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Evaluation on Teratogenicity of Withania Somnifera and Carbamazepine in Drosophila Paralytic Mutant

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Abstract:- Epileptic patients need to take medicine for the rest of their lives. Teratogenic effect of antiepileptic drugs must be extensively screened and investigated utilizing animal model organisms. *Drosophila* is a great model for efficiently screening prospective therapeutic compounds and determining whether or not they are teratogenic. Children exposed to antiepileptic drugs (AEDs) during pregnancy had a greater percentage of malformations than those who were not. Carbamazepine (CBZ) is one of the most widely prescribed antiepileptic drugs (AEDs). Congenital and developmental malformations have been related to CBZ therapy during pregnancy. Plant extracts are widely used in traditional medicine to form the foundation of health care and to cure various illnesses. *Withania somnifera* (*W. somnifera*) is an anticonvulsant herbal medicine. The present study is intended to evaluate the teratogenicity of *W. somnifera* and CBZ in *Drosophila* paralytic mutant. As a wild-type control, *Drosophila* Oregon-R strain were employed. The results indicate that CBZ administration increases the frequency of wing deformities in both *Drosophila* strains in a dose-dependent manner. However, *W. somnifera* at various concentrations is a safe therapeutic potent with no teratogenicity while treating epilepsy in *Drosophila* paralytic mutants.

Keywords:- *Teratogenicity, Drosophila paralytic mutant, Withania somnifera, carbamazepine.*

I. INTRODUCTION

Epilepsy disorder, a broad category of common neurodegenerative disorders defined by recurring unprovoked seizures, is a serious public health issue due to the vast number of people afflicted and the absence of effective drugs[1]. Because epileptic patients are required to take medicine for the remainder of their lives, teratogenicity and other long-term implications must be properly screened and examined utilizing animal test models[2].

Drosophila melanogaster plays an important part in mutation research and genetic toxicity for its comprehensive genetic knowledge and considerable experimental expertise with this organism, as well as genetic similarities to mammals[3].The possibility of using mutants as a platform for the development of novel epilepsy medications, such as anti-epileptic drugs(AEDs), is one of the most enticing properties of *Drosophila*-based models [4].

Anti-epileptic medicines are reported to be present in one out of every 200 pregnancies (AEDs). There has been a surge in public awareness of birth malformations caused by prenatal AED exposure in recent years [5]. Since the late 1960s, studies on the malformation rates of children exposed to AEDs during pregnancy have consistently shown that those who were exposed have a larger percentage of malformations than those who were not [6].

Carbamazepine (CBZ), a commonly prescribed AED, is often used as a first-line therapy and is generally successful and safe; nevertheless, 30–40% of patients with epilepsy do not react well to it, and it can have major side effects in some people [2]. Congenital and developmental malformations such as neural tube defects, cardiovascular and urinary system anomalies, and cleft palate have been associated to CBZ treatment during pregnancy [7, 8].

Aside from AEDs' widespread availability, allopathic drugs have substantial drawbacks in the treatment of epilepsy, including prescription costs, physician access, and side effects [9, 10].

Herbal therapy has the potential to be an effective treatment choice for epileptic patients all around the globe since it is both cost-effective and culturally acceptable. *Withania somnifera* (*W. somnifera*) is a complementary and alternative medicine (CAM) that is used to treat epilepsy [11]. It possesses an antioxidative mechanism that enhances (gamma-aminobutyric acid) GABA and cortical muscarinic acetylcholine (Ach) levels in the brain and increases neurite regeneration [12]. The active compounds in W. somnifera's roots, withanolides, are essential for pharmacological action [13].

There have recently been concerns that certain medicinal herbs are carcinogenic or induce genotoxicity [14].Future regulatory systems and risk assessment recommendations will be required for herbal medicine, which is usually considered to be safe [15].It's also difficult to discriminate between the effects of AEDs and the consequences of maternal epilepsy.As a result, it's vital to continue and expand the testing of CBZ and *W. somnifera* for teratogenicity in order to find the most effective and safest epilepsy medication alternative.In the current study, teratogenicity was assessed by administering three different dosages of *W. somnifera* or CBZ to the *Drosophila* paralytic mutant and wild-type *Drosophila* (Oregon-R) and analyzing the congenital malformations.A gain of function mutation in an allele of the voltage-gated sodium (Na+) channel gene (*para* gene) on chromosome X causes the $para^{bss1}$mutant strain of *Drosophila* paralytic bang-senseless1 flywhich is semi-dominant in phenotype[16]. All voltage-gated Na+ channels in humans and flies have the same structure and function [17].Due to the $para^{bss1}$mutant's high seizure sensitivity and resistance to AED therapy, this mutant is frequently used as a model for intractable epilepsy [16]. In

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the present study, the teratogenicity of *W. somnifera* and CBZ were evaluated in homozygous females and hemizygous males of *Drosophilapara*^{bss1}mutant and Oregon-R strains.

II. MATERIALS AND METHODS

A. Chemical and herbal medicine

An antiepileptic drug, i.e., carbamazepine(CAS 298-46-4; EC number 206-062-7; Synonym: 5H-Dibenz[b, f]azepine-5-carboxamide) and herbal medicine, *Withania somnifera* were tested in current study. CBZ was procured from Sigmae Aldrich, India. *W. somnifera* root extract standard powder (Withanolides, 2.57 %; Withaferin A, 2.38 %) procured from M/s Sami Labs Ltd., Bengaluru, India. Alkaloids (isopelletierine, anaferine, cuseohygrine, anahygrine, and others) and steroidal lactones (withanolides, withaferins)are among the physiologically active chemical elements of *W. somnifera* [18].

B. Standardization of drug dosage and treatment

Mohammad's modified methodology has been used to standardize drug doses [19].Following seven days of exposure to various doses of *W. somnifera* or CBZ in standard wheat flour-agar media, adult flies (*para*^{bss1} and Oregon-R) were evaluated for 50 percent mortality, or lethal concentration (LC_{50}). *W. somnifera* standardized powder at 0.005, 0.01, and 0.05% w/w [13] or CBZ at 5, 10, and 40 μg/ml [3] dissolved in distilled water were added to the flies' standard wheat flour-agar media.

The control cultures of both *Drosophila* strains were fed the identical diet with distilled water added to the media, but they were not given any compounds [3]. All of the experiments were carried out in triplicate.

C. Fly strains

At a constant temperature of (22 1 °C) and relative humidity of 70–80%, *D. melanogasterpara*^{bss1} and Oregon-R strains were cultured on standard wheat flour-agar media. The *D. melanogaster para*^{bss1} mutant employed in this study obtained from the National Centre for Biological Sciences (NCBS) fly facility department in Bangalore, India. The University of Mysore in Mysore, India, provided the *Drosophila* Oregon-R strain.

D. Teratogenicity Testing (Morpho phenotypic malformation)

After light anesthesia with diethyl ether for about 1 minute, 20 freshly eclosed virgin female and 20 unmated male *Drosophila para*^{bss1}mutant and Oregon-R strains were collected in an uncrowded condition and reared in 10 glass vials (25 x 100 mm) contained media without (control) and with (treated) three dosages of *W. somnifera* or CBZ. Adult flies (parental generation =P) were allowed to deposit eggs for 24 h on the media. Then, adult flies (P) were removed from those glass vials. When emergence of first generation (F1) started, 50 males and 50 females offspring (F1) from each compound dosage, were chosen and etherized and observed using Olympus stereo microscope examination for teratogenicity, i.e., observing external Morpho phenotypicmal formations [20].

E. Statistical analysis

Statistical analysis was carried out using GraphPad Prism version 8.4.3 (GraphPad Software, San Diego, CA).Analysis of variance (ANOVA) was used in the wing deformities analysis assay.

III. RESULTS

The aim of the present study was to evaluate the deleterious effects of*W. Somnifera* and CBZ on morpho phenotypic malformations (teratogenicity) in the F1 offspring of *Drosophila para*^{bss1}mutant and Oregon-R strains under Olympus stereo microscope observation. The only morpho phenotypic malformations observed in the present study were wing deformities (Fig. 1).Table 1 shows the frequency of morpho phenotypic malformations in both control and treated *Drosophilapara*^{bss1} and Oregon-R strains with *W. somnifera*or CBZ.

Following exposure of *Drosophila para*^{bss1}mutant and Oregon-R strains to three different doses of *W. somnifera*, Olympus stereo microscope observation revealed a very low frequency of morpho phenotypic malformations, i.e. wing deformities, indicating no significant differences in the frequency of wing deformities in treated flies compared to the frequency of wing deformities in control flies(Table 1).

The frequency of wing deformities in *Drosophilapara*^{bss1}and Oregon-R strains (F1) exposed to 5 μg/ml CBZ was 7% and 2%, respectively, which is not statistically significant when compared to the frequency of wing deformities in control flies (Table 1).

There were significant increases in the frequency of wing deformities in *Drosophilapara*^{bss1} flies supplemented with 10 μg/ml and 40 μg/ml CBZ. Table 1 shows that the frequencies of wing deformities on exposure to 10 μg/ml and 40 μg/ml CBZ were 14% (**p = 0. 0018) and 29% (****p< 0.0001), respectively.

The frequencies of wing deformities in *Drosophila* Oregon-Ron exposure to 10 μg/ml and 40 μg/ml CBZ were 9% (**p = 0. 0045) and 21% (****p< 0.0001), respectively. These results indicate that in Oregon-Rflies supplemented with 10 μg/ml and 40μg/ml CBZ, there were significant increases in the frequency of wing deformitiescompared to control flies (Table 1).

According to the present research, the frequency of *para*^{bss1}flies with wing deformities was greater than the frequency of Oregon-R flies with wing deformities in either control or treated flies with three different doses of *W. somnifera* or CBZ.This indicates that the frequency of morpho phenotypic malformations was higher in *Drosophila* paralytic mutants, i.e., *para*^{bss1}, than in Oregon-R flies.

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Compound	**Percentage of *para*^{bss1} flies (F1) born with wing deformities (n= *100*)**	***p value***	**Percentage of *Oregon-R* flies (F1) born with wing deformities (n= *100*)**	***p value***
Control	6%	-	2%	-
W. somnifera (0.005%)	5%	0.7456	1%	0.7251
W. somnifera (0.01%)	7%	0.5619	2%	0.8667
W. somnifera (0.05%)	6%	0.9678	3%	0.7245
CBZ (5 µg/ml)	7%	0.8264	2%	0.5618
CBZ (10 µg/ml)	14%	*0.0018*	9%	0.0045
CBZ (40 µg/ml)	29%	$p < 0.0001$	21%	< 0.0001

Table 1: Total frequency of wing deformities in Drosophila*para*^{bss1} mutant and Oregon-R strains on exposure to Withania somnifera and carbamazepine

Data was analyzed by one-way ANOVA. (n= 100, n: Total number of flies for each compound dosage). CBZ in *para*^{bss1} flies: (p = **0.0018, ****p< 0.0001); CBZ in *Oregon-R*flies: (p = **0.0045, ****p < 0.0001).

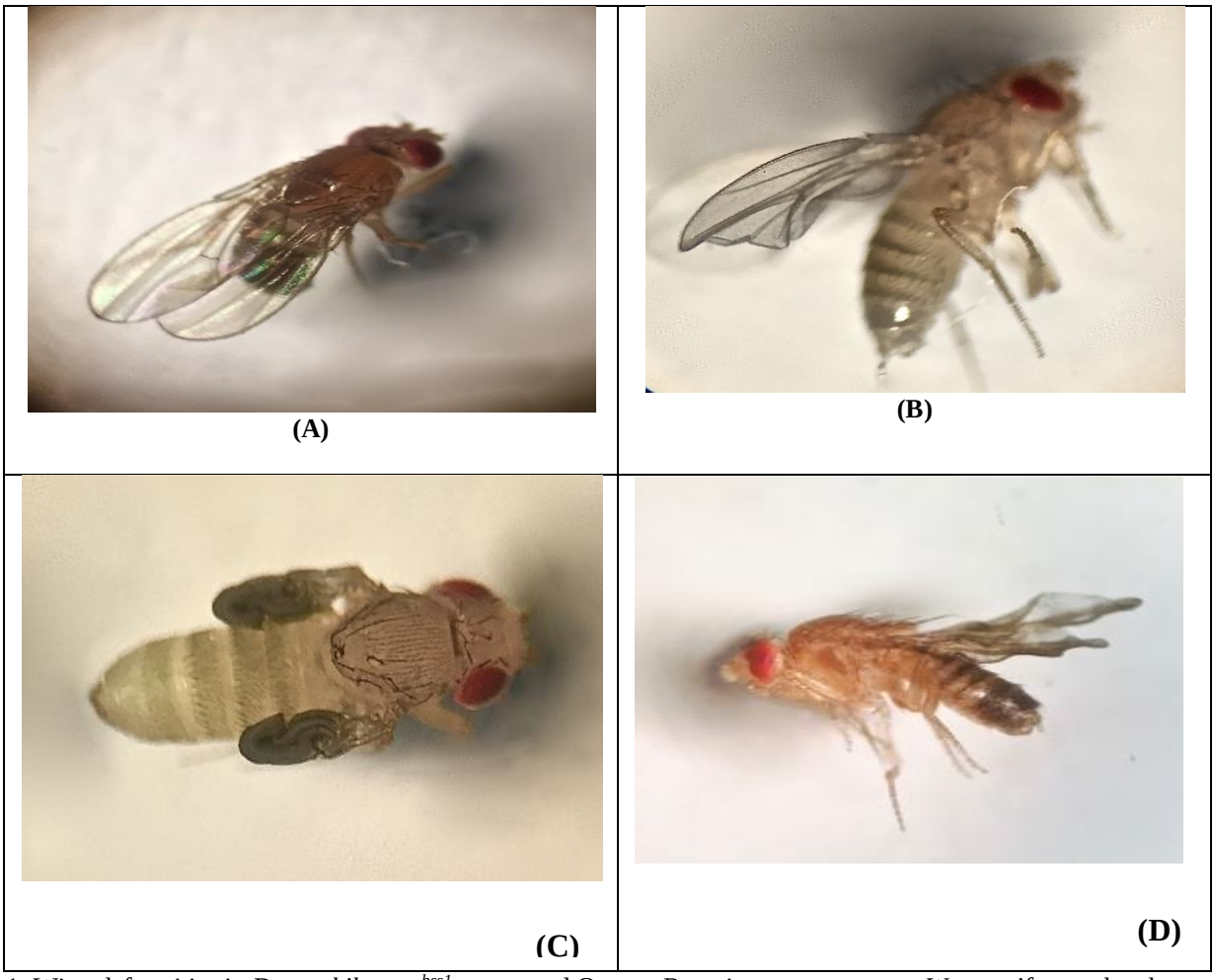

Fig. 1: Wing deformities in *Drosophila para*^{bss1}mutant and Oregon-R strains on exposure to *W. somnifera* and carbamazepine. **(A)** wild-type wing with no deformity; **(B)** Marginal folded wings; **(C)** Unexpanded wings; **(D)** Vertically twisted wing.

IV. DISCUSSION

Drosophila is a great model for identifying suitable medicinal compounds promptly [21]. The main goal of this study was to evaluate the teratogenicity in *Drosophila para*^{bss1}mutant and Oregon-R strainsfollowing treatment with different concentrations of *W. somnifera*(0.005%, 0.01%, 0.05%w/w), an herbal ingredient, and CBZ (5, 10, and 40 µg/ml), a common antiepileptic drug.

About one in every 200 pregnancies is estimated to include AEDs.In order to avoid the adverse effects of seizures, most women with epilepsy require AED treatment during pregnancy. However, exposing an embryo to AEDs while still in the womb increases the chance of congenital malformations. Research comparing the malformation rates of children exposed to AEDs during pregnancy has frequently demonstrated that these children have a higher percentage of malformations than those who were not[6].

Many of the congenital malformations reported in the offspring of epileptic patients are tied to the mother's genetic background, and AEDs only enhance the occurrence of malformations in those who are genetically susceptible. The present study showed that the frequency of wing malformations in *Drosophila* paralytic mutants (*para*^{bss1}) which are genetically epileptic flies, is greater than the frequency of wing deformities in *Drosophila* wild-type (Oregon-R).This indicates that epileptic flies' genetics and AED exposure both generate morpho phenotypic malformations in flies.

The current study indicated that in both *Drosophilapara*^{bss1}and Oregon-R strains, mid-dose (10 µg/ml) and high-dose (40 µg/ml) of CBZ operated as teratogens and induced a significant increase in the frequency of morpho phenotypic malformations, i.e., wing deformities, compared to control flies(Fig. 1, Table 1). According to the literature, CBZ is a recognized teratogen that should be avoided during pregnancy [22, 23].Offspring of mothers who were exposed to CBZ during pregnancy have been found to have a higher incidence of cardiovascular abnormalities[24, 25, 26].Kohl et al.,reported that carbamazepine has a dose-dependent detrimental effect on embryonic development, with a substantial influence on neural tube development [27].

The current study revealed that*W. somnifera* did not induce any significant differences in the frequency of morpho phenotypic malformations, i.e., wing deformities in both *Drosophila* strains in comparisons to control flies.Herbal medicines have been utilized to treat various diseases in many parts of the world for ages. It has previously been shown that a considerable proportion of women took a variety of herbal medicines for various purposes throughout their pregnancy [28]. Herbal usage during pregnancy is generally discouraged owing to the potential for adverse effects on the fetus [29]. Some research found that herbal extracts had morphological and toxic impacts on animal offspring, whereas others did not [30,31]. On the other hand, clinicians are not always aware of the risks of utilizing herbal medicines during pregnancy and nursing and while breastfeeding [32]. Undoubtedly, all herbal products should be taken with extreme caution during pregnancy, as there are no established safe intake limits. To validate the safe dosages of herbal medicines for embryos, more methodologically rigorous research on model organisms are needed. According to the literature, *W. somnifera* root extract had no effect on parental females' body weight, corpora lutea number, implantations, viable fetuses, or external, skeletal, or visceral malformations[33].

In conclusion, the current findings indicate that mid-dose (10 µg/ml) and high dose (40 µg/ml) of CBZ, a commonly used synthetic AED, caused high frequencies of morpho phenotypic malformations, i.e., wing deformities in both *Drosophila bara*^{bss1} and Oregon-R strains and acted as teratogen agents. On the other hand, *W. somnifera* did not exhibit any significant variations in the frequency of wing deformities in any of the *Drosophila* strains in comparison to control flies. The outcome of the present study supports the idea that *W. somnifera* is a safe therapeutic potent in neurodegenerative disorders, including epilepsy.

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