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Pharmacological Study

Effect of *Drakshavaleha* in cyclophosphamide induced weight loss and reduction in crown-rump length in developing mice embryo

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Abstract

Being an anti-mitotic and apoptosis inducing agent, Cyclophosphamide (CP) causes stunting in size and loss of body weight of the pups on intra-peritoneal injection (10 mg/kg) to pregnant mice on day 11 of gestation. Loss of body weight due to CP administration could be minimized by feeding *Drakshavaleha* (16 g/kg) orally to pregnant mice from day "0" to day "18" of their gestation. Recovery observed in terms of body weight of the pups was statistically significant ($P < 0.001$) in *Drakshavaleha* treated pups. *Drakshavaleha* also recovered the crown-rump length of the pups occurred due to CP administration. Growth retardation with decreased fetal weight was observed in all CP treated pups when compared with the controls.

Key words: Cyclophosphamide, *Drakshavaleha*, mice, *Rasayana*, vitamins, weight-loss

Introduction

Programmed cell death is a part of normal development of an individual during his intra-uterine life. Altered rate of programmed cell death at a critical period of development may lead to serious structural defects. Likewise, altered rate of cell proliferation may also induce malformations.[1] Thus agents that interfere with the cell proliferation or differentiation cause congenital malformations. Cyclophosphamide (CP) is one of such agents.

Rasayana therapy is one of the eight branches of *Ayurveda*, constitute a group of single or polyherbal preparations made from plant extracts, commonly used to improve health and longevity. It has been reported that *Rasayanas* have immunomodulatory, anti-oxidant and anti-tumor functions improve memory, intelligence, youthfulness, luster, complexion, and efficiency.[2] It helps to preserve harmony in three psychobiological dimensions known as *Doshas*[3] and biological rhythms, which regulate the entire functioning of the physiology.[4] *Drakshavaleha* has been indicated to treat *Pandu* (anemia) and *Kamala* (jaundice).[5] Due to anti-anemic properties and nutritional values, it has been used as a *Naimittika Rasayana* (promoter of specific vitality in specific disease) by some of the Ayurvedic physicians in Rajasthan to a woman during her pregnancy expecting a good health of both mother and her offspring.

Prenatal supplementation with multiple micronutrients has a greater positive impact on birth weight than supplementation with iron/folic acid.[6] Keeping this fact in mind, the objective of the study was to investigate the protective effect of *Drakshavaleha* against CP induced growth retardation in mice pups in terms of body weight and crown-rump (CR) length. As most of the teratogens act by causing hypoxia, the protecting nature of the *Drakshavaleha* may neutralize their damaging effects by its *Rasayana* functions (oxygenating/anti-oxidant nature).

Materials and Methods

Avaleha or *Leha* intended for internal administration is a semi solid preparation of drugs by addition of *Sharkara* (sugar), decoctions, juices, etc., Its acceptance is more as compared to other classical Ayurvedic drugs. *Drakshavaleha* (one such *Avalehas*) used in the study was prepared as per the procedure of the *Avaleha Kalpana*.[7]

Ethical guidelines for use and care of laboratory animals were followed as per the guidelines of Indian Council of Medical Research, New Delhi ICMR.[8] Eighty mice (40 male and 40 female) with an average age of 50 days weighing 25 ± 5 g used in the study were procured. All mice were kept in isolation for 19 days to confirm that they are non-pregnant and had a normal estrous cycle. Mice found in proestrous phase of the

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cycle were caged with normal males of the same strain overnight in an air cooled room at 75°F with 50% humidity. Animals were provided standard food pellets and tap water *'ad libitum'* throughout the study. Mating was accomplished by placing one male and one female mouse in the polypropylene cage. Vaginal smear of the female mice were examined for spermatozoa at 8 a.m. next morning. Mating was confirmed by the presence of sperms in the vaginal smear. Mid night of the day of mating was designated as day "0" of the embryo development and subsequent 24 h period was considered as day "1" of the gestation. Sperm positive females were isolated and divided into the following groups:

Group I (Control): Pregnant mice (n = 10) received 0.2 ml of vehicle (distilled water) intra-peritoneal on day 11 of pregnancy. Group II (*Drakshavaleha*): Pregnant mice (n = 10) received *Drakshavaleha* (16 g/kg) orally from day "0" to day "18" of pregnancy. Group III (CP): Pregnant mice (n = 10) received CP (10 mg/kg) intra-peritoneal on day 11 of pregnancy. Group IV (CP + *Drakshavaleha*): Pregnant mice (n = 10) received CP (10 mg/kg) intra-peritoneal on day 11 under cover of *Drakshavaleha* (16 g/kg) orally from day "0" to day "18" of pregnancy [Table 1].

On day 19 of pregnancy i.e., 1 day prior to full term (20 days) mice were put under ether anesthesia and hysterectomy was done. The uterine sacs were inspected and recorded for sites of resumptions and viable fetuses. The fetuses were removed from the uterus and dried by wiping on a blotting paper. Every pup was weighed and measured (CR length) with the help of weighing machine (least count of 10 mg) and slide Vernier caliper respectively. These pups were examined under magnifying lens and dissecting microscope for external malformations.

Dose of *Drakshavaleha* was 16 g/kg body weight. CP manufactured by Biochem Pharmaceutical Industries Ltd, Mumbai, India with a trade name of "Inj. Cycloxan" was used in the experiment.

Statistical analysis

All the results expressed as mean ± Standard Deviation SD intergroup comparisons have been done by one way ANOVA followed by *Post-hoc* test Statistical Package for the Social Sciences (SPSS, Version 13).

Observations and Results

As seen in Table 2, the pups which were administered after *Drakshavaleha* throughout the period of gestation, average weight was 1.27 ± 0.10 g (93 pups) as against controls which had weight of 1.17 ± 0.17 g (67 pups). Its statistical correlation was highly significant ($P < 0.001$). The comparison was suggestive of significant gain of the weight of pups following *Drakshavaleha* administration to the mother mice. The CR length was also of same conclusion i.e., 2.47 ± 0.12 cm versus 2.35 ± 0.16 cm in the *Drakshavaleha* and controls respectively [Table 3]. It

Table 1: Grouping of the animals and posology

Group		CP (11th day)	*Drakshavaleha* (0-18 days)	Day of fetal collection
I	Control	Vehicle only	-	19
II	*Drakshavaleha*	Vehicle only	16 g/kg	19
III	CP	10 mg/kg	-	19
IV	CP+*Drakshavaleha*	10 mg/kg	16 g/kg	19

CP: Cyclophosphamide

Table 2: Effect of *Drakshavaleha* on weight of pups

Group	No. of mother mice	No. of fetuses	Weight in g (mean±SD)	Significant pairs (by *Post-hoc* test)
Control	10	67	1.17±0.17	Group I versus Group III
Drakshavaleha	10	93	1.27±0.10	Group I versus Group IV
CP	10	61	0.83±0.20	Group II versus Group III
CP+*Drakshavaleha*	10	76	1.00±0.16	Group II versus Group IV
				Group III versus Group IV
One-way ANOVA	*F*=75.97			

CP: Cyclophosphamide, SD: Standard deviation

Table 3: Effect of *Drakshavaleha* on crown-rump length of pups

Group	No. of mother mice	No. of fetuses	CR length in cm (mean±SD)	Significant pairs (by *Post-hoc* test)
Control	10	67	2.35±0.16	Group I versus group III
Drakshavaleha	10	93	2.47±0.12	Group I versus group IV
CP	10	61	1.97±0.22	Group II versus group III
CP+*Drakshavaleha*	10	76	2.24±0.18	Group II versus group IV
				Group III versus group IV
One-way ANOVA	*F*=75.35			

CP: Cyclophosphamide, CR: Crown-rump, SD: Standard deviation

was therefore concluded beyond doubt that *Drakshavaleha* increases size and weight of pups born to mothers who were fed *Drakshavaleha* during pregnancy. In CP group these two observations (weight and CR length) showed a significant decrease i.e., 0.84 ± 0.20 g (61 pups) and 1.97 ± 0.22 cm as compared with controls in which mean weight and CR length was 1.17 ± 0.17 g (67 pups) and 2.35 ± 0.16 cm respectively. The difference between two groups, i.e., CP versus control shows a significant decrease in size of pups ($P < 0.001$). In the group where mother mice were administered CP undercover of *Drakshavaleha* (group IV) the loss in the weight and size of the pups showed a significant recovery ($P < 0.001$). However, size remained smaller as compared with controls and animals fed with *Drakshavaleha* alone. The average weight of the pups of CP + *Drakshavaleha* group was 1.00 ± 0.16 g versus control and *Drakshavaleha* alone group, i.e., 1.17 ± 0.17 g and 1.27 ± 0.10 g respectively. The CR length showed recovery from an average of 1.97 ± 0.22 cm in CP group to 2.24 ± 0.18 cm in CP + *Drakshavaleha* group [Figure 1]. Though, there is obvious recovery from the CP group, the weight and size still remained smaller as compared to controls and *Drakshavaleha* groups.

In the present investigation, the effect of CP causing growth retardation and decreased fetal weight were seen [Table 3, Graph 1] when the drug was injected on 11th day of gestation in CP group. Among litters whose mothers received the drug (CP) on the same day in same dose under *Drakshavaleha* cover (group IV) the pups were seen with significant weight gain, which however, was lower as compared to the pups born to mothers with administration of *Drakshavaleha* alone (group II). CR length was also of same conclusion, i.e. 2.47 ± 0.12 versus 2.35 ± 0.16 cm in group II and group I respectively [Graph 2]. The above observation suggests that *Drakshavaleha* (alone) increases the size and weight of pups when given to mother mice during gestation period and the difference between the two groups is statistically significant ($P < 0.001$).

Table 4: Formulation composition of *Drakshavaleha*

Sanskrit name	Botanical name	Part used	Quantity
Draksha	*Vitis vinifera* Linn.	Fruit	3 kg
Kumkum	*Crocus sativus* Linn.	Stamen	12 g
Gojihva	*Onosma bracteatum* Wall.	Leaf	100 g
Yashtimadhu	*Glycyrrhiza glabra* Linn.	Root	25 g
Taruni pushpa	*Rosa centifolia* Linn.	Flower	50 g
Chandanam	*Santalum album* Linn.	Heart wood	25 g
Mangalya-kusuma	*Convolvulus pluricaulis* Linn.	Whole parts	100 g
Brahmi	*Bacopa monnieri* Linn.	Whole plant	100 g
Maricha	*Piper nigrum* Linn.	Fruit	50 g
Ela	*Elettaria cardamomum* Maton.	Seed	25 g
Sarkara	*Saccharum officinarum* Linn.	Sugar	5 kg

Discussion

CP is known to reduce fetal weight drastically and is an established teratogen. This reduction of weight is due to intensive anti-mitotic activity of CP. It also increases the rate of apoptosis. Teratogenic doses of CP on day 11 after copulation is associated with a dose-related increase in embryo lethality, gross morphological abnormality and cephalic

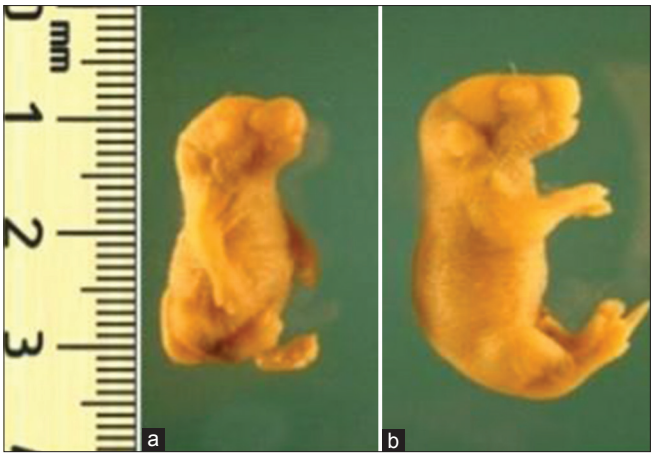
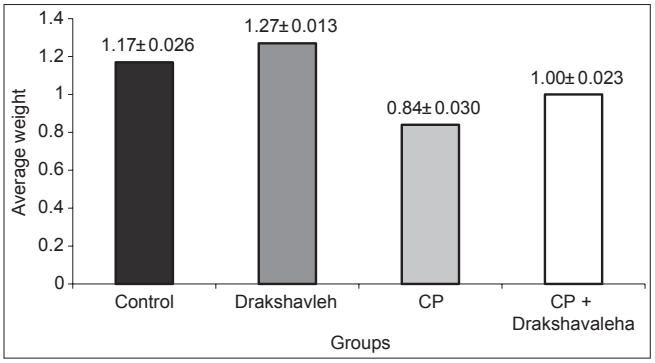
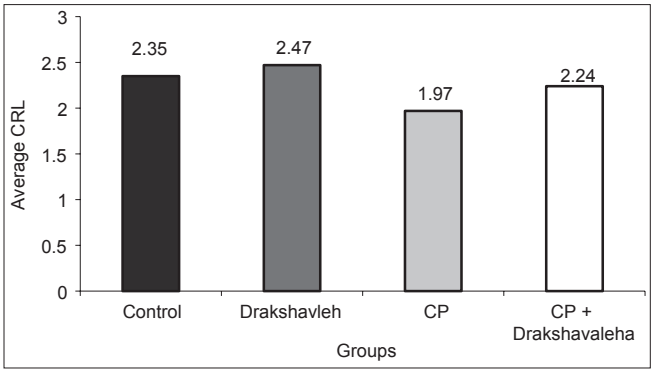


Figure 1: Pups of mice collected on day 19 of pregnancy. (a) Treated with cyclophosphamide (CP) (10 mg/kg on 11th day) showing the crown-rump length as compared with (b) Treated with both CP (10 mg/kg, 11th day) under cover of *Drakshavaleha* (16 g/kg, 0-18 days)



Graph 1: Mean weight (g) ± SE of control and treated pups



Graph 2: Mean crown-rump length (cm) of control and treated pups

Deoxyribonucleic acid DNA strand breaks and a reduction in fetal weight DNA is the primary target of embryo-toxic mechanism of CP.[9]

The precise reasons for the protective effects of *Drakshavaleha* against any teratogen induced malformation are not clear till date however, the possibilities can be considered.

Multivitamin supplementation use among pregnant women is as effective as iron-folic acid in improving anemia status and appears to have other benefits for maternal and child nutritional status.[10] Ascorbic acid (vitamin C) is an important intracellular reducing agent. Its anti-oxidant properties protect against the adverse effects of free radicals reactions. Ascorbate inhibits CP induced sister-chromatids exchanges in mice.[11] Vitamin C supplementation may help to reduce the risk of pregnancy complications like pre-eclampsia, intrauterine growth restriction and maternal anemia.[12] Vitamin *P* enhances the action of vitamin C and for this reason they should be taken together. (Rhyme for ancient wanderer.) Large, transient increases in the total anti-oxidant capacity of plasma have often been observed after the consumption of flavonoid rich foods by humans.[13] Vitamins and minerals referred to collectively as micronutrients have important influences on the health of pregnant women and the growing fetus.[14] To prevent teratogen induced DNA damage, optimum dose of vitamins is required to be given during their intra-uterine life than more doses of the vitamins.

By Ayurvedic concept of physiology, it may be presumed that a *Rasayana* agent promotes nutrition through one of the following modes:[15] (a) By direct enrichment of the nutritional quality of *Poshaka Rasa*, i.e., the nutrient plasma. (b) By promoting nutrition through improving the *Agnivyapara* i.e., digestion and metabolism. In general, all *Rasayanas* are nutrition promoters but some are specific to certain organs or diseases called *Naimittika Rasayana*,[16] for example, *Hridaya Rasayana* for heart, *Twachya Rasayanas* for skin, *Chaksusya Rasayanas* for eyes and others. Due to rich in nutritional values and iron supplements *Drakshavaleha* can been used as a *Naimittika Rasayanas* pregnancy which may help to promote the health of the pregnant women and facilitates full growth and development of progeny in the womb.

Conclusion

In the present context, *Drakshavaleha* [Table 4] can be conceived to be a *Naimittika Rasayana* during pregnancy to obtain a healthy progeny. Whether a single ingredient of *Drakshavaleha* such as vitamin C, vitamin P, folic acid, minerals, etc., was effective in the current research or the result was a collective outcome is such difficult to conclude at this level. *Rasayana* as a whole has been the approach by *Ayurveda* and the current research follows this trend while being less analytical.

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हिन्दी सारांश

द्राक्षावलेह का साइक्लोफॉस्फेमाईडजनित मूषक भारहानि एवं लम्बाई अल्पता पर प्रभाव

सुनील कुमार, गजेन्द्र सिंह, के. आर. सी. रेड्डी

साइक्लोफॉस्फेमाईड एक एण्टी–माइटोटिक एवं एपोप्टोसिस उत्प्रेरक है । गर्भावस्था के ११वें दिन जब इसे १० मि.ग्रा/कि.ग्रा. की मात्रा में अन्तर्पर्युदर्या कला के माध्यम से मूषक में दिया गया तो इसके कारण पप्स की लंबाई एवं वजन में ह्रास हुआ । लंबाई एवं वजन में हुआ यह ह्रास, माइस के गर्भावस्था के '० से १८' दिन तक द्राक्षावलेह १६ ग्रा/कि.ग्रा. की मात्रा में सेवन करने से दूर हो गया । लंबाई एवं वजन में हुआ यह लाभ सांख्यिकीय मत से सार्थक रहा। वजन में ह्रास के साथ वृद्धि–मन्दता सामान्य की तुलना मे साइक्लोफॉस्फेमाईड से उपचारित पप्स के सभी वर्गों में देखा गया ।