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Influence of Indian snake (*Naja naja*) venom on cognition and biochemical functions in N- Nitrosodiethylamine treated *Drosophila melanogaster*

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ABSTRACT

The flies (*Drosophila melanogaster*) were segregated into four groups. 1^{st} Group is normal-control, 2^{nd} group is wild type flies administered with 0.01% NDEA, 3^{rd} group is wild type flies administered with 0.01% NDEA + 0.01% *Naja naja* snake venom (NNV) and 4^{th} group is flies administered with 0.01% NNV alone were administered via food medium for 21 days. After the experimental period, the behavioural changes were analyzed. The behavioural assays include negative geotaxis, phototaxis, smell chemotaxis, taste chemotaxis, thermotaxis, and hygrotaxis were carried out in all groups of flies. The behavioural changes were found to be deteriorated in NDEA administered flies when related to the control but the behaviour is likely to regularise in NDEA+NNV administered flies. The protein carbonyl levels and levels of thiobarbuturic acid reactive substance (TBARS), protein thiol and lipid peroxides were noticeably elevated in NDEA administered flies as matched to control flies and similarly be likely to regularize in the NDEA+NNV group. Likewise, the levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione-S-transferase (GST) and reduced glutathione (GSH) were diminished in the group of NDEA administered and were noticeably more in the group of NDEA+NNV administered group. NNV has been stated to possess pharmacologically active components such as disintegrins, cobratoxin, hannalgesin, cytotoxin II, etc. which could possess antioxidant, antibacterial, hypotensive, cancer suppressive, anticoagulant, and analgesic activities. Our present study provides evidences that these components could normalize cognitive behaviour and attenuate oxidative stress in a genetically important model organism *D. melanogaster*.

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INTRODUCTION

Drosophila melanogaster generally called as a fruit fly, is one of the regularly employed model organisms for pharmacological research. For more than one hundred years, the low cost, fast multiplication, and enviable genetic tools have made the fly crucial for basic biomedical research [1]. The fly has been shown to be useful to investigate the underlying mechanisms in aging, oxidative stress, immunity, cancer, diabetes and drug abuse. The genome of *Drosophila* is 60% similar to the genome of humans, mostly less dispensable and about 75% of the genes

is highly accountable for human diseases is complementary in flies. These characteristics, along with a rapid generation time, less cost of maintenance, and the existence of strong genetic tools, which make the fruit fly more suitable for the investigation of complicated pathways related to cancer research [2].

Further, around the past ten years, *Drosophila* has been used to study cognitive function and/or intellectual ill-health, which has given a huge quantum of disease-related scientific findings [3]. A series of analysis has been employed to evaluate the cognitive behaviour in *Drosophila* including phototaxis, negative geotaxis, smell and taste chemotaxis, hygrotaxis and thermotaxis. There are various types of cancer which are known to weaken cognitive behavioural characteristics [4]. In *D. melanogaster* age-associated defects in behavioural characteristics has also been documented. It is well known that in mammals including humans with cancer could experience psychological and oxidative stress. Several researchers investigated the molecular and cellular mechanisms of emotional/cognitive distress during malignancy growth [5].

Yellow (*y*) is the gene coding for melanin and could define the patterns of melanin on the skin of *D. melanogaster*. The Yellow protein coded by *a yellow* gene is necessary to synthesize black pigment melanin and is expressed in a pattern that draws a parallel with the distribution of this pigment. In the *y* mutant, all the black pigments were removed and the fly is yellow in colour. The genetic changes *in the yellow* gene can simply be acknowledged by the peculiar yellow pigment noticed in the cuticle of the adult flies and the mouth portion of the 3^{rd} instar larva. The *yellow* gene's function is miscellaneous and is liable for modifications in cognitive functions. The yellow gene is an excellent gene to examine the genetic behaviour since it is evidently seen that, when an organism containing *Y* gene, helping to easily identify the pathway of developing DNA to progeny.

Oxidative stress is tightly linked to every stage of cancer, from carcinogenesis to the tumour-bearing state and also, from remedy to avoidance. Cancer and oxidative stress form a crucial cycle; when oxidative stress surpasses the capability of the body's oxidation-reduction system, genetic changes might also additional arise, and/or intracellular signal transduction and transcription elements could be influenced immediately or through antioxidants, leads to cancer. Likewise, the tumour formation is stated to be oxidative stress-related with excessive active oxygen production and irregular oxidation-reduction regulation. The oxidative stress is upraised, despite the fact that tumour bearing tissues endure lower free radical load because of out-of-control and excessive production of cells, within the body of the tumor-bearing host [6].

N-nitrosodiethylamine (NDEA) is an important cancer-causing agent and it could promote tumours in several organs of numerous animal model systems [7]. This carcinogen is found in various foods such as soya beans, fish (smoked, dried and salted) cheese, meat and alcoholic beverages. Individuals are rendered to low concentrations of N-nitrosodimethylamine in occupational settings, via the ingestion of meals and cigarette smoke. NDEA is also found in buns, rolls, muffins, ham and oysters. NDEA is known to cause oxidative and cellular damages by promoting the synthesis of free radicals. The metabolic conversion of NDEA by the action of cytochrome P450 enzymes ends in the formation of ethyl-acetoxyethyl-nitrosamine that is further conjugated by the action of phase II biotransformation enzymes to non-poisonous compound. This activation of NDEA by P450-catalyzed-hydroxylation is known to produce unsteady intermediates that would alkylate the DNA and consequently produce cancer.

Snake venom is an exciting natural toxin source used in toxicological and pharmacological research. Snake venoms possess a high amount of pharmacologically active small peptides and components such as disintegrin, 3FTX (cardiotoxin), 3FTX (neurotoxin), natriuretic peptide, Kunitz-type inhibitor, crotamine, BPPs, waglerin, and sarafotoxin and with considerable clinical and medical prospective. Specifically, *Naja naja* venom contains cytotoxins, cardiotoxins, hemorrhagins, neurotoxins, coagulants/anticoagulants, disintegrins, myotoxins, nephrotoxins, hannalgesin, cobratoxin and cytotoxin II which are known to possess cytotoxic activites and could prevent carcinogenesis [8].

In addition, researchers are examining the possibility of the use of snake venom proteins for the remedy against cancer and to find out the effects of snake venom constituents in the prevention of circulatory system-based diseases [9]. Snake venom components have the capability to interrupt the features of endothelial cells which are present inside the blood vessels. This should results in the cells to split from one another, which results in their death leading to the prevention of endothelial tumor. In addition, this influence of snake venom toxin can also be used to disturb the blood flow in the tumor and accordingly could inhibit the tumor growth. In earlier research, the treatment of cancer in mice by using cobra venom was investigated.

However, in the case of *in vitro* investigation, venom reveals powerful cytotoxic and apoptogenic influences on human leukemic cells (U937/K562) by diminishing the rate of cell proliferation. The toxins of snake venom have been additionally examined as blockers of metastasis or cancer [10].

Currently, corresponding tools and techniques are used to study the potential applications of toxin components as molecular probes, and potential templates for the detection of drugs and drug design analysis. This kind of research approaches indicates that peptides present in snake venom are commonly strong molecules, which is capable to withstand in the tough proteolytic environment [11]. When these molecules are inducted in the venom glands by disulfide bonds formation and/or post-translational modifications, the constancy of these molecules is achieved [11]. Researchers are investigating the development of novel drugs against cancer from organic and natural resources all over the world. As snake venom compounds illustrate distinct structural and functional properties resulting in the inhibition of platelet accumulation, cytotoxicity, hemolysis, and cardiac arrest, they could be promising candidates in the prevention of cancer. In this context, the present study has been performed in the prevention of behavioural abnormalities and oxidative stress by the *N. naja* venom during carcinogenesis (by the action of N-nitrosodiethylamine) in *Drosophila.*

MATERIALS AND METHODS

Experimental protocol

In the present study, wild-type (WT) of *D. melanogaster* was used. WT flies (received from *Drosophila* Stock Centre, University of Mysore, Mysore, India) were retained at 20-25º C and cultured on standard in Light-Dark (12:12) condition. The experimental flies were segregated into 4 groups, WT (group 1 – control, group 2 – NDEA (0.01%), group 3 – (snake venom + NDEA treated), and group 4 – snake venom (0.01%)) delivered through food medium for 21 days. Flies were cultured in yeast, maize powder, and sucrose diet with nepagin as an anti-fungal agent at 21±2°C in a 12:12 (light: dark) cycle. At the end of the 21^{st} day, flies (n = 30/group) were collected from all four groups and were used for further analysis.

Haemolymph and tissue homogenate Collection

Suitable holes in a 0.5 ml eppendorf tube were made and placed into a 1.5ml eppendorf tube with a removed lid. Flies (30 nos.) were dissected by removing legs and wings. The tubes of 1.5 ml containing 0.5 ml tubes were centrifuged at 3200 rpm for 12 minutes. In the bottom of the 1.5 ml tube, hemolymph was collected and was mixed with cold phosphate-buffered saline. Head and intestine tissues were dissected from wild-type flies (n = 30). Using 0.1 M sodium phosphate buffer (pH 7.4) the respective tissue homogenates were prepared and then centrifuged at 4800 rpm for 12 minutes at 4ºC. The supernatant was filtered by using a sieve along with nylon mesh (pore size 10 mm) and thereafter employed for biochemical assays.

Behavioural assays to assess cognition in flies

The behavioural assays such as (i) negative geotaxis, (ii) phototaxis, (iii) smell chemotaxis, (iv) taste chemotaxis, (v) thermotaxis and (vi) hygrotaxis was carried out on WT and NDEA-treated WT flies by using the methods of [12, 13] with minor changes.

Negative geotaxis

Flies were kept in a vertical glass column (15 × 1.5 cm) which was secured at one side. By gently tapping the vial, flies were trapped at the bottom of the column. After 1 minute, count the flies individually, which are seen at the top of the column, and flies which rested at the lowest.

The result was reported as a number of flies escaped over the least interval of 13 cm in 60 s. Adult flies (30 nos.) for every evaluation were used and the experiment was done three times and mean±SD values were calculated (Figure 1).

Figure 1: Negative geotaxis

Phototaxis

In this study, two vials (15 × 1.5 cm) were attached with one another with translucent tape and had

been split into 3 same partitions (I, II, and III, compartment I became closer to a light source). About 30 flies were introduced in the vial and sealed with a cotton plug. The flies were kept in darkness for 30 minutes. This endorsed adaptation of the flies to the darkness. Then, the flies were introduced to the light source (emergency lamp, Philips, India) and maintained 5 cm away from the vial.

After one minute, the flies present in each compartment were counted and the results were noted as a percentage of flies. The test was repeated for three separate sets of flies and mean±SD was calculated (Figure 2).

Figure 2: Phototaxis

Smell chemotaxis

In this study, a potent repellent benzaldehyde was used. About 30 flies were introduced into two vials (15 × 1 cm) joined collectively using an obvious tape and is marked in three identical compartments (1, 2, and 3). The test tube (compartment 3 adjacent to the cotton plug) was closed with a cotton plug which was dipped in 1 ml of 100 mM benzaldehyde. After one minute, the number of flies, found in every compartment was measured and the end result was noted as a percentage. The test was repeated for separate three sets of flies and mean±SD was calculated (Figure 3).

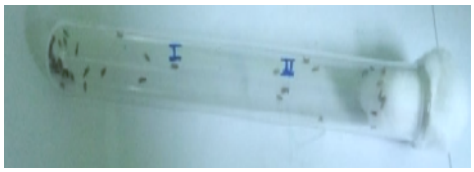

Figure 3: Smell chemotaxis

Taste chemotaxis

In this study, non-volatile attractant sucrose was used. About 30 numbers of flies were placed inside in a test tube (18 cm × 1 cm) and is separated into three equidistant divisions. The cotton plug was dipped in 1 ml of 0.1% sucrose and was plugged in the test tube. After one minute, the variety of flies, found in every division was numbered and the end result had been noted as a percentage. The test was repeated for separate three sets of flies and mean±SD was calculated (Figure 4).

Thermotaxis

In this study, two vials (15 × 1.5 cm) were used. The first vial was heated to a temperature of 45º C and

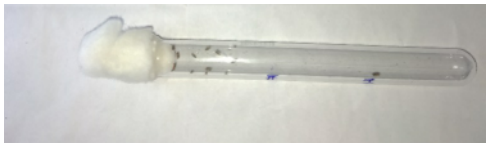

Figure 4: Taste Chemotaxis

was directly linked to another vial (at room temperature) with a transparent tape containing about 30 flies. The connected vials were compartmentalized into three equal zones (I, II and III – compartment III heated zone). After one minute, the number of flies present in each compartment was counted and the result was noted in the percentage of total flies present. The test was repeated for separate three sets of flies and mean±SD was calculated (Figure 5).

Figure 5: Thermotaxis

Hygrotaxis

In a vial (15 × 1.5 cm), 1 ml of distilled water was taken, closed with a parafilm cover, and was kept overnight. After 12 hours, about 30 flies were taken into another vial (15 × 1.5 cm). After removing parafilm and water from the first vial, two vials were connected by using transparent tape. The connected vials were separated into 3 similar zones (I- moisturized zone, II and III). After one minute, the total number of flies present in every zone was noted and the final result was denoted as a percentage of the total number of flies. Further, the test was repeated for separate three sets of flies and mean±SD was calculated (Figure 6).

Figure 6: Hygrotaxis

Biochemical assays

Estimation of indices of redox homeostasis

The content of protein carbonyl was determined. The sample was distributed into 2 portions containing 1-2 mg protein, and then an equal quantity of 2N HCl was added and incubated for 60 minutes at 25 °C. The mixture was precipitated with 10% TCA after incubation and centrifuged. The precipitate was blended with ethanol and ethyl acetate (1:1) mixture and was diluted in 1 mL of 6 M guanidine HCl. The distinction in absorbance between the

DNPH treated and HCl treated sample was determined at 366 nm. By the method of Niehaus and Samuelsson [14], the levels of TBARS in tissues and plasma were measured. In this method, malondialdehyde and other thiobarbituric acid reactive substances (TBARS) were quantified by their activity with thiobarbituric acid (TBA) in an acid state to develop a pink coloured chromophore, which has been measured at 535nm. Further, an assay of free protein thiol groups is carried out by derivatization with 5, 5 ′dithiobis (2- nitrobenzoic acid) (DTNB) The measurement depends on the appearance of a coloured thiolate ion mixture that can be determined spectrophotometrically at 410 nm. The thiol group assay is often carried out on soluble protein fractions, by homogenization in a buffer containing a detergent such as sodium dodecyl sulfate (SDS).

Based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole (R1), with malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE) at 40°C, this lipid peroxidation assay is developed. MDA or 4-HNE reacts with N-methyl-2-phenylindole to form a stable chromophore with an O.D. measured at 580 nm. By Habig method, the activity of glutathione-S-transferase (GST) was assayed in tissue homogenate using CDNB as substrate, results in an increase in absorbance at 340 nm. By the Kakkar method, superoxide dismutase in the erythrocytes and tissues was estimated. This assay depends on the prevention of NADH-phenazine methosulphate, nitroblue tetrazolium formazon formation, and this reaction was originated by the introduction of NADH. After incubating for 90 seconds, the reaction was stopped by adding glacial acetic acid. After the end of the reaction, the color developed was extracted from the n-butanol layer and the absorbance was measured at 520 nm.

The activity of catalase in hemolymph/tissue homogenate was assayed. Along with 0.9 ml of phosphate buffer, 0.4 ml of hydrogen peroxide, and 0.1 ml of tissue homogenate/hemolymph and were added. Then 2.0 ml of the dichromate-acetic acid mixture was added and the reaction was stopped after 15, 30, 45, and 60 s. This mixture was kept under a boiling water bath for 10 minutes, then cooled and the colour formation was measured at 610 nm. The specific activity was stated as mmol of H_2 O_2 used / min/ mg of protein for tissues or mmol. By using the Rotruck method the GPx activity in the erythrocytes and tissue was studied. In the presence of GSH, a known quantity of enzyme preparation was made to react with H_2O_2 for a particular time period and then the required GSH content was calculated. By Ellman (1959) method, reduced glutathione was estimated. This method shows that, when 5, 5'-dithiobis (2-nitrobenzoicacid) (DTNB) was added to the compounds containing sulphydryl groups, the yellow colour formation was noticed.

Statistical analysis

The percentage of volume contribution was noted as mean ± SD. To analyze the data, the Statistical Package for Social Sciences (SPSS) version 22.0 (IBM Corporation, New York, NY, USA) was used. The test of homogeneity was employed to estimate the sample distribution of the data set.

RESULTS

The negative geotaxis value was decreased significantly after NDEA treatment. In NDEA and snake venom-treated group, the value was increased ($p>0.05$) when related to normal control. In group IV (snake venom alone treated), the value was more or less similar when related to the normal (control) group ($p>0.05$).

Most commonly, a larger number of flies tend to move nearer to the light source (compartment I), Table 1. Nonetheless, this response was considerably decreased ($p<0.05$) when compared to the normal (control). Similarly, a large number of NDEA treated flies were observed in compartment II when related to the normal - control ($p<0.05$). When NDEA + snake venom treated flies were compared with those of the NDEA treated, the response was considerably raised in compartment I ($p<0.05$).

A larger number of control flies were perceived to diverge from the pungent smell of the chemical benzaldehyde from 1^{st} compartment to 3^{rd} compartment when correlated with NDEA treated flies ($p<0.05$, Table 1). Significantly increased movements were noticed in snake venom + NDEA treated flies to compartment III ($p<0.05$). More number of control flies were tend to move closer to the cotton-plug dipped with sugar solution (compartment I) when related to the mobility of NDEA treated flies towards compartment III ($p<0.05$). The response of snake venom-treated flies was closer to the movement of control flies ($p>0.05$, Table 1).

As far the thermotaxis assay, a large percentage of control flies move to diverge from the hot surface of the 3^{rd} compartment to the 1^{st} compartment when related to NDEA treated flies ($p<0.05$).

Meanwhile, the trend shown averted in NDEA + snake venom treated flies compared to group II flies ($P<0.05$). At the same time, an insignificantly noticed presence of snake venom alone treated flies was noticed in the 3^{rd} compartment when related to control flies. The hygrotaxis assay revealed an increased proportion of control flies related

Table 1: Negative geotaxis Drosophila melanogaster. Values with a * indicate significant variation at p<0.05 by Duncan's Multiple Range Test (DMRT)

Negative Geotaxis (%±SD) (WT-Control)	NDEA treated	NDEA + snake venom	Snake venom treated
93.6±15.8	81.2±14.6*	72.3±13.8*	94.5±16.3*

to NDEA treated flies which had moved towards the humid region in the 1^{st} compartment (p<0.05, Table 2).

The percentage of NDEA+snake venom treated flies were considerably increased in the 3^{rd} compartment when related to NDEA treated flies (p<0.05). Invariably snake venom treated flies illustrated the behavioural activities of negative geotaxis, smell and taste chemotaxis, phototaxis, hygrotaxis, and thermotaxis alike to those that were noticed in the control group (p>0.05).

The variations in the amount of lipid peroxidation products in normal-control and experimentally treated flies are given in Table 3. In NDEA administered flies TBARS, protein carbonyl and lipid peroxides were considerably increased (p<0.05) when compared to the normal control. The range of TBARS, protein carbonyl and lipid hydroperoxidases were insignificantly decreased (p>0.05) after the execution of snake venom when related to the normal-control flies.

The level of phase I cytochrome p450 (phase I enzyme) was noticeably reduced whereas the cytochrome p450 (phase I enzyme) was significantly elevated in tumor-bearing flies as compared to normal-control flies (p<0.05). Administration of snake venom to NDEA treated flies significantly decreased and raised phase II enzyme activities compared to group III.

Table 3 illustrates the levels of enzymatic and non-enzymatic antioxidants such as SOD, CAT, GPX, vitamin C and E, and GSH in the head, intestine, and hemolymph of normal-control and treated flies. A noticeable reduction (p<0.05) in the actions of enzymatic and non-enzymatic antioxidants in group II flies was noticed. In the snake venom administered group, the levels were noticeably increased (p<0.05) when compared to NDEA administered flies.

DISCUSSION

Flies treated with NDEA tend to build up oxidative stress during tumorigenesis. This, in turn, could inhibit normal negative geotaxis behaviour and to shorten the duration of sleep [15]. However, possibly, the improvement of cognitive function by snake venom in NDEA treated *D. melanogaster* is the first study. The physiological, molecular, and signaling mechanisms underlying for the abnormalities in behavioural indices are to be investigated. However, the normalization of ROS levels and inhibition of carcinogenesis under snake venom treatment could normalize the behaviour in flies.

Our results clearly suggested that during tumorigenesis the behaviours (negative geotaxis, thermotaxis, smell, and taste chemotaxis, phototaxis, and hygrotaxis) are clearly altered. Defects in cognition are reported widely in a wide range of cancers [4]. Our findings also added additional evidences that the cognitive behaviors could have been affected owing to carcinogenesis in flies. Our results also indicated that snake venom could nullify the harmful effects of NDEA and thus tend to bring back the flies behaviours to near normal. There are several complex molecules of biologically active proteins, enzymes, peptides, organic and inorganic compounds present in the snake venom. Venoms isolated from the snake is an important agent which helps in curing various types of cancers [16]. The earliest investigation using snake venom against tumor cells was the defibrination process. The study reveals that Ancrod, a polypeptide from *Agkistrodon rhodostoma* (Malayan pit viper), treated with cyclophosphamide, could produce defibrination, which could reduce the weight of tumor cells by fibrin clot or the product of coagulation is broken down and involved in both separations and will reduce the spread of a various type of tumors. However, the results showed that, in addition to the defibrination process, platelet aggregation, was another complex process which could be tangled in the process. Snake venom usually contains integrin, which can be a factor for the emergence of medicines for the remedy of cancer. Disintegrins primarily isolated from viperid snake venoms, normally contain integrin which has low molecular weight molecules with variations in their effectiveness, organization of various structures, and distinct nature.

Disintegrins are the elongated peptides that are precisely joined to the cell surface integrins on various types of cells, inclusive of tumour cells, and these integrins may act as competing barriers. Integrins were significant surface adhesion molecules and cell signaling receptors which can manage the prolifer-

Table 2: Behavioural assays in *D. melanogaster.* Values not sharing a common superscript numerical vary significantly at p<0.05 by Duncan's Multiple Range Test (DMRT)

% of flies present in compartment Behavioural assay	Compartment I (mean±SD)	Compartment II (mean±SD)	Compartment III (mean±SD)
Phototaxis			
WT (control)	87.6±4.0[1]	22.3±3.6[1]	6.4±1.2[1]
NDEA treated	72.8±4.5[2]	26.2±2.8[2]	10.6±2.0[2]
NDEA+snake venom	83.4±3.2[1]	27.6±3.2[1]	8.3±1.7[1]
Snake venom alone	92.4±3.4[1]	24.2±4.5[1]	6.1±1.2[1]
Smell chemotaxis			
WT (control)	25.6±5.0[2]	16.8±4.3[1]	90.8±3.2[1]
NDEA treated	18.3±1.8[2]	24.5±3.1[2]	70.3±4.5[2]
NDEA+snake venom	4.8±1.4[1]	18.3±3.7[1]	88.1±3.7[1]
Snake venom alone	2.5±0.9[1]	12.7±4.6[1]	92.6±2.8[1]
Taste chemotaxis			
WT (control)	75.8±12.4[1]	19.4±5.3[1]	10.4±4.7[1]
NDEA treated	68.7±7.6[2]	27.0±4.9[2]	17.3±6.8[2]
NDEA+snake venom	86.2±6.3[1]	20.8±3.7[1]	12.8±4.9[1]
Snake venom alone	84.6±8.9[1]	16.5±4.8[1]	10.5±4.1[1]
Thermotaxis			
WT (control)	76.5±15.27[1]	7.4±1.1[1]	1.2±0.6[1]
NDEA treated	62.6±6.5[2]	20.9±4.1[2]	8.5±2.9[2]
NDEA+snake venom	79.1±6.2[1]	6.3±0.9[1]	1.6±0.8[1]
Snake venom alone	76.2±7.2[1]	7.2±0.9[1]	1.2±0.5[1]
Hygrotaxis			
WT (control)	60.0±15.0[1]	21.6±6.8[1]	7.3±2.3[1]
NDEA treated	70.5±6.7[2]	26.3±5.2[2]	9.8±2.1[2]
NDEA+snake venom	87.6±7.1[1]	22.7±5.4[1]	6.5±1.8[1]
Snake venom alone	92.5±8.3[1]	20.6±5.8[1]	7.1±2.1[1]

ation of cells, cell survival, and migration. Owing to this critical role, disintegrins were intended as the therapy of some cancer molecules which focussed on cell-to-cell and cell-to-matrix interactions with results on most cancer development, metastasis, and strategies which includes cancer cell aggression, cell motility, and multiplication. Our findings are dependable with the report on relaxing oxidative stress by snake venom in cell lines of *Drosophila* signifying that elevation of TBARS level in NDEA induced flies could be attenuated by snake venom. This could be owing to excessive generation of ROS and with an early event associated with hypoxia [17]. *D. melanogaster* had a various number of antioxidative defense mechanisms to prevent cellular damage induced via reactive oxygen species (ROS).

Investigations have demonstrated that an exciting beneficial approach to deal with tumors is that CR-LAAO has the biological property to stimulate the production of H_2O_2 and also includes induction of apoptosis. The antioxidative defense mechanism could search ROS and performs a key role in the suppression of lipid peroxidation and consequently, plays a defensive role in the development of cancer.

SOD and CAT include an equally protective set of enzymes against ROS. This defence mechanism functions over enzymatic components such as CAT, SOD, GST, and GPx, and non-enzymatic constituents.

In NDEA exposed flies, enzymatic and non-enzymatic antioxidants levels were lowered.

In this study, the augmented points of TBARS and lipid hydroperoxides in hemolymph and tissues such as the intestine and brain were noticed might be owing to NDEA triggered synthesis of free radical, membrane impairment, and cellular distruption. Improvement of lipid peroxidation is noticed in snake venom-administered flies because of the improved activity of antioxidants. The antioxidant

Table 3: Biochemical assays in *D. melanogaster.* Values not sharing a common superscript numerical vary significantly at p<0.05 by Duncan's Multiple Range Test (DMRT)

S. No.	Biochemical Parameter	Group	Hemolymph	Head	Intestine
Redox homeostasis					
1.	Protein carbonyl (nmole/mg protein)	WT (control)	5.9±1.05[1]	4.1±0.92[1]	2.6±0.10[1]
		NDEA treated	9.2±2.46[2]	3.2±0.63[2]	1.5±0.06[2]
		NDEA+snake venom	6.4±0.09[1]	4.3±0.65[1]	2.4±0.09[1]
		Snake venom alone	5.6±1.01[1]	3.0±0.76[1]	2.2±0.07[1]
2.	Thiobarbituricacid reactive substances (TBARS) (nmole/mg protein)	WT (control)	12.8±2.63[1]	8.4±1.30[1]	7.2±1.74[1]
		NDEA treated	18.0±3.85[2]	6.5±0.90[2]	5.8±1.26[2]
		NDEA+snake venom	12.1±1.99[1]	7.6±1.20[1]	6.2±1.31[1]
		Snake venom alone	13.7±3.95[1]	12.6±4.41[1]	9.6±2.20[1]
3.	Protein thiol (mmol/mg protein)	WT (control)	38.7±6.21[1]	28.4±4.81[1]	24.8±3.95[1]
		NDEA treated	48.6±3.12[2]	20.2±2.85[2]	16.3±1.38[2]
		NDEA+snake venom	37.2±5.08[1]	26.9±2.58[1]	23.1±2.98[1]
		Snake venom alone	37.6±5.36[1]	26.3±3.81[1]	24.5±3.08[1]
4.	Lipid peroxides (nmole/mg lipid)	WT (control)	35.6±5.37[1]	27.9±5.13[1]	26.7±3.73[1]
		NDEA treated	43.2±4.02[2]	28.7±3.08[2]	16.6±2.07[2]
		NDEA+snake venom	34.5±3.16[1]	24.7±3.26 [1]	22.8±2.31[1]
		Snake venom alone	35.0±0.12[2]	23.5±1.08[2]	21.2±0.79[2]
5.	Superoxide dismutase (SOD) (Unit[a] nmole/mg protein)	WT (control)	12.1±2.01[1]	7.8±1.21[1]	6.5±1.24[1]
		NDEA treated	11.6±2.15[2]	10.05±2.7[2]	8.0±1.06[2]
		NDEA+snake venom	18.3±3.02[1]	12.4±2.46[1]	9.1±2.04[1]
		Snake venom alone	20.8±3.29[1]	13.2±1.47[1]	11.0±1.18[1]
6.	Catalase (CAT) (Unit[b] /min/mg protein)	WT (control)	165.3±2.57[1]	140.3±13.59[1]	110.7±10.22[1]
		NDEA treated	141.3±12.68[2]	119.6±9.78[2]	92.3±5.87[2]
		NDEA+snake venom	167.4±18.72[1]	140.2±10.85[1]	106.4±9.24[1]
		Snake venom alone	164.3±22.42[1]	145.4±12.84[1]	118.5±12.62[1]
7.	Glutathione-S-transferase (Unit/100mg protein)	WT (control)	12.6±0.14[1]	8.5±0.71[1]	6.7±0.53[1]
		NDEA treated	8.5±0.08[2]	5.9±0.54[2]	4.±0.32[2]
		NDEA+snake venom	12.7±1.05[1]	8.2±0.92[1]	7.2±0.61[1]
		Snake venom alone	15.4±2.86[1]	13.1±1.85[1]	12.3±1.62[1]
8.	Glutathione peroxidase (GPx) (Unit[c]/mg protein)	WT (control)	11.8±4.32[1]	6.5±0.95[1]	5.6±0.84[1]
		NDEA treated	8.5±1.24[2]	4.2±0.63[2]	2.9±0.54[2]
		NDEA+snake venom	10.6±3.24[1]	5.4±0.91[1]	3.9±0.62[1]
		Snake venom alone	11.4±3.86[1]	6.2±0.92[1]	4.8±0.81[1]
9.	Reduced glutathione (GSH)	WT (control)	16.6±8.14[1] (μl/ml)	10.4±2.11[1] (μl/mg tissue)	9.5±1.54[1] (μl/mg tissue)
		NDEA treated	11.4±3.12[2]	7.6±0.18[2]	6.4±0.51[2]
		NDEA+snake venom	15.6±6.35[1]	10.4±1.41[1]	9.6±1.32[1]
		Snake venom alone	16.2±7.28[1] (μl/ml)	12.6±1.28[1] (μl/mg tissue)	8.6±1.25[1] (μl/mg tissue)

nature of the polyphenolic compounds could maintain the fly's resistance against NDEA derived free radical damages. The notable elevation in GSH level in snake venom treated flies expresses the ability of the venom to sustain the level of GSH by preventing the toxicity of glutamate and stimulating cystine (cysteine is the precursor of GSH and an outstanding source for thiol group) absorb into the brain by its free radical foraging and cell-protective properties. The reasonable mechanism by which snake venom caused its protective effect was due to the properties of free radical scavenging, and by sustaining the cellular integrity of cells in *D. melanogaster*.

The constituents of TBARS, protein carbonyl, protein thiols, and lipid peroxides (the products of extreme oxidative stress) were increased in hemolymph (>0.01) although they are evidently reduced in the head and intestine tissues of flies. During tumorigenesis, the administration of reactive oxygen species (ROS) levels is a key factor as the augmented levels of ROS can be damages the cells. Therefore, the tumour cells show the mode of actions, for instance, peroxide scavenging system to sustain the ROS balance to identify the proliferative state of cells. Moreover, earlier reports stated that the fast-dividing tumour cells in the tissues of the head and intestines could utilize elevated concentrations of ROS [18].

Likewise, in this study, the results showed that as the levels of ROS were reduced in the tumour cells, and ultimately could result in the reduction in the end-products of oxidative stress in these tissues. Similarly, there is a constant reduction in the amount of antioxidants – CAT, SOD, GST, GSH, and GPx in tissues and hemolymph; this might be due to the accelerated utilization of antioxidants by the cancer cells [19]. Substances with potent antioxidant activity, such as ascorbic acid are known to prevent hepato carcinogenesis. As stated previously, snake venom is well recognized for its activities of antioxidants, such as through the removal of toxins present in reactive oxygen and reactive nitrogen species. Through the interaction with hydrogen peroxide, the snake venom-stimulated toxicity pathways may form a network of proteins antioxidant defense by modulating oxidative stress. The administered dose of snake venom might have diminished the oxidative stress, thereby, retreating the effects of pro-oxidation of fly by representing a notable modulation in most of the redox homeostasis indices. Particularly, the noticeable decreased level of lipid peroxides in hemolymph tissues of snake venom-administered *D. melanogaster* flies denotes a decrease in the levels of lipid peroxidation of lipids.

The mechanisms of action have been determined to study a cardiotoxin (a component in snake venom) that triggers programmed cell death in K562 cells through the dysfunction pathway of ROS-independent mitochondria and the mechanism (caspase-dependent) of Bax/Bcl-2 ratio in human colorectal Colo205 cancer illustrated the possibility of destroying breast cancer tissue using snake venom by inhibiting the DNA and RNA synthesis. This investigation reveals that snake venom intensely suppressed nucleic acid formation in breast cancer tissues. It could cause a reduction in the proliferation of cells which protect the most susceptible brain and intestine tissues of carcinogenic flies.

The considerable reduced level noticed in the SOD activity, catalase, and GPx in carcinogenic flies might be owing to disruption of cells results from the activation of cysteine proteases family called as caspases. Caspases are inactive proenzymes, which may cleave, on activation of various substrates in the cytoplasm or nucleus and could lead to morphological changes and cell death. Most of the enzymes of the redox pathway in carcinogenic flies or mutant flies are known to be decreased (including SOD, catalase, and GPx) [20].

The proportional increment in GSH level in snake venom-treated flies denotes the capacity of the venom to increase the concentration of GSH in the brain by promoting cystine (GSH precursor uptake into the brain and could directly protect from NDEA's actions by its free radical scavenging and cytoprotective effects.

CONCLUSION

Venom from snakes is an important agent for curing many types of cancers. This study shows that snake venom strongly inhibited the formation of nucleic acids in cancer tissues. It may cause a decrease in cell proliferation. Flies exposed to NDEA (500 μM) for 7 days as evidenced by the marked elevation in oxidative stress markers coupled with a significant decrease in activities of antioxidant enzymes such as SOD and CAT which suggested an increased generation of ROS and toxic aldehydes.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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