.

III. RESULTS AND DISCUSSION

Cytogenetic procedures are very helpful to evaluate the clastrogenic activity of chemicals which are chief components of chemotherapy drugs that are responsible for genotoxicity. The Sperm Morphology Assay functions as an important and sensitive indicator in assuming reproductive genotoxicity. They can be used to estimate the spermatogenic impairment, fertility and heritable genetic alterations. In the present investigation Sperm Morphology Assay was conducted following the criteria of Wyrobek and Bruce (1975). As numerous types of mutations can lead to abnormal sperm morphology, this test is treated as more sensitive test in detecting germ cell mutagens than other germinal mutagenicity assays [18]. Sperm morphology assay is also said to provide a quantitative technique for locating genetic impairment in male germ line cells. In our laboratory numerous drugs have been tested for the induction of sperm head abnormalities and published elsewhere [19].

Several in vitro and in vivo experimentations revealed the therapeutic potential of Piperine, an active ingredient of *Piper nigrum* and defensive effects of piperine. However in our investigation we intended to evaluate the modulating effects of crude extract of *Piper nigrum* against adriamycin induced genotoxic impairment. An effort has been made in the present experimentation to evaluate whether such toxic effects induced by adriamycin are counteracted or counter balanced by administration of Piper nigrum fruit extract .

In the present examination the higher occurrence of sperm abnormality induced by adriamycin is a measure of genetic impairment produced at the spermatogonial stage of the mouse germ cells. In the present examination the results on the incidence of sperm head abnormalities in Piper nigrum fruit extract treated animals are represented in Table 1. There is a slight rise in the percentage of abnormal sperms that is 2.00% in controls to 2.33, 2.80, 3.20 in 200, 300 and 400 mg/kg body weight *Piper nigrum* fruit extract in treated animals respectively. The differences in the occurrence of sperm abnormalities were found to be statistically insignificant when compared between control and *Piper nigrum* fruit extract treated groups. Hence, the results clearly indicate the antimutagenic nature of *Piper nigrum* fruit extract. The results on the percentage of sperm abnormalities in *Piper nigrum* fruit extract + Adriamycin treated mice are presented in Table 2.

There is a rise in the percentage of abnormal sperms that is 13.00% when adriamycin alone is administered in a group compared to control group that is 2.00% . There is a gradual reduction in the percentage of abnormal sperms that is 11.20%, 9.86%, 8.13% in16mg/kg ADR, 200 +16 mg/kg B.wt, 300+16 mg/kg B.wt and 400+16 mg/kg B wt. *Piper nigrum* fruit extract + Adriamycin treated animals respectively when compared to adriamycin alone treated group that is 13.00%. There is a significant reduction in the incidence of sperm head abnormalities in groups primed with *Piper nigrum* fruit extract (**P<0.01, *P<0.05).There is an increase in the percentage of inhibition in the percentage of abnormal sperms with increase in the dose of *Piper nigrum* fruit extract in *Piper nigrum* fruit extract +Adriamycin treated animals. The percentage of inhibition by *Piper nigrum* fruit extract in 200 +16 mg/kg B.wt, 300 +16 mg/kg B.wt and 400 +16 mg/kg B wt. doses of *Piper nigrum* fruit extract +Adriamycin treated animals is 13.75%, 26.25 % and 42.50% respectively (Table.2; Graph.1). Various types of Sperm abnormalities were shown in the Figures(1-8).

Table 1. Frequency of sperm head abnormalities in mice administered with various doses of *Piper nigrum* Fruit extract (PFE).

Treatment dosage in mg/kg/bw	Normal sperms		Abnormal sperms	
		%		%
Control	1470	98.00	30	2.00
200 PFE	1465	97.66	35	2.33
300 PFE	1458	97.20	42	2.80
400 PFE	1452	96.80	48	3.20

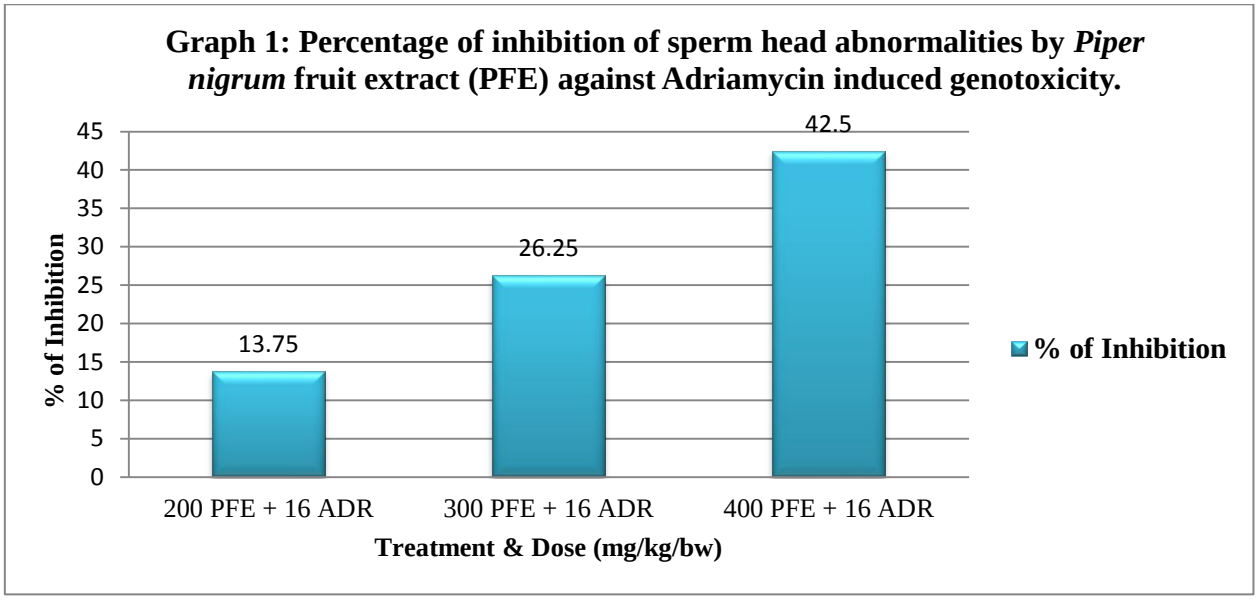
The $p>0.05$ level, hence the difference is considered to be statistically insignificant.

Table 2. Frequency of sperm head abnormalities in Adriamycin (ADR) treated mice primed with of *Piper nigrum* Fruit extract (PFE).

Group	Treatment &Dose (mg/kg/bw)	Normal sperms		Abnormal sperms		% of Inhibition
			%		%	
Group I	Control	1470	98.00	30	2.00	
Group II	16 mg/kg ADR	1310	87.00	190	13.00**	
Group III	200 PFE + 16 ADR	1332	88.80	168	11.20	13.75
Group IV	300 PFE + 16 ADR	1352	90.13	148	9.86*	26.25
Group V	400 PFE + 16 ADR	1378	91.86	122	8.13*	42.50

The $p^{**}<0.01$ level, hence the difference is considered to be statistically very significant.

$p^{*}<0.05$ level , hence the difference is considered to be statistically significant.



Various types of Sperm Abnormalities.

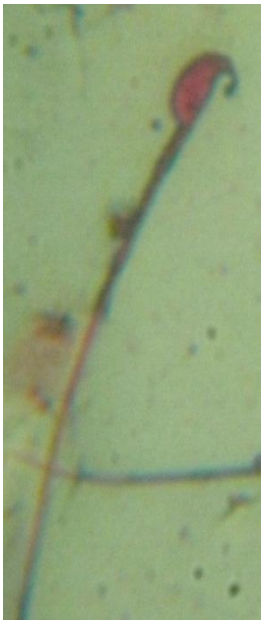


Figure 1. Normal Sperm

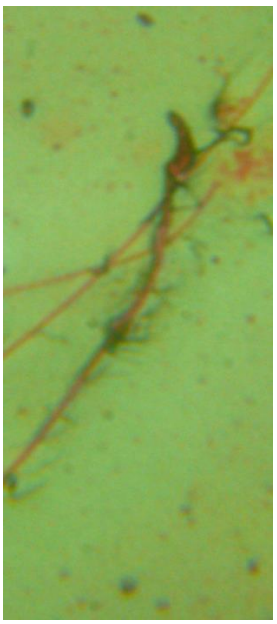


Fig:ure 2. Sickle shaped

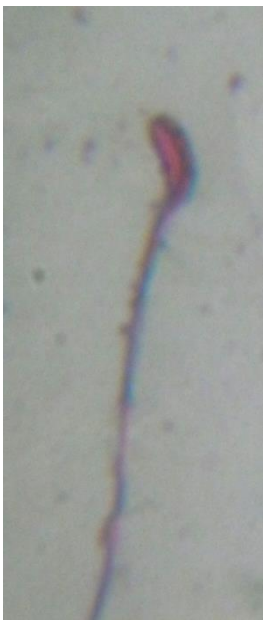


Figure 3. Banana shaped

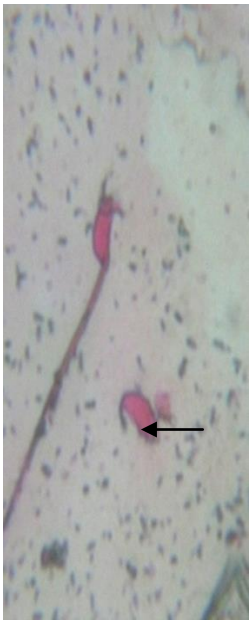


Figure 4. Head detached

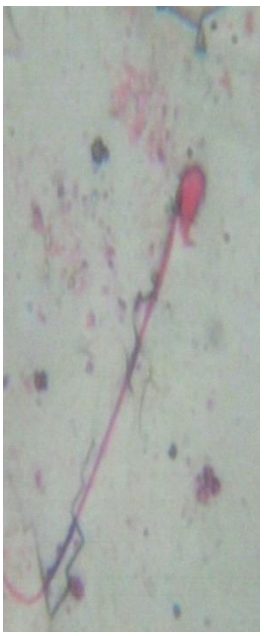


Figure 5. Neck bent

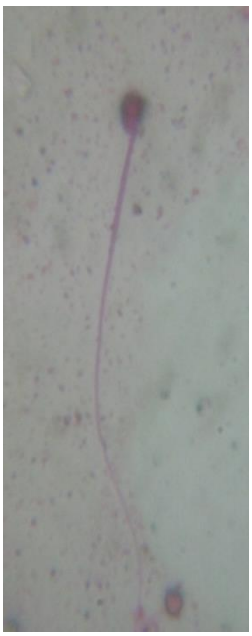


Figure 6. Pin headed

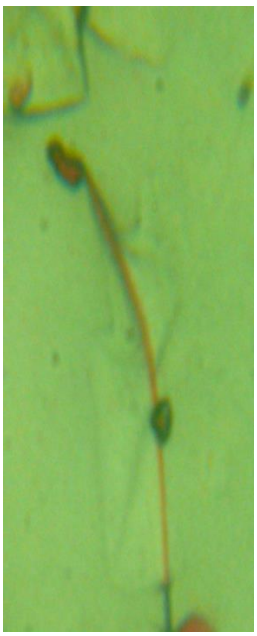


Figure 7. Hammer headed

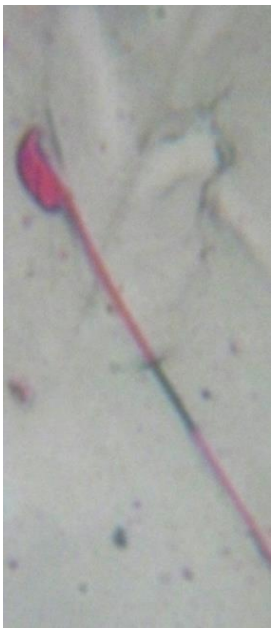


Figure 8. Hookless sperm

The main genetic effect of adriamycin and related compound is binding to DNA. It is known that adriamycin and other anthracyclines induce peroxide production in a variety of tissues. Cellular enzymes are capable of converting adriamycin into free radical metabolites , adriamycin cytotoxicity may be mediated by free radicals derived from this drug [20.] The present results are comparable with that of Meistrich et al [21.] who has studied sperm production and fertility in male mice treated with adriamycin (ADR) at 6 or 8 mg/kg body weight. Testicular sperm production and epididymal sperm counts were markedly lowered after adriamycin treatment. Gradual improvement of counts occurred but sperm counts have not reached control levels even more than 1 year after treatment. Epididymal sperm morphology indicated that adriamycin treatment induced morphological abnormalities throughout the

test; the frequencies of sperm with detached tails and the frequencies of sperm with morphologically abnormal heads continued to raise for about 2-3 fold above control.

Similarly Mestrich *et al*, [22.] established that the mutagenic effect of Adriamycin on mouse spermatogonial stem cells is tested by assessment of spermatocyte chromosome and of dominant lethality transmitted through the spermatozoa. The effect of adriamycin on mutation, cytotoxicity and sperm head abnormalities were compared with that of radiation. The cytotoxic effect of 6GY of gamma-radiation on stem spermatogonia was equivalent to about 4-5 mg/kg b.wt. adriamycin. Chromosomal translocations were observed in 6% of spermatocyte of mice treated with adriamycin.

Piperine and related compounds have been shown to reduce cell proliferation, angiogenesis and metastasis in breast cancer cells. Piperine enhances the anti-cancer effects of curcumin (the most biologically active component of turmeric) [23.]. Piperine has been found to increase the cytotoxicity of Adriamycin in drug resistant hormone receptor positive (ER+/PR+) breast cancer cells. Now a new study has reported that piperine increases the cell-killing effects of Taxol (paclitaxel) in HER2/neu overexpressing (HER2+) breast cancer cells. [23.]

in and Han,[24.] investigated the enhanced oral exposure of fexofenadine (10 mg/kg) in rats in the presence and absence of piperine (10 or 20 mg/kg, given orally). Results of study indicated that combination of piperine increases the oral exposure (AUC) of fexofenadine by 180% to 190% and bioavailability approximately by 2-folds. They concluded that this effect of piperine likely due to the inhibition of P-glycoprotein-mediated cellular efflux during the intestinal absorption [24.]

Janakiraman and Manavalan, [25.] aimed to include Piperine (bioenhancer) as a formulation additive in oral formulations of Ampicillin Trihydrate. Physical mixture of Ampicillin Trihydrate and Piperine (1:1) was tested for their compatibility and stability study. The above studies proved that Piperine can be used as a formulation additive for bioenhancing effect in oral formulations of Ampicillin Trihydrate [25.]

Black pepper is the world's most traded spice. It is one of the most common spices added to European cuisine and its descendants. The spiciness of black pepper is due to the chemical piperine. Black Pepper (or perhaps long pepper) was believed to cure illness such as constipation, diarrhea, earache, gangrene, heart disease, hernia, hoarseness, indigestion, insect bites, insomnia, joint pain, liver problems, lung disease, oral abscesses,sunburn, tooth decay, and toothaches. As a medicine, pepper appears in the Buddhist Samannaphala Sutta, chapter five, as one of the few medicines allowed to be carried by a monk [26.] Piperine and other components from black pepper may also be helpful in treating vitiligo, [27.] However, extracts from black pepper have been found to have anti-carcinogenic effects, antioxidant ,anti inflammatory and antiulcer properties [28,29.]respecially when compared to chili [30.] Anti inflammatory [31.] Antidepressent nature [32.], antioxidant properties [33.], anti diabetic nature[34.], Protective effects [35.] Nephroprotective [36.]. It has been shown that piperine can dramatically increase absorption of selenium, vitamin B,beta- carotene and curcumin as well as other nutrients it means it works as a Bioenhancer [37, 38, 39.]

IV. CONCLUSION

Animals when treated with different doses of crude *Piper nigrum* fruit extract revealed antimutagenic effect and the percentage of sperm head abnormalities were nearly equal with that of control values. In the present investigation Adriamycin, a chemotherapy drug presented significant rise in the frequency of abnormal sperm morphology, but when animals primed with *Piper nigrum* Fruit extract , a significant

inhibition of genotoxicity was observed in adriamycin treated animals. Thus the overall results indicate the modulating effects of *Piper nigrum* Fruit extract against drug induced damage in swiss albino male mice.

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