



ORIGINAL RESEARCH ARTICLE

ROLE OF DOOSHIVISHARI AGADA ON TERATOGENIC EFFECT OF CYFLUTHRIN IN EXPERIMENTAL MODEL W.S.R. FETAL WEIGHT AND HEIGHT

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Abstract:

Background: Pesticides are known for their toxicity on the mammalian system including embryonic development. Cyfluthrin (Trade name: Baygon and Solfac) is used commonly in India and reported to be a teratogenic agent and reported for low birth weight and stunned fetuses (short height), and dooshivishari agada (DVA) commonly use in conditions like garavishajanya dooshivisha. **Aims:** To evaluate the efficacy of dooshivishari Agada in cyfluthrin induced Teratogenicity with respect to body weight and height. **Materials & Methods:** The 30 pregnant swiss albino mice were divided in to 3 groups of 10 each and 1st group was control administered with normal saline, 2nd group given cyfluthrin 32mg/kg body wt, 3rd group cyfluthrin(32mg/kg body wt) With DVA 1.2gm/kg body wt orally during the organogenesis period i.e. from 5th to 14th day. **Statistical analysis used:** It was analyzed using student t test using software graph pad prism version 6. **Results:** There weights in 1st and 3rd groups are nearly equal and showed the increase of weight and height in 3rd group i.e. treated group, weight: Group1-1.24±0.15, Group2-0.68±0.13, and group 3-1±0.11. Height: Group1-2.03±0.23, Group2-1.22±0.28, Group3-1.78±0.21 (Mean± SEM). Comparing the group 2 with group 3 the p value<0.05 in both the criteria's. **Conclusion:** The dooshivishari agada has shown the significant effect in increasing the weight and height of the fetuses which were exposed to the teratogenic agent

Key words: Teratogenicity, Dooshivishari agada, Dooshivisha, Garavisha

Introduction:

The most common problems among the newborns in human beings is the low birth weight¹ annually about 23.8% of total birth showed the low birth weight,² this low birth may be due to the insufficient food intake, stress, use of some drugs and chemicals, exposure to environmental factors etc during the pregnancy.³ Some of these drugs, chemicals, environmental and genetic factors fall under the category called

teratogens.⁴Teratogens commonly affect the fetus in the organogenesis stage itself.⁵ Organogenesis refers to that period of time during development when the organs are being formed. After an egg has been fertilized, damage to any of the organ systems of the body which may ultimately result in some type of birth defect usually strikes during this time frame.⁶

Cyfluthrin [cyano (4-fluoro-3-phenoxy-phenyl) methyl 3-(2, 2 dichloroethenyl)-2, 2-

dimethylcyclopropane carboxylate], a type II synthetic parathyroid is the active ingredient of non systemic insecticides primarily used for the control of chewing and sucking insects and also in public health situations (Adams et.al, 2002);⁷ cyfluthrin is known to produce loss of fetal weight and stunned fetuses (reduced height) (syed et.al 2009).⁸ It is commonly used household insecticides in India sold under the trade names Baygon and Solfac. (Thompson 1992).⁹ Synthetic pyrethroids, including cyfluthrin, have a similar mode of action as organochlorines. They act on the membrane of nerve cells blocking the closure of the ion gates of the sodium channel during re-polarization. This strongly disrupts the transmission of nervous impulses.¹⁰

In ayurveda there is a type of poisoning called dooshivisha which is defined as when the poison after treating if remains in the body in little amount then when it get exposed to the dusita desha (polluted environment), dusita kala(rainy season), dusita anna (spoiled food) and does the diwaswapna (day sleep) then the symptoms will be produced called as dooshivisha. And other concept called Garavisha (artificial poison) is the combination of different parts of body, excreta of different animals, incompatible drugs, chemicals, ashes, and poisonous substances act as low potent poison.¹¹and some types of teratogen fall under the category of Garavisha (artificial poison), and when these artificial poisons get accumulated in the body and produces the condition called garavishajanya dooshivisha (low potent poison. In dooshivisha the symptoms will be facial paralysis, anorexia, emaciation of the person, loss of sperm and ovum, vomiting, fainting, slurred speech etc.^{12 13}

Dooshivishari Agada is one of the formulation explained in the context of sthavara, jangam and kritrima visha and commonly used to treat dooshivisha condition.¹⁰ The formulations have shown

nephro-protective, hepato-protective,¹⁴ antioxidant, immuno-modulatory actions¹⁵ most of the drugs in this are having antioxidant, immunomodulatory, rasayana and vishagna properties.^{16, 17}

As cyfluthrin is a poison of artificial origin, and accumulates daily in body as slow poison because the cyfluthrin is used as household insecticide. Hence it is considered as garavisha as gains entry in to body in to low doses acts like dooshivisha.

So keeping this in the mind an attempt is made to analyze the effect of dooshivishari agada (DVA) over cyfluthrin induced teratogenicity these and teratogenicity in this context is considered as the garavishajanya dooshivisha.

Aims and Objectives

To evaluate the efficacy of dooshivishari Agada in cyfluthrin induced Teratogenicity with respect to body weight and height.

Materials and methods:

Trial Drug Details

Dooshivishari Agada

Dooshivishari Agada (DVA)^{18 19 20 21} the contents of Dooshivishari agada are mentioned in the table below.^{22, 23} The dooshivishari Agada available in the market of vaidyaratnam pharmacy taken suvarchika as Tribulusterrestris Linn, and previous two dissertation also taken suvarchika as Tribulusterrestris Linn.

Preparation of Dooshivishari Agada

All drugs for preparation of dooshivishari Agada was collected from available sources, and authenticated by the central research facility, and preparation of dooshivishari Agada was carried out in Bhaishaja kalpana department following the SOP's.

Dosage: Human dosage of Dooshivishari Agada mentioned in classics is 12 gm²⁴ and Animal Dose will be calculated by dose conversion table of Paget and Barnes (1964)

Table 1: Showing the drugs present in the Dooshivishari Agada

Sl.no	Ingredients	Botanical Name	Useful parts	Karma
1.	Pippali	<i>Piper longum Linn.</i>	Phala(Fruit)	Kasahara, shulaprashamana, shirovirechana.
2.	Pippalimool a	<i>Piper longum Linn.</i>	Mula (root)	
3.	Dhyamaka	<i>Cymbopogon martinii (Roxb.) Wats.</i>	Patra(Leaves)	Stanyajanana.
4.	Jatamamsi	<i>Nardostachys JatamamsiDC.(N. grandiflora)</i>	Mula(Root)	Sanjnasthapana.
5.	Lodra	<i>Symplocos racemosa Roxb.</i>	Twak (Stem Bark)	Shonithasthapana. Sandhaniya.
6.	Ela	<i>Elettaria cardamomum Maton</i>	Phala(Fruit)	Shwasahara. Angamardaprashamana
7.	Suvarchika	<i>Tribulus terrestris Linn.</i>	Phala(Fruit), Mula(Root)	Mutravirechaniya, Shothahara. Krimighna.
8.	Kutannatam	<i>Oroxylum indicum (Linn) Benth.Ex Kurz.</i>	Mula twak (Root bark)	Shothahara. Sheetaprashamana.
9.	Natam	<i>Valeriana wallichii D.C.</i>	Mula(Root)	Sheetaprashamana.
10.	Kusta	<i>Saussurea lappa C.B. Clarke.</i>	Mula(Root)	Shukrashodhana. Lekhaniya.Asthapanopaga.
11.	Yastimadhu	<i>Glycyrrhiza glabra Linn.</i>	Mula (Root)	Shonitasthapana, Kantya, Jeevaniya, Varnyashonithasthapana, Kandugna.
12.	Chandana	<i>Pterocarpus santalinus Linn. f.</i>	Khandasara (Heartwood)	Vishagna, Kandugna, Varnya, Dahashamana.
13.	Gairika	<i>Red ochre</i>		Vishgna, netrya,

Experimental Study

As per guidelines of OECD 414,423 & ICH S5 (R2) the study has been carried out, and the permission was obtained from the Institutional Animal Ethical Committee for carrying the animal study. Cyfluthrin was obtained from the sigma Aldrich, Bangalore, and the animal study was conducted following the 30 female mice and 10 male mice of 8 weeks old were purchased from authorized Animal breeder (Venkateswara pvt ltd Bangalore) and were bred and housed

in an air cooled animal house with natural day light of 12-24 hours and fed with tap water and pellets. Female and male mice were housed for mating in the ratio of 3:1. The females were checked every morning for vaginal plugs. The day a vaginal plug was seen was counted as 0 day of pregnancy. Thirty inseminated females were randomly selected and divide into 3 groups. Each group contains 10 fertilized mice. First group i.e. control receives the normal saline, 2nd group receives cyfluthrin i.e. 32mg/body

weight, 3rd group receive cyfluthrin 32mg/kg body weight and Dooshivishari Agada 1.5gm/kg body wt.

Female Swiss albino mice was randomly divided into following groups & receive the treatment as mentioned in the study design as below:

Study design

Table 2: study design

Sl.no	Groups	Intervention	Observation
1	Fertilized mice(FM)n=10	Fertilization Normal saline	Till 18 th day
2	FM + cyfluthrin n=10	32mg/kg body wt of cyfluthrin from 5 th to 14 th day of gestation.	Till 18 th day
3	FM +cyfluthrin +Dooshivishari Agada	32mg/kg body wt of cyfluthrin from 5 th to 14 th day of gestation.+ dooshivishari Agada 1.2gm/kg body wt from 5 th to 18 th day of gestation	Till 18 th day

Females of all the groups were weighed on every alternate day throughout pregnancy. They were sacrificed on the 18th day of pregnancy. The live fetuses and placenta were removed and their wet weights with the calibrated weighing machine and the height

with the venire’s caliper were recorded. The data was analyzed using students t test.

Results:

Results are explained with statistical analysis was done using software graph pad prism version 6

Table 3: Influence over the weight and height of the fetus

Group	Parameters	Fetal body weight (Mean ±S.E)	Fetal body height (Mean ±S.E)
Group 1		1.24±0.15	2.03±0.23
Group 2		0.68±0.13	1.22±0.28
Group 3		1±0.11	1.78±0.21

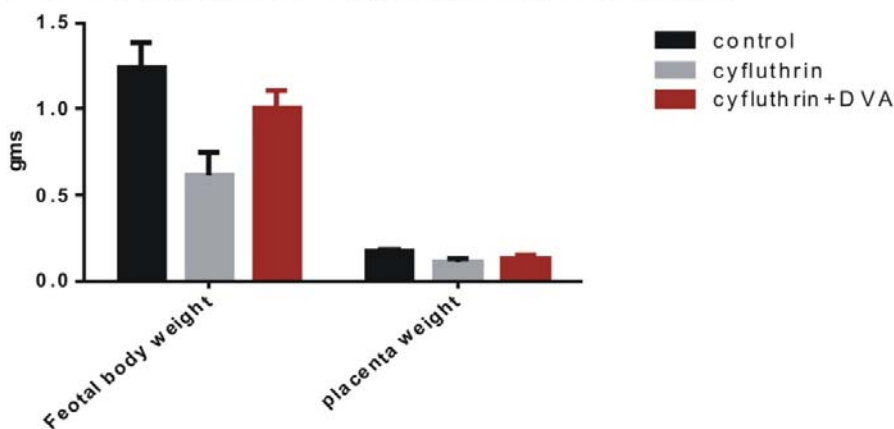
Table 4: Students t-test analysis

Comparison	Fetal body weight			Fetal body height		
	Mean difference	t value	P value	Mean difference	t value	P value
Group 1 vs Group 2	-0.62	3.12	0.0067*	-0.81	2.23	0.0382*

Group 3 vs Group 2	0.41	2.3	0.031*	0.5	1.6	0.120
Group 3 vs Group 1	-0.20	1.07	0.2	-0.25	0.8	0.4
*Significant (P < 0.05), **highly significant (p<0.005), ***Extremely Significant (P< 0.001), non significant (P>0.05)						

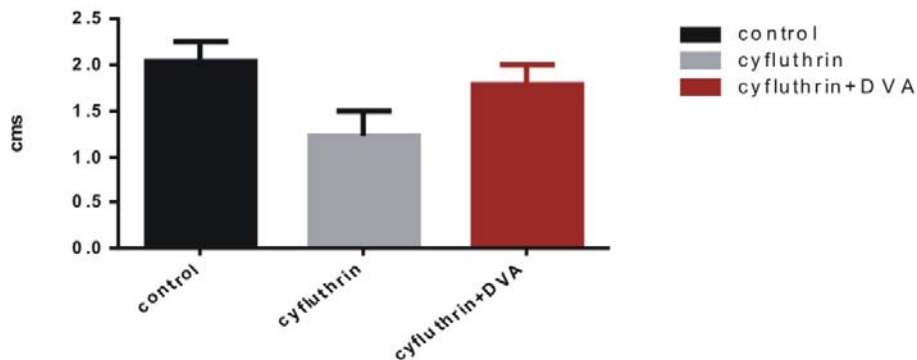
Graph1: Showing fetal body weight and weight of placenta:

Data showing feotal body weight and placenta weight



Graph 2: showing the height of the fetuses

Data showing the height of fetuses in three groups



Discussion:

Dushivisha is a typical Visha (poison) that manifests its effects after sometime. Visha (poison) which is devoid of two or three

gunas (properties) out of classical ten properties attains a latent or hidden stage in the body called dooshivisha (Latent poison). This low potency of the poison usually won't

cause sudden death because of the enveloping (avarana) action by Kapha, this low potency poisons is retained in the body for long period without producing any grave or fatal symptoms.²⁵

Dooshivisha is state where poison originating from inanimate (plant) or animate (animal) sources or any artificial poison (kritrima visha) retained in the body after partial expulsion or which has provisionally undergone detoxification by the anti-poisonous drugs or battered by forest fire, wind or the sun.^{25 26}

The conceptual thoughts about dooshivisha is that all vishas (poisons) being not completely eliminated from the body or partially detoxified due to incomplete metabolism lose its original gunas (properties) and gets converted in to low potency due to exposure to heat, flame, fire, sunlight etc or naturally less potent poison after entering the body without elimination due to some conjugation and after a secondary cause causes several diseases, depends where the poison deposited.

Poisoning in the body currently happens in the condition that is due to poisonous bites-inanimate poison exposure-virrudhahara and ahitaahara-fast foods and cola beverages like colas-alcohol, tobacco etc. Drugs like quinine, NSAIDs, steroids etc. Pesticides, Metals, Minerals, pollutants etc. Drugs using for long period for Blood pressure, Diabetes mellitus, Despirine, ARTs, Phenobarbitone.²⁷ with these types of poisoning which currently occurs and accumulates in the body slowly and act as cumulative poison and considered as Doosivisha.

In dooshivisha all the rasadi dhatu dusti takes place and will lead to the shukra (sperm or ojus or saptadhatu) kshayathere will be denaturing of the shukra (stree and pum shukra) and there will be pratiloma dhatu kshaya. Agnimandya is one of the symptom of dooshivisha, further leads to the dhatu agni mandya then all the nourishment of

dhatu will be lost so there will be effect as mutagenic and even affect of this dooshivisha over the pregnant woman lead to the even dushana(destruction) of the fetus,²⁸ so it can be considered as Teratogenicity. So the cyfluthrin known for teratogenic agent fall under pesticides so act as the dooshivisha. Dooshivishari agada has been explained in the context of dooshivisha as a treatment aspect.²⁹

In the present study there was reduction in the maternal weight and height with the cyfluthrin. A similar dose related reduction in maternal weight gain on administration of heptachlor and chlorpyrifos during pregnancy has been reported by Purkerson-Parker et al. (2001)³⁰ and Farag et al. (2003)³¹ respectively. The high dose also lowered the average fetal weight and height. This reduction in the fetal weight may be associated with reduction in the maternal weight gain (Kavlock et al., 1981; Narotsky and Kavlock, 1995)^{32, 33}. It may be presumed that damage to cells and organ system due to pesticide treatment caused embryoletality. Similar results were also obtained by Farag et al. (2006)³⁴ and Tian et al. (2005) who witnessed depressed maternal body weights accompanied by reduced fetal weight and height in rats treated with other pesticides like dimethoate and chlpyrifos respectively.³⁵

In the DVA group the no of live fetuses were increase compared to the cyfluthrin. This may be due to the variation in the maternal weight gain among the cyfluthrin group and DVA group as DVA group the maternal weight gain is more. And same is with the fetal weight can be considered and in DVA due to the anabolic activity the nutrient supply may be increased. In DVA there is presence of iron the extra supplementation of iron during the pregnancy results in the maternal and fetal weight gain.

Conclusion:

The Dooshivishari Agada has shown the significant effect in increasing the weight and height of the fetuses which were exposed to the teratogenic agent.

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