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ORIGINAL ARTICLE **OPEN ACCESS**

Genoprotective Nature of Isolated D-Pinitol from Glycine Max L Merr. Plants Against Doxorubicin-Induced Genotoxicity Evaluated by *In Vivo* Sperm Shape Abnormality Assay

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ABSTRACT
This research study examined the genoprotective effect of D-Pinitol (D-P) against the genotoxicity caused by Doxorubicin (DOX) in albino mice. Sixty male albino mice were divided into ten groups. For 15 days, 0.9% normal saline was administered to Group I (Control). On 1st, 8th and 15th days, intraperitoneal injection of DOX (5 mg/kg) was administered to Group I (Positive control). For 15 days, D-P 100 mg/kg, 200 mg/kg, 300 mg/kg & 400 mg/kg orally was administered to Group III, IV, V & VI respectively. DOX (5 mg/kg) and D-P 100 mg/kg were administered to Group VII. DOX (5 mg/kg) and D-P 200 mg/kg were administered to Group VIII. DOX (5 mg/kg) and D-P 300 mg/kg were administered to Group IX. DOX (5 mg/kg) and D-P 400 mg/kg were administered to Group X. The role of D-P on DOX-induced genotoxicity was assessed using in vivo Sperm shape abnormalities test. In the animals treated with DOX, a significant (P<0.001) rise in sperm shape abnormalities and a significant decrease in sperm count were observed. In comparison to the control group, the groups treated with D-P alone did not change the abnormalities in sperm shape or sperm count. When D-P is combined with DOX, there is a dose-dependent reduction in sperm shape abnormalities and an increase in sperm count compared to DOX alone treated group (Group II). Through its antioxidant, free radical scavenger, and anti-inflammatory properties, D-P was effective in providing protection. Since D-P has exhibited protection against DOX-induced genotoxicity, it can be utilized as a genoprotective agent.
Keywords: *D-Pinitol, Doxorubicin, Protective effect, Genotoxicity, Genoprotective agent.*

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INTRODUCTION

Neoplasms appear to have increased in prevalence in economically prosperous nations as a consequence of population, ageing and growing, as well as a rise in the acceptance of cancer-related lifestyle changes like cigarette use, inactivity, and sophisticated eating practices [1]. One of the main causes of death in the globe now is neoplasm [2]. Doxorubicin (DOX), an effective chemotherapeutic medication, is an anthracycline antibiotic that is used to treat a number of human malignancies [3]. By intercalating into DNA, blocking topoisomerase II, and halting the production of DNA and RNA, DOX is able to exert its therapeutic benefits [4]. Apparent toxicity to normal tissues apart from its cytotoxic action to tumor cells restrict DOX's therapeutic use because of inflammatory responses, free radical generation, and oxidative stress [5][6]. As DOX plays a significant role in cancer treatment, it is crucial to lessen its toxicity to normal cells. This can be done by administering simultaneously, such as free radical scavengers, antioxidants and anti-inflammatory agents. Hence, a possible therapeutic approach to DOX-induced toxicity is to reduce oxidative stress, and inflammation [5]. A naturally occurring cyclitol molecule called D-Pinitol (D-P) [7] has been utilized in the traditional practice of Ayurveda medicine for many years [8]. D-P is widely distributed in all regions of soybean plants (Glycine max L. Merr., a member of the Leguminosae family), where it is the most abundant soluble carbohydrate [9] [10]. D- P's ability to decrease or inhibit oxidative stress [11] and the inflammatory process [12] is responsible for its most therapeutic actions such as cancer preventive [13], cardioprotective [14], hepatoprotective [15] and renal protective effect [16]. So, the purpose of this investigation was to ascertain if isolated D-P could attenuate the genotoxic effects of DOX in normal tissues.

MATERIAL AND METHODS

In vivo Sperm shape abnormality assay:

Materials Required:

Doxorubicin HCL (CIPLA, India), Giemsa stain (Hi-media, India), and Microscope (Olympus Optical Co., Germany).

Animal Care and Handling:

Swiss albino mice (Sex: Male) weighing 25 to 30 g were housed in cages with a twelve - hour light/dark cycle. The animals were acclimated according to CPCSEA criteria before the study began [17]. According to studies conducted by Hajra et al. and Navaaro et al., the DOX and D-P dosages were chosen, respectively [5][18].

Methodology:

According to the treatment protocol (Table 1), animals received D-P for 15 days and DOX for 3 days (on first day, eighth day, and fifteenth day) [19]. D-P treated 30 minutes prior to the DOX administration. After twenty-four hours of the last treatment, the animals were sacrificed by exposing them to carbon dioxide inhalation. The epididymis was extracted through laparotomy, and the sperm suspension was made by chopping up the epididymis in 1 ml of normal saline. After staining with 1 percent Giemsa for 30 minutes, the solution was filtered through 80 mm nylon mesh to prepare smears to assess sperm shape abnormalities. A microscope was used to count the morphological abnormalities in sperm shape at 100 X magnification. One thousand sperms were examined for morphological damage in each animal, and the results were represented as a percentage of total abnormalities. Neubauer's hemocytometer was used to count the sperm in the epididymis. The results were expressed as the number of sperm per milligram of epididymis weight [20].

Statistical Analysis:

One-way ANOVA for this research was performed statistically using GraphPad Prism software version 8.01. Statistics were evaluated to be significant at P values under 0.05 ($P<0.05$).

RESULTS AND DISCUSSION

The number of different types of sperm abnormalities is shown in both Table.2. & Figure.1. The percentage of abnormal sperm is shown in Table.3. and Figure.2. and the total number of sperm count is represented in Table.3. and Figure.3. Amorphous head, Headless Sperm, Tailless Sperm, Bent at the cephalocaudal region, Bent tail, and other abnormalities such as two-tailed sperms were all taken into account while looking for sperm abnormalities (Figure.4.). A few aberrant sperms per thousand sperms were found in both the vehicle control and D-P solely treatment groups. On the alternative side, the positive control, DOX, demonstrated a significant rise in the abnormalities in sperm shape. The D-P and DOX groups exhibited a significant ($P<0.001$) decline in sperm abnormalities in a dose-dependent manner as compared to the DOX-only treated group. Both the vehicle control and D-P alone treated groups of mice had normal sperm counts. However, compared to the vehicle control mice, animals treated with DOX had a significantly ($P<0.001$) reduced sperm count. When supplemented with DOX, D-P significantly ($P<0.001$) raised sperm count in a dose-dependent manner.

Genotoxic studies are useful for understanding the extent of DNA damage caused by medication. Genotoxic substances can impair a cell's genetic makeup [21][22]. DOX, a genotoxic agent is possible inducers of sperm cell morphology changes because they can affect the normal events of gametogenesis [23]. In support of the earlier result, the current investigation revealed that DOX treatment, by its capacity to trigger oxidative stress and inflammatory activity, significantly increased sperm shape abnormalities and lowered sperm count [24]. D-P did not exhibit any abnormalities in the shape of sperm and sperm count when tested for genotoxicity. From the Table.2. & Table.3. and Figure.2. & Figure.3., it is also revealed that administration of DOX resulted in an abnormal reduction in the sperm shape abnormality and an increase in sperm counts. While our findings explicitly showed that pre-administration of D-P with DOX decreased genotoxicity in germ cells caused by DOX in mice, as demonstrated by reduced sperm shape defects and improved sperm count.

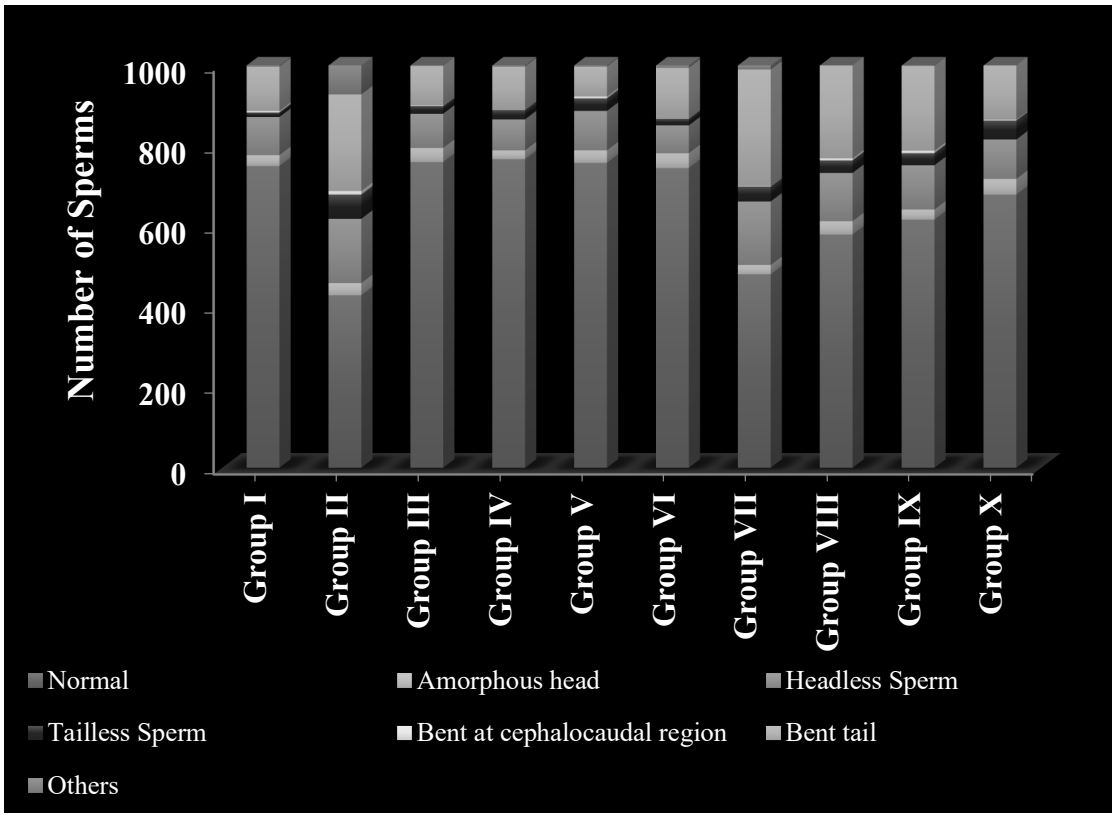

Figure.1. Histogram - Number of Sperm abnormalities that occurred in treated groups of mice

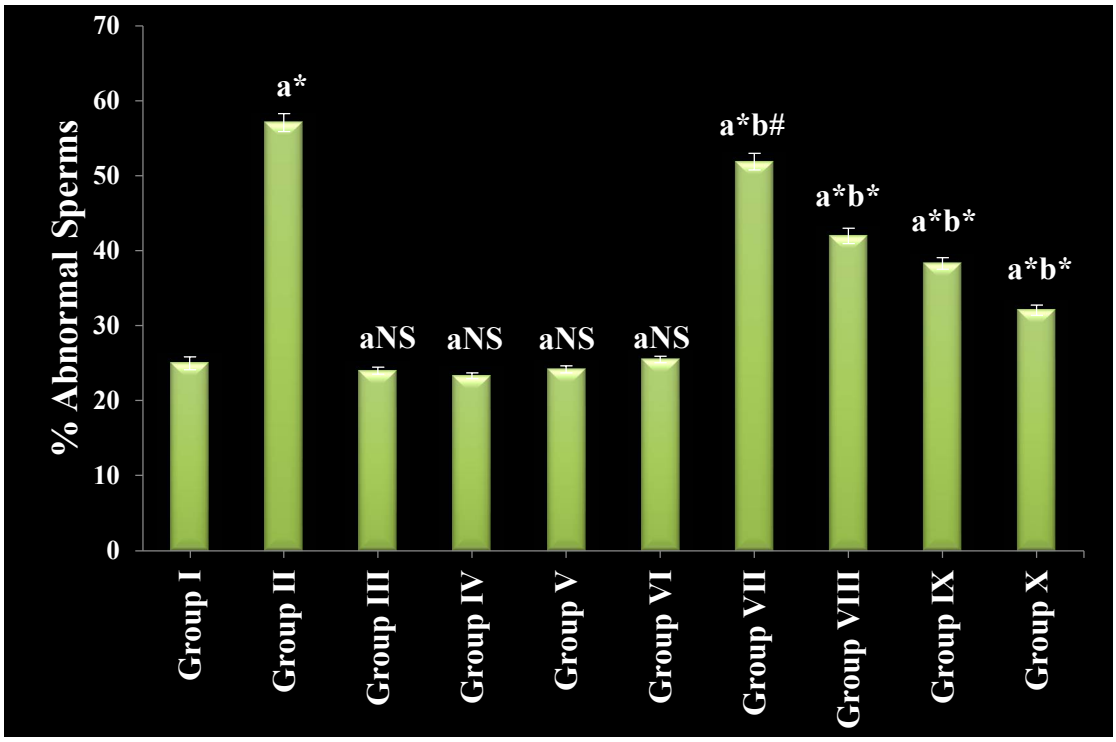

Figure.2. Histogram - Percentage of abnormal sperms

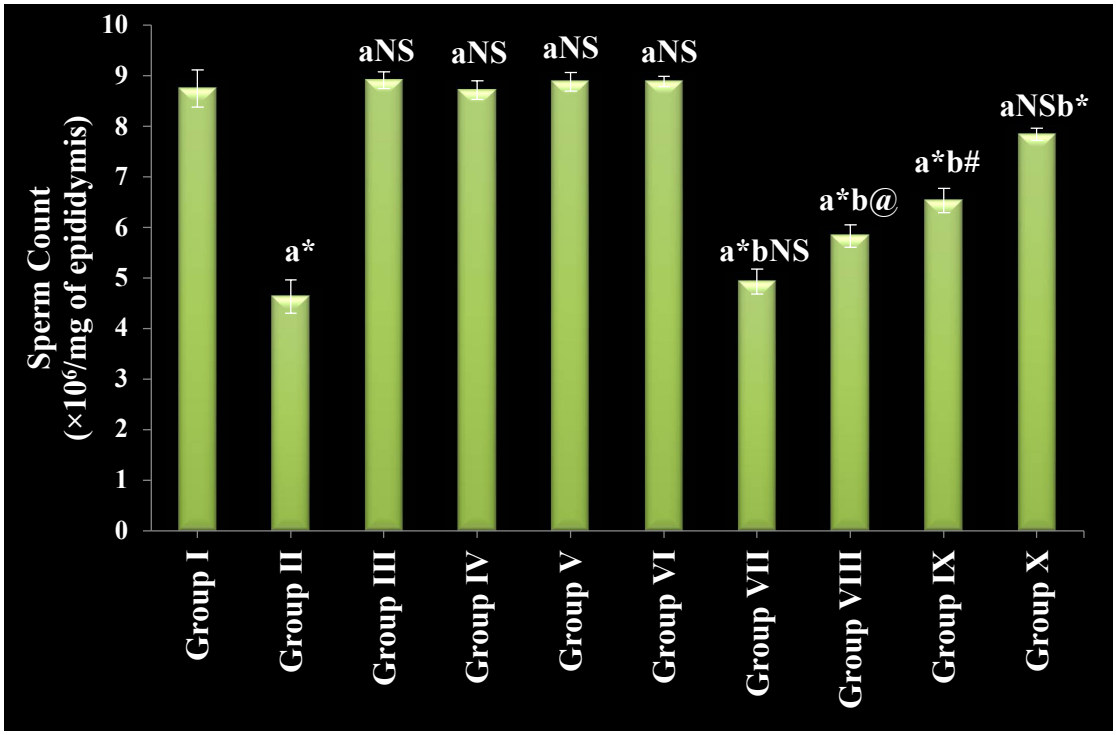

Figure.3. Histogram - Total Number of Sperm Count

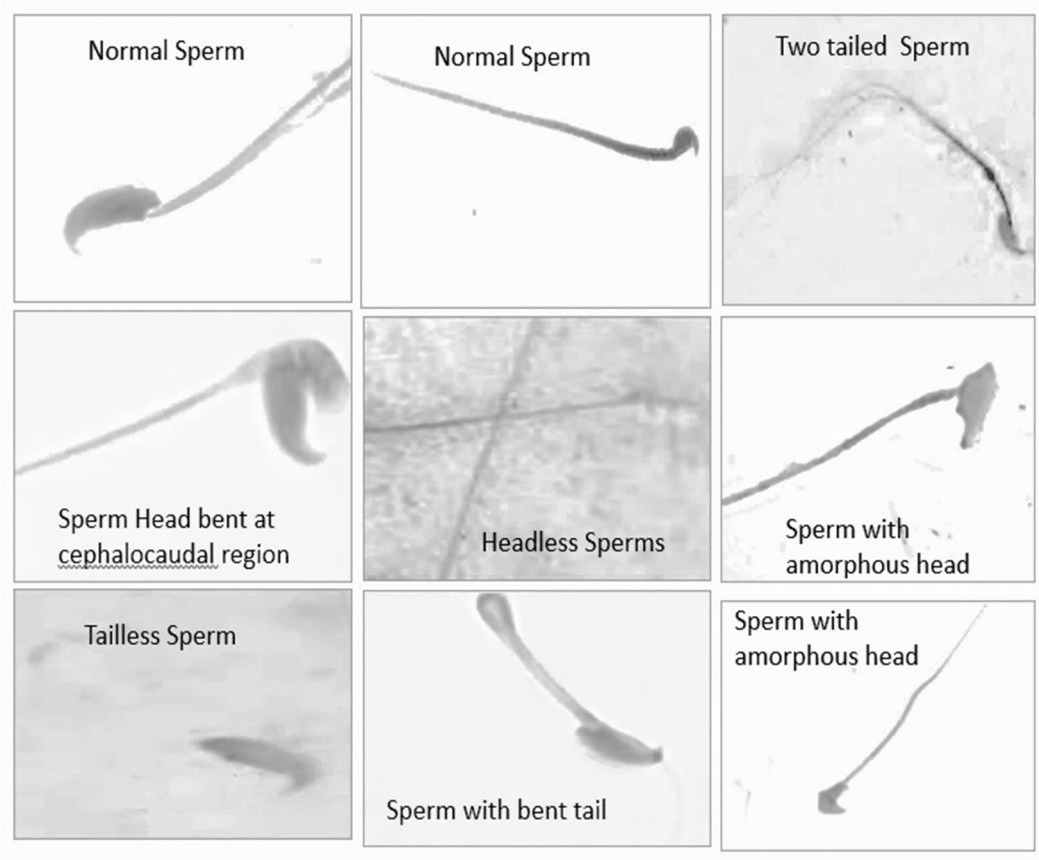

Figure.4. Abnormal Sperms in the treated groups of mice

Table.1. Treatment Protocol

Groups	Labeled	Treatment
I	Vehicle Control	0.5 ml of 0.9% normal saline
II	Positive Control	Doxorubicin (5 mg/kg), i.p. on 1st, 8th and 15 th days (Positive Control)
III	Test	D-Pinitol (100 mg/kg), p.o. daily
IV		D-Pinitol (200 mg/kg), p.o. daily
V		D-Pinitol (300 mg/kg), p.o. daily
VI		D-Pinitol (400 mg/kg), p.o. daily
VII		Doxorubicin (5 mg/kg), i.p. on 1st, 8th and 15 th days+ D-Pinitol (100 mg/kg), p.o. daily
VIII		Doxorubicin (5 mg/kg), i.p. 1st, 8th and 15 th days + D-Pinitol (200 mg/kg), p.o. daily
IX		Doxorubicin (5 mg/kg), i.p. 1st, 8th and 15 th days + D-Pinitol (300 mg/kg), p.o. daily
X		Doxorubicin (5 mg/kg), i.p. 1st, 8th and 15 th days + D-Pinitol (400 mg/kg), p.o. daily

Table.2. Number of different types of sperm abnormalities that occurred in treated groups of mice

Nature of Sperms	Group I (Control- 0.9% Normal saline)	Dose in mg/kg								
		Group II (DOX 5)	Group III (D-P 100)	Group IV (D-P 200)	Group V (D-P 300)	Group VI (D-P 400)	Group VII (DOX 5+ D-P 100)	Group VIII (DOX 5+ D-P 200)	Group IX (DOX 5+ D-P 300)	Group X (DOX 5+ D-P 400)
Normal	750± 13.66	429± 19.14	760± 10.33	767± 18.32	758± 14.19	745± 13.84	481± 16.56	580± 24.18	617± 15.67	679± 16.71
Amorphous head	27± 2.78	30± 2.898	35± 2.098	22± 1.265	31± 1.549	37± 3.55	23± 2.62	33± 2.295	25± 3.386	39± 2.733
Headless Sperm	95± 5.209	160± 5.663	85± 2.394	77± 3.337	98± 3.204	69± 2.543	158± 2.781	120± 4.803	110± 4.524	98± 3.044
Tailless Sperm	11± 1.033	60± 3.941	19± 1.528	23± 1.183	31± 1.751	16± 1.211	37± 1.77	31± 1.528	30± 3.493	47± 4.597
Bent at cephalo -caudal region	4± 0.816	9± 0.632	1± 0	0	5± 1.342	0	1± 0	5± 1.238	6± 1.633	2± 0.365
Bent tail	110± 2.978	240± 6.557	99± 2.921	108± 3.256	74± 4.107	127± 3.367	290± 5.145	231± 6.218	211± 6.126	135± 3.483
Others	3± 0.73	72± 3.215	1± 0	3± 6.831	3± 8.944	6± 9.309	10± 1.183	0	1±0	0

Mean ± SEM, n=6.

Table.3. Effect of DOX and D-P on Sperm abnormalities in mice

Criterion	Group I (Control- 0.9% Normal saline)	Dose in mg/kg								
		Group II (DOX 5)	Group III (D-P 100)	Group IV (D-P 200)	Group V (D-P 300)	Group VI (D-P 400)	Group VII (DOX 5+ D-P 100)	Group VIII (DOX 5+ D-P 200)	Group IX (DOX 5+ D-P 300)	Group X (DOX 5+ D-P 400)
% Sperm abnormalities	25± 0.877	57.1± 1.194 a*	24± 0.492 aNS	23.3± 0.398 aNS	24.2± 0.46 aNS	25.5± 0.432 aNS	51.9± 1.088 a*b#	42± 0.996 a*b*	38.3± 0.789 a*b*	32.1± 0.695 a*b*
Sperm Count (×10⁶/mg of epididymis)	8.75± 0.363	4.633± 0.326 a*	8.917± 0.162 aNS	8.717± 0.183 aNS	8.883± 0.18 aNS	8.883± 0.101 aNS	4.933± 0.249 a*bNS	5.833± 0.223 a*b@	6.533± 0.243 a*b#	7.85± 0.12 aNSb*

Mean ± SEM, n=6, where a - Group II, III, IV, V, VI, VII, VIII, IX, X compared with Group I. b - Group VII, VIII, IX, X compared with Group II. * P < 0.00, # P < 0.01, @ P < 0.05.

CONCLUSION

D-P has a genoprotective effect against DOX-induced genotoxicity in germ cells. The genotoxic evaluation of D-P revealed that it did not induce any genotoxic effects. The antioxidant and anti-inflammatory properties of D-P would be the foremost reason for its genoprotective effect.

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LIST OF ABBREVIATIONS

DOX – Doxorubicin
D-P – D–Pinitol

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest for this study.

INFORMED CONSENT

The Institutional Animal Ethics Committee (IAEC) of Adhiparasakthi College of Pharmacy (Reg. No. 409/PO/Re/S/01/CPCSEA) approved the experimental protocol for in vivo chromosomal aberration assay. The approval number was APCP/IAEC/2019-2020/1.

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