Dinesh Kumar et al /J. Pharm. Sci. & Res. Vol. 12(2), 2020, 243-251

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Evaluation of Antioxidant Properties of *Tinospora Crispa* from Physiochemical and Enzymatic Behavior of *Drosophila melanogaster*

Dinesh Kumar
Research scholar, Department of Biotechnology, UIET, Maharshi Dayanand University, Rohtak, India

Veer Bhan*
Assistant Professor, Department of Biotechnology, UIET, Maharshi Dayanand University, Rohtak, India

Abstract
Tinospora crispa is a native of the rainforests and deciduous forests of Asia. It is widely used as traditional medicines to treat various health problems. Various studies have reported that *Tinospora crispa* is a rich source of flavonoids and alkaloids which have strong antioxidant properties. Studies based on DPPH, FRAP and TBA assays the aqueous plant extracts have rich antioxidant behavior compared with BHT and Vitamin C (Zulkairi et al., 2009).
In this study, we examined the role of *Tinospora crispa* aqueous plant extracts for evaluation of antioxidant properties in the model organism *Drosophila melanogaster*. The different plant extracts (PEs) was used across a concentration gradient and increasing age of fly on biomarker of oxidative stress in the standard regime of *Drosophila melanogaster*. The results from the *Tinospora crispa* plant extracts approach of antioxidant behavior were confirmed by various experimental parameters of physiology (Survivorship, Fecundity, SOD, Catalase, MDA, Phenoloxidase, and Protein carbonylation) and physical fitness (Chill Coma Recovery Time and Negative Geotactic Movement). Also, these studies may be helpful to control oxidative damage in the body that may help to improve the healthy lifespan.
Keywords: *Tinospora crispa*, *Drosophila melanogaster*, oxidative stress and Aging

INTRODUCTION

Oxidative damage by free radicals has been well investigated within the context of oxidant and antioxidant balance. In fact, oxidative stress responds various deleterious processes resulting from an imbalance between the excessive formation of reactive oxygen species and nitrogen species and limits the antioxidant defense mechanism of the organism. The theory Free Radical Theory of Aging (FRTA) suggested that the oxidative stress is produced by the free radicals through reaction catalyzed by molecular oxygen (Harman.,1956). Although body's own antioxidant enzyme systems can dismutase the deleterious effect of oxidative stress. The oxidative stress may include free radicals like superoxide like free radical, other reactive oxygen species and reactive nitrogen species. But in the mitochondrial matrix where oxidative phosphorylation occurs consumes about 90% of intracellular oxygen. When the metabolic rates becomes high and also generation of free radicals becomes high then body's endogeneous reserves of antioxidants (SOD, Catalase) becomes insufficient to scavenge the free radicals and lead to the production of oxidative stress (Harman.,1972). That oxidative stress may be the cause of diseases like cardiovascular disease, neurodegeneration and also for aging, age related illness.
Herbs are major sources of many drugs acts as potential source of antioxidant from ancient times. Pharmacological evaluations of herbs may lead to the discovery of new natural antioxidant for treatment of diseases. *Tinospora crispa* is the one of the herb that have medicinal properties. *Tinospora crispa* known as "akar patawali" in Malaysia and "Andawali" in India is an herbaceous climbing plant that is widely distributed in South East Asia, particularly in Vietnam, Thailand, Malaysia, Indonesia and India belongs to the family menispermaceae. This medicinal herb has been used in the Thai traditional medicine due to its anti-pyretic, anti-diabetic, anti-inflammatory, anti-malarial and health maintaining properties.
This study shows the potential antioxidant role of *Tinospora crispa* and enlights the different phytochemicals have been used against number of diseases also been effective in scavenging of free radicals and prolongs the lifespan of *Drosophila melanogaster*.

MATERIAL AND METHODS

Fly strain and Husbandry

Drosophila melanogaster wild type flies were used for this study. The flies were collected from the fruit market of Shimla (Himachal Pradesh, India) through Trap Bait Method and the net sweeping method and identified thoroughly. The basal media i.e. cornmeal sugar medium vehicle for refined the flies comprised of corn flour (72 g), sugar (64 g), yeast (40 g), and agar-agar (15 g) and water 1400 ml for liter. The bactericidal propionic corrosive (3 ml) and the fungicidal sodium benzoate (1 gm) were included and blended completely. In the wake of cooking, media were permitted to cool to room temperature and Media cooked as slurry was filled the containers or vials and was permitted to set before the exchange of grown-up flies or pupae. Stock flies were kept up in bottles while culture vials were utilized for support of littler populaces or exploratory gatherings. Flies were permitted to sustain not obligatory and developed at 21°C with 60% moistness in 12 h day–night cycle.

Plant collection and its Extraction

The plant, *Tinospora crispa* personally collected from the botanical garden of Maharshi Dayanand University, Haryana (India) and were kept for air dry after initial washing with double distilled water on the same day. The dried stem were ground to fine powder using laboratory blender on medium speed. The 1:20 ratio of powder and solvent (ethanol + water, 50:50) was transferred into reflux apparatus and maintained for six hours at boiling temperature. The extracted solvent was allowed to evaporate to dryness under vacuum on a rotary evaporator at 40°C. All the extracts were used to filter through a Whattman filter No 1 and were stored at -20°C till usage. The obtained viscous dried extracts were used directly as 2mg/ml, 4mg/ml, 6mg/ml and 8mg/ml in standard food medium.

Survivorship assay

It is a proportion of an individual getting by until a given age, and these information were communicated as extent endurance for each test gathering. In this investigation virgin flies were explicitly isolated and set in 10 groups each gathering contains 10 flies (N=100) for both control and PEs treated flies. The endurance information was recorded from the principal day of endurance. The vials were supplanted from new one in at regular intervals. The dead flies were expelled from vial and information was recorded. Factual examination of the contrasts between the survivorship of exploratory gatherings was finished utilizing the log-rank test, which utilizes an aggregate measurement to dole out a P-worth to evaluate invalid speculations. Contrasts were viewed as noteworthy if the P-value was less than 0.05.

Fecundity assay

Fecundity was estimated as the amount of eggs each female replacement above duration of 24 hours. Approximately, for 18 hours transferring to new bottles of food due to continuous inspection, the amount of eggs placed in these fresh vials was checked. Tallying was eliminated by the eye in a stereo zoom microscope. Contraindications in egg lying between conditions were dissected using nonparametric tests.

Developmental Time

Eggs were allowed to grow on different diet regimes (control food and different concentration with plant extract) for 16 hours, then permitted to mature at 21ºC. Time egg to adult was scored to count developmental time.

Superoxide Dismutase (SOD) Enzyme assay

SOD are the class of enzymes that catalyses the dismutation of superoxide (O_2^-) into oxygen (O_2) and Hydrogen peroxide (H_2O_2). It is initially discovered by McCord and Fridovich in 1969, SOD has been seen as pervasive in every single vigorous creature from to human. Four sort of SOD have been distinguished based on their metal cofactors and conveyance. The copper zinc structure (Cu-Zn SOD) is generally normal with an essential dissemination in cytoplasm of eukaryotic cells. Another mainstream structure of SOD is Mn-SOD which is found in mitochondria. SOD is a family of metalloproteins that catalyzes the dismutation of O_2^- to form H_2O_2.

$$O_2^- + O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

The approximate 100 flies were homogenized in 1 ml cold 0.1 M phosphate buffer (pH-7.4) solution and crushed by using mortar-pestle tissue homogenizer, then, centrifuged at 8000 g for 10 min. The supernatant was collected for enzyme activity. In the method of SOD enzyme estimation, the reduction of NBT (nitroblue tetrazolium) by addition SOD enzyme (tissue homogenized) under aerobic condition was observed. We have made a cocktail of four solutions containing 50 µM of sodium carbonate (Na_2CO_3, pH - 10), 96 µM of NBT, 0.6% of triton X-100 and 2 mM of hydroxylamine hydrochloride (pH-6), then added 0.1 ml of enzyme supernatant. The enzyme activity was expressed as **units/min/mg of protein** at A- 560 nm, where one unit of SOD enzyme was expressed as amount of inhibition with rate of reaction by 50 %.

Catalase assay:

CAT movement in the control and plant separate encouraged flies will be estimated by following the capacity of the catalyst to part H_2O_2 inside 1 min of hatching time according to convention controlled by Aebi in 1984 with certain alterations. The investigation will be done in 15 mL bird of prey tube. The test blend comprised of 1 mL of 0.01 M sodium phosphate cushion (pH 7.0), 25 µL of test 10% homogenate. The last volume will be made 1 mL by including refined water and the cylinders will be vortexed. 500 µL of 0.2 M Hydrogen peroxide (1:3 by volume) will be included trailed by 2 mL dichromate acidic corrosive (5% of $K_2Cr_2O_7$ with icy acidic corrosive). Cylinders will be vortexed again and will be kept for bubbling for 15 min and will be cooled under faucet water before estimating the optical thickness (OD). OD will be estimated at 240 nm.

For spectrophotometric estimations wavelength was set to 240 nm and cuvette was placed into test holder of spectrophotometer. To set auto zero 3 ml hydrogen peroxide + PBS was utilized. The unit meaning of catalase is that one unit will disintegrate 1.0 µM of hydrogen peroxide in pH 7.0 at 25 °C. The time was recorded for the absorbance A-240 to diminish from 0.45 to 0.40 absorbance units.

Lipid Peroxidation (LPO) assay

The Malondialdehyde, a lipid peroxidation finished result in fly homogenate, was estimated by the strategy portrayed by Wills (1969) with some minor modifications. A 200 mL aliquot of fly (without head and wing) homogenate was blended in with 2 ml of thiobarbituric corrosive (TBA) – trichloroacetic corrosive (TCA) reagent (0.375 and 15%, individually). The volume was made up to 3 ml with refined water and bubbled on a water shower at 95 ^oC for 20 min. The arrangement was then cooled under faucet water. The response item (TBA–MDA complex) was separated by including 3 ml of n-butanol to the above arrangement. The absorbance of the pink shaded

concentrate in n-butanol was estimated at 532 nm utilizing a spectrophotometer. The measure of MDA was determined utilizing a molar termination coefficient of 1.56 * 105 M-1 cm-1 and communicated as nmol of MDA shaped per mg of protein.

Protein estimation assay

An aliquot of fly cytosol or mitochondrial protein concentrations were determined by using Lowry's reagent (2% Na_3CO_3 in 0.1N sodium hydroxide containing 1% copper sulphate and 2% sodium potassium tartarate) and incubated for 10min at room temperature. Following addition of 0.1ml of Folin-Ciocalteu reagent (1N), the reaction mixture was allowed to stand for 20min at room temperature. The absorbance was measured at 750nm and the concentration of protein was determined using bovine serum albumin as the standard.

Protein carbonylation assay

It is the measurement of protein oxidation in the form of protein carbonyls. These are the result of oxidative cleavage of protein. This assay of Protein Carbonylation is for recognizing and evaluating the oxidative modification of proteins. The compound DNPH responds with proteins to shape hydrazones that can be recognized spectrophotometrically at the absorbance 370 nm. The protocol was elaborated by Levine., 2002 used with some modifications.

Phenoloxidase (PO) Assay

Phenoloxidase is the enzyme finally formed in the cascade of melanization which may be the result of antimicrobial and antifungal activity in insects inclinding *Drosophila melanogaster*, in insects it is the key compound for immunity, wound healing and for the melanization in the exoskeleton of flies. This is cytotoxic so insects store it in inactive form proPhenoloxidase.

The most preferable method used to quantify PO is a colorimetric assay by measuring the conversion of the compound L-DOPA to Dopachrome at 492 nm. In this reaction the test solution becomes dark; the conversion of L-DOPA to Dopachrome is depend upon the amount of PO present in Hemolymph. In this reaction, L-DOPA must be freshly prepared and protected from light until used.

Chill Coma Recovery Time (CCRT)

It comprises the transfer of control and treated flies which were devoid of anesthesia in immaculate glass vials (50 flies per vial) for about 3 hr in a bucket which constitutes melting ice to persuade Chill Coma. After that in large petriplates flies were kept. A was started once the flies were placed in petriplates and only those flies were considered recovered merely when they stand on their legs. It should be well eminent that recurrence use of flies should not be done in the duration of treatment experiment as a result of reported consequence of exposure to cold on longevity as well as immunity. The calculation of CCRT was done by taking mean time.

Negative Geotactic Movement

In this experiment vertical movement of flies is a recurrent evaluation of locomotory events in fruit fly individuals. The control and PEs treated flies which were devoid of anesthesia, 50 flies were transferred into 10 cm tall clean glass vial and waited for 10 min after completing the transfer of all participating *Drosophila* fly, to allow them to become fully awake and active. The individuals, they didn't become active were removed after waiting for an additional 5 min. After wards, the respective frame was tapped 3 times. The climbing time was recorded by a stop watch at 10 sec, 30 sec and 60 sec post startle. All work was done on sex basis. Finally flies were shifted back into their rearing vials without anesthesia.

RESULTS

Survivorship Analysis

In this experiment the survival curves was examined of *Drosophila melanogaster* fruit flies male and female separately which was treated with PEs gradient (i.e. 2, 4, 6, and 8 mg/ml) of *Tinospora crispa*. The flies were reared at 21^{o}C in standard diet regime of *Drosophila*. The wild type of *Drosophila melanogaster* from Shimla, India was reared for survivorship analysis. The survival of flies was recorded until all the flies in the vial become died. The lifespan of *Drosophila* consists of three rounds i.e. Healthy Lifespan, Transitory Lifespan and the aged or senescent lifespan. The three replicates were used to show the survival fitness in the fly.

The statistical analysis of the survival curves of male and female control flies were performed through Kaplan-Meier Survival Analysis method that explored the mortality rate during the aging process of life of *Drosophila* from the huge mass of population of flies. The survivorship was analyzed statistically by the computer software GraphPad Prism 8.30 by using log rank, (Mental cox test and Gehan-Breslow-Wilcoxon test).

The male flies were treated with PEs concentration gradient (i.e. 2, 4, 6, and 8 mg/ml) of *Tinospora crispa*. By using the PEs concentration of *Tinospora crispa* in the standard food medium of *Drosophila* the lifespan was extended accordingly the raised in the concentration of PEs. The maximum lifespan of *Drosophila* male flies in the PEs concentration of *Tinospora crispa* of (i.e. 2, 4, 6, and 8 mg/ml) was of 105 days, 115 days, 121 days and 97 days respectively. Also, the female flies were treated with PEs concentration gradient (i.e. 2, 4, 6, and 8 mg/ml) of *Tinospora crispa* in the standard food medium of *Drosophila.* The lifespan was extended accordingly the raised in the concentration of PEs. The maximum lifespan of *Drosophila* female flies in the PEs concentration of *Tinospora crispa* (i.e. 2, 4, 6, and 8 mg/ml) was of 105 days, 115 days, 121 days and 97 days respectively (Fig. 1). The result showed that the PEs concentration of *Tinospora crispa* successfully extended the median lifespan and maximum lifespan of male and female *Drosophila melanogaster.*

Fecundity Analysis

The per day fecundity was analyzed of flies that was treated with different PEs concentration of *Tinospora crispa* (i.e. 2, 4, 6, and 8 mg/ml). The fecundity was analyzed for 21 days. The total 250 female flies were observed i.e. 50 flies of each PEs concentration.

Table 1: The One Sample t-Test of Fecundity analysis of Control Flies of *Drosophila melanogaster*, Shimla 21°C against the different PEs concentration of *Tinospora crispa* i.e. (2, 4, 6, and 8) mg/ml

One sample t Test	Control	2 mg/ml PEs Concentration	4 mg/ml PEs Concentration	6 mg/ml PEs Concentration	8 mg/ml PEs Concentration
t, df	t=31.27, df=49	t=29.91, df=49	t=34.73, df=49	t=16.90, df=49	t=27.74, df=49
P-Value (two tailed)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SD of discrepancy	2.700	3.700	2.700	4.700	3.400
SEM of discrepancy	0.3818	0.5233	0.3818	0.6647	0.4808

Table 2: This table shows the analysis Ordinary Two Way ANOVA of the effect of PEs of *Tinospora crispa* on SOD, Catalase, LPO, Phenoloxidase and Protein Carbonylation behaviors of different concentration i.e. (2, 4, 6, and 8) mg/ml against the three age groups i.e. (7, 21, and 35) days old male flies

Two Way ANOVA	ANOVA Table from Interaction	SS	DF	MS	DF(DFn, DFd)	P-Value
SOD	Male	592.38	8	74.04	F (8, 135) = 5.271	P<0.0001
	Female	416.1	8	52.02	F (8, 135) = 4.552	P<0.0001
Catalase	Male	108.9	8	13.61	F (8, 135) = 3.730	P=0.0006
	Female	89.44	8	11.18	F (8, 135) = 2.788	P=0.0069
LPO	Male	0.02613	8	0.003267	F (8, 135) = 1.124	P=0.3512
	Female	0.01213	8	0.001517	F (8, 135) = 0.5617	P=0.8076
Phenoloxidase	Male	0.007867	8	0.0009833	F (8, 135) = 0.6172	P=0.7623
	Female	0.004400	8	0.0005500	F (8, 135) = 0.3452	P=0.9466
Protein Carbonylation	Male	757.1	8	94.64	F (8, 135) = 3.514	P=0.0010
	Female	237.7	8	29.71	F (8, 135) = 1.103	P=0.3650

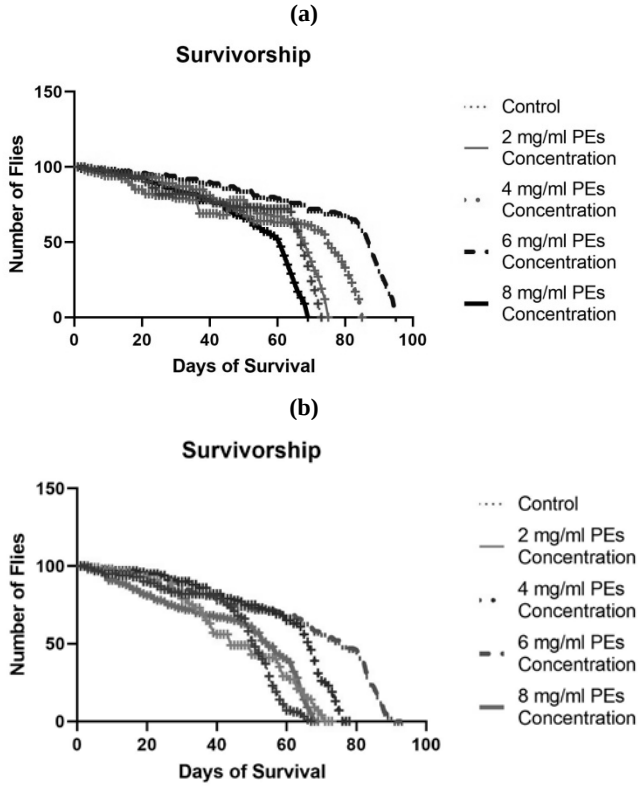

Fig 1: The figure shows the Survivorship Curve for (a) male and (b) female flies of *Drosophila melanogaster* treated against the PEs of *Tinospora crispa*

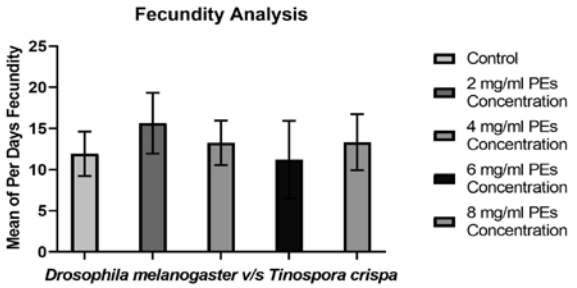

Fig 2: The Bar Diagram shows the mean 21 days Fecundity analysis of Control Flies of *Drosophila melanogaster*, Shimla 21°C against the different PEs concentration of *Tinospora crispa* i.e. (2, 4, 6, and 8) mg/ml

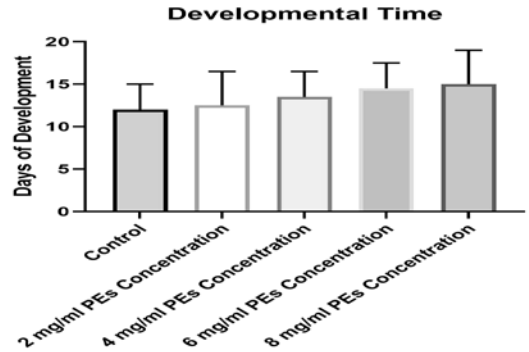

Fig 3: The Developmental Time of Control flies of *Drosophila melanogaster*, Shimla 21°C against the different PEs concentration of *Tinospora crispa*i.e. (2, 4, 6, and 8) mg/ml

The mean fecundity of 21 days was observed as Control (11.94 ± 2.7), 2 mg/ml (15.65 ± 3.7), 4 mg/ml (13.26 ± 2.7), 6 mg/ml (11.23 ± 4.7) and 8 mg/ml (13.34 ± 3.4) (Fig 2). The PEs concentration of 2 mg/ml showed very significant result in respect of egg laying more than control flies but other PEs concentration didn't showed any significant change. All values are analyzed by one sample t Test (P-Value ≥ 0.0001) (Table 1) by software GraphPad Prism 8.3

Developmental Time

The use of plant extract of *Tinospora crispa* at concentration 2mg/ml, 4mg/ml, 6mg/ml and 8mg/ml in the standard food regime of *Drosophila* the developmental time (Time taken from egg to adult) was increased as 12 ± 3 (Control), 12.5 ± 3 (2 mg/ml), 13.5 ± 4 (4 mg/ml), 14.5 ± 4 (6 mg/ml) and 15 ± 3 (8 mg/ml) days respectively. There will be a developmental delay, according to above said PEs concentrations of *Tinospora crispa* i.e. from 12 days to 15days (Fig 3). Hence, the developmental time of fly was positively correlated with the increase in the concentration of plant extract concentration of *Tinospora crispa* in food medium and also physical health span.

Superoxide Dismutase (SOD) Activity Analysis

In the present study the activity of enzyme Superoxide Dismutase (SOD) was observed against the PEs concentration of *Tinospora crispa* (i.e. 2, 4, 6, and 8 mg/ml). All the PEs concentration was applied on three different age groups (i.e. 7, 21, and 35 days) old male and female flies.

The outcome of SOD of male and female flies of *Drosophila melanogaster* were analyzed by two way ANOVA processed in software GraphPad Prism 8.3. The mean SOD activity of flies with SD values was observed. The significant result was observed from male flies by the PEs concentration of 4 and 6 mg/ml in male flies i.e. the analysis from the two way ANOVA the percentage of total variation from interaction was observed 1.927 (P≥ 0.0001, significant) and DF(DFn, DFd) was observed from their interaction F (8, 135) = 5.271. Also, the significant result was observed by the PEs concentration of 4 and 6 mg/ml i.e. the analysis from the two way ANOVA the percentage of total variation from interaction was observed 1.623 (P≥ 0.0001, significant) and DF(DFn, DFd) was observed from their interaction F (8, 135) = 5.990 (Fig 4 and Table 2). Thus, the results show that the PEs concentration of *Tinospora crispa* of 4 and 6 mg/ml was successfully improved the SOD activity in the male and female flies.

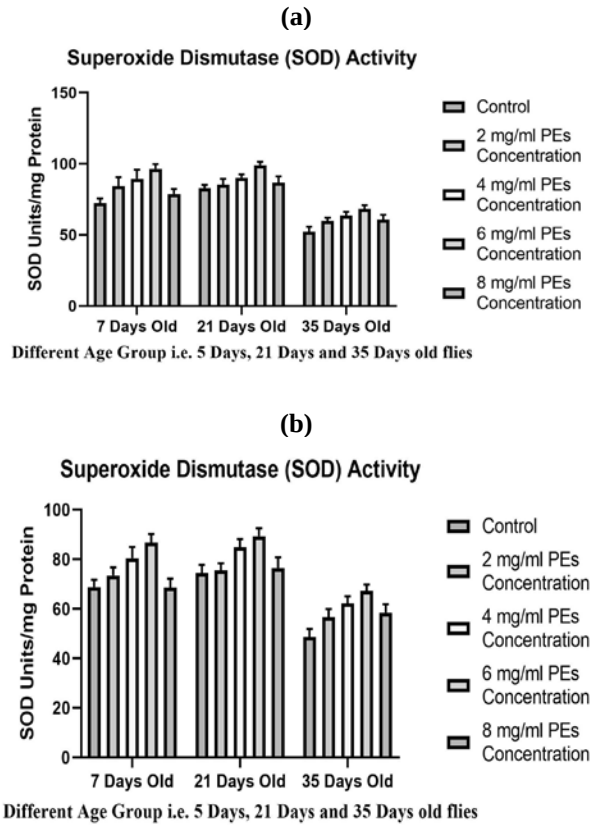

Fig 4:The bar diagram represents the SOD analysis of Control (a) male and (b) female flies of *Drosophila melanogaster*, Shimla 21°C of different age groups i.e. (7, 21, and 35) days against the different PEs concentration of *Tinospora crispa* i.e. (2, 4, 6, and 8) mg/ml

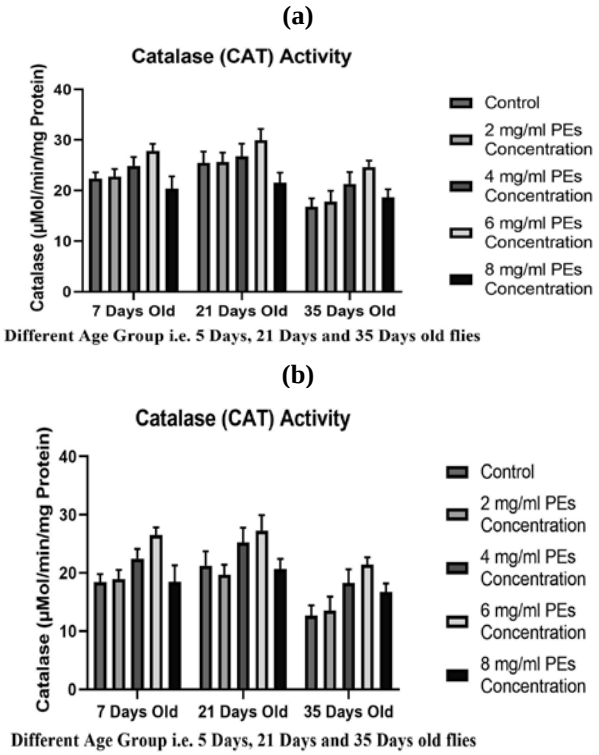

Fig 5: The Bar Diagram shows the CAT analysis of Control (a) male and (b) female flies of *Drosophila melanogaster*, Shimla 21°C of different age groups i.e. (7, 21, and 35) days against the different PEs concentration of *Tinospora crispa* i.e. (2, 4, 6, and 8) mg/ml

Catalase (CAT) Activity Analysis

In the present study the activity of enzyme Catalase (CAT) was observed against the PEs concentration of *Tinospora crispa* (i.e. 2, 4, 6, and 8 mg/ml). The activity of catalase enzyme was depends up on the activity of SOD enzyme activity. Actually SOD converts the superoxide free radicals into the less reactive product Hydrogen peroxide (H_2O_2) and the catalase enzyme converts the Hydrogen peroxide (H_2O_2) into non reactive species O_2 and H_2O. All the PEs concentration was applied on three different age groups (i.e. 7, 21, and 35 days) old male flies.

The observations of CAT activity of male and female flies of *Drosophila melanogaster* were analyzed by two way ANOVA processed in software GraphPad Prism 8.3. The mean CAT activity of flies with SD values was observed. The significant result was observed from male flies by the PEs concentration of 4 and 6 mg/ml i.e. the analysis from the two way ANOVA the percentage of total variation from interaction was observed 4.341 (P≥ 0.0006, significant) and DF(DFn, DFd) was observed from their interaction F (8, 135) = 3.730. Also, the significant result was observed by the PEs concentration of 4 and 6 mg/ml i.e. the analysis from the two way ANOVA the percentage of total variation from interaction was observed 4.341 (P≥ 0.0006, significant) and DF(DFn, DFd) was observed from their interaction F (8, 135) = 3.730 (Fig 5 and Table 2). Thus, the results show that the PEs concentration of *Tinospora crispa* of 4 and 6 mg/ml was successfully improved the CAT activity in the male and female fly.

Lipid Peroxidation (LPO) Quantification Analysis

In the present study the quantification of Lipid Peroxidation (LPO) was observed against the PEs concentration of *Tinospora crispa* i.e. Control (2, 4, 6, and 8) mg/ml. All the PEs concentration was applied on three different age groups i.e. (7, 21, and 35) days old aged flies of male and female respectively.

The result outcome of LPO through quantification of Malondialdehyde (MDA) of flies of *Drosophila melanogaster* were analyzed by two way ANOVA processed in software GraphPad Prism 8.3. The mean Lipid Peroxidation (LPO) quantized values with their SD values were observed. The significant result was observed from the male flies by the PEs concentration of 4 mg/ml and 6 mg/ml i.e. the analysis from the two way ANOVA the percentage of total variation from interaction was observed 1.109 (P≥ 0.3512, NS) and DF(DFn, DFd) was observed from their interaction F (8, 135) = 1.124. And, the analysis from the female flies, the two way ANOVA the percentage of total variation from interaction was observed 0.6002 (P≥ 0.8076, NS) and DF(DFn, DFd) was observed from their interaction F (8, 135) = 0.5617 (Fig 6 and Table 2). Thus, the results show that the PEs concentration of *Tinospora crispa* of 4 mg/ml and 6 mg/ml was successfully controlled the peroxidation of cell membrane lipids both in the male and female fly.

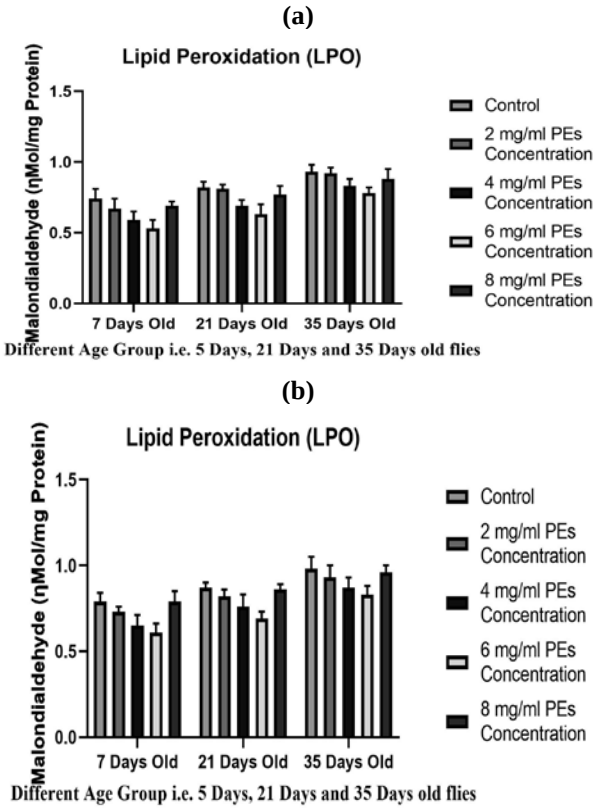

Fig 6: This Bar Diagram shows the quantitative analysis of Malondialdehyde the product of peroxidation of lipids (LPO) of Control (a) male and (b) female flies of *Drosophila melanogaster*, Shimla 21°C of different age groups i.e. (7, 21, and 35) days against the different PEs concentration of *Tinospora crispa* i.e. (2, 4, 6, and 8) mg/ml

Phenoloxidase (PO) Activity Analysis

The phenoloxidase is the enzyme which shows the host specific immune response specially in the cuticular membrane of the insects (Arthropods). The pathogens like fungus, bacteria whenever enters in the body of insects the enzymatic cascade of phenoloxidase through melanization. Actually the enzyme Phenoloxidase (PO) converts the L-DOPA to Dopachrome which leads to the process of melanization a response against the surface pathogens.

In the present study the quantification of Phenoloxidase (PO) activity was observed against the PEs concentration of *Tinospora crispa* i.e. Control (2, 4, 6, and 8) mg/ml. All the PEs concentration was applied on three different age groups i.e. (7, 21, and 35) days old aged flies of male and female respectively.

The observations of PO through quantification of Phenoloxidase (PO) activity of male and female flies of *Drosophila melanogaster* were analyzed by two way ANOVA processed in software GraphPad Prism 8.3. The mean Phenoloxidase (PO) activity with their SD values was observed. The significant result was observed from male flies by the PEs concentration of 4 mg/ml and 6 mg/ml i.e. the analysis from the two way ANOVA the percentage of total variation from interaction was observed 2.152 (P≥ 0.7623, NS) and DF(DFn, DFd) was observed

from their interaction F (8, 135) = 0.6172. Also, the analysis from female flies, the two way ANOVA the percentage of total variation from interaction was observed 1.174 (P≥ 0.9466, NS) and DF(DFn, DFd) was observed from their interaction F (8, 135) = 0.3452 (Fig 7 and Table 2). Thus, the results show that the PEs concentration of *Tinospora crispa* of 4 mg/ml and 6 mg/ml was successfully improved the Phenoloxidase (PO) Enzyme activity both in the male and female flies.

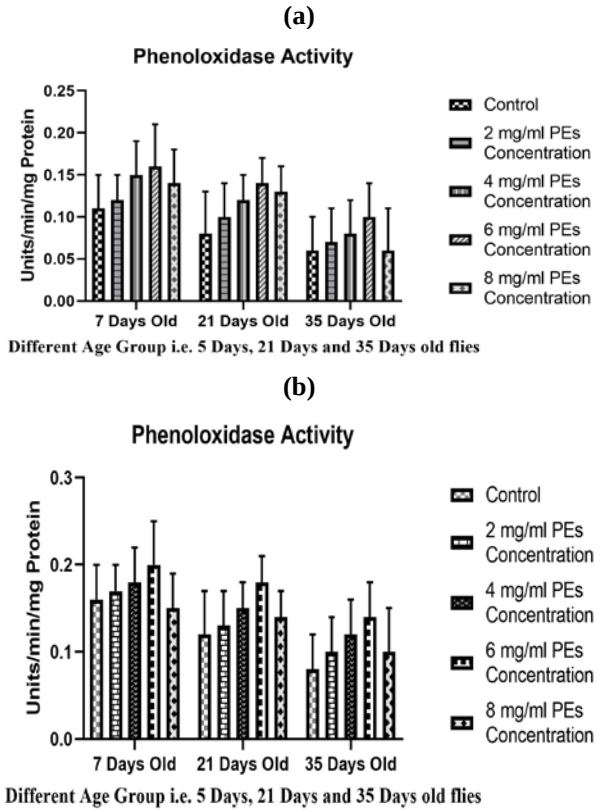

Fig 7: The Bar Diagram shows the quantitative analysis of Phenoloxidase Activity of Control (a) male and (b) female flies of *Drosophila melanogaster*, Shimla 21°C of different age groups i.e. (7, 21, and 35) days against the different PEs concentration of *Tinospora crispa* i.e. (2, 4, 6, and 8) mg/ml

Protein Carbonylation Analysis

In this study the level of Protein Carbonyls was quantified in the three different age groups and PEs concentration gradient i.e. (2, 4, 6, and 8) mg/ml. of *Tinospora crispa* in *Drosophila melanogaster* in-vivo. As studied, the effect of increasing age on the levels of Protein Carbonyls was observed as the age of the fly going to increase due to oxidative stress the production of Protein Carbonyls was also increased. So from the studies based on the antioxidant behavior of *Tinospora crispa* (Chaudhary *et al*., 2010), the PEs of this plant was used to overcome the production of the Protein Carbonyls by reducing the oxidative stress.

The results from the Protein Carbonylation of male and female flies of *Drosophila melanogaster* were analyzed by two way ANOVA processed in software GraphPad Prism 8. The mean value of Protein Carbonyls in nMols of flies with SD values was observed. The significant result was observed from male flies by the PEs concentration of 4 mg/ml and 6 mg/ml of *Tinospora crispa* i.e. the analysis from the two way ANOVA the percentage of total variation from interaction was observed 3.924 (P≥ 0.0010, significant) and DF(DFn, DFd) was observed from their interaction F (8, 135) = 3.514. Also, the analysis from female flies the two way ANOVA the percentage of total variation from interaction was observed 1.248 (P≥ 0.3650, NS) and DF(DFn, DFd) was observed from their interaction F (8, 135) = 1.103 (Fig 8 and Table 2). Thus, the results show that the PEs concentration of 4 mg/ml and 6 mg/ml was successfully improved the physical health and the flies from oxidative stress of aging in all three age groups of male and female flies.

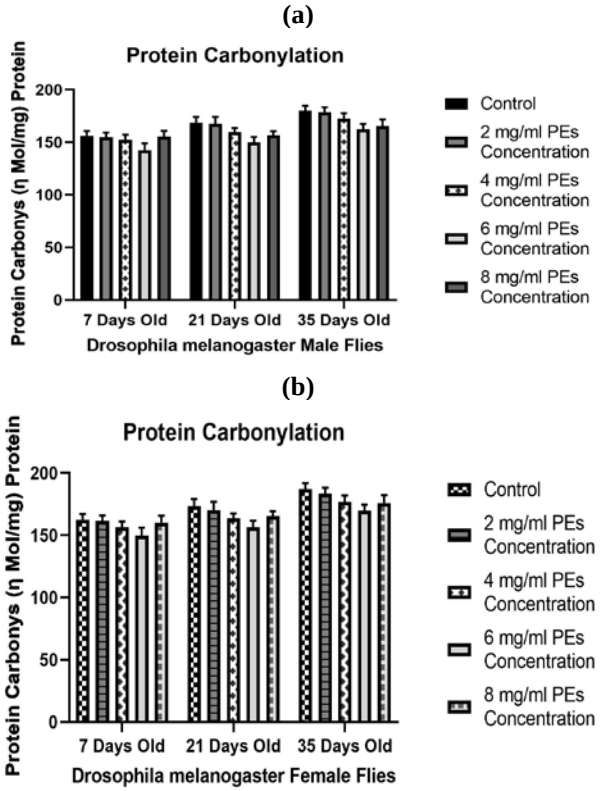

Fig. 8: The Effect of PEs of *Tinospora crispa* of different concentration i.e. (2, 4, 6, and 8) mg/ml shown in the bar Diagram against the three age groups i.e. (7, 21, and 35) days old (a) male and (b) female flies

Chill Coma Recovery Time (CCRT)

The CCRT is the time taken in seconds (s) by flies to recover from Chilled Coma Condition reared at different environmental conditions. The chilled coma condition is a very significant parameter to describe the physical health of the fly. Until the fly couldn't stand up on its legs the data was not recorded.

The results from the CCRT of male and female flies of *Drosophila melanogaster* were analyzed by two way ANOVA processed in software GraphPad Prism 8.3. The mean CCRT of with their SD values was observed. The significant result was observed from male flies by the PEs

concentration of 4 mg/ml and 6 mg/ml of *Tinospora crispa* i.e. the analysis from the two way ANOVA the percentage of total variation from interaction was observed 1.787 (P≥ 0.0001, significant) and DF(DFn, DFd) was observed from their interaction F (8, 735) = 39.16. Also the analysis from female flies the two way ANOVA the percentage of total variation from interaction was observed 4.468 (P≥ 0.0001, significant) and DF(DFn, DFd) was observed from their interaction F (8, 735) = 48.73 (Fig 9). Thus, the results show that the PEs concentration of 4 mg/ml and 6 mg/ml was successfully improved the physical health and the flies from oxidative stress of aging in all three age groups of male and female flies.

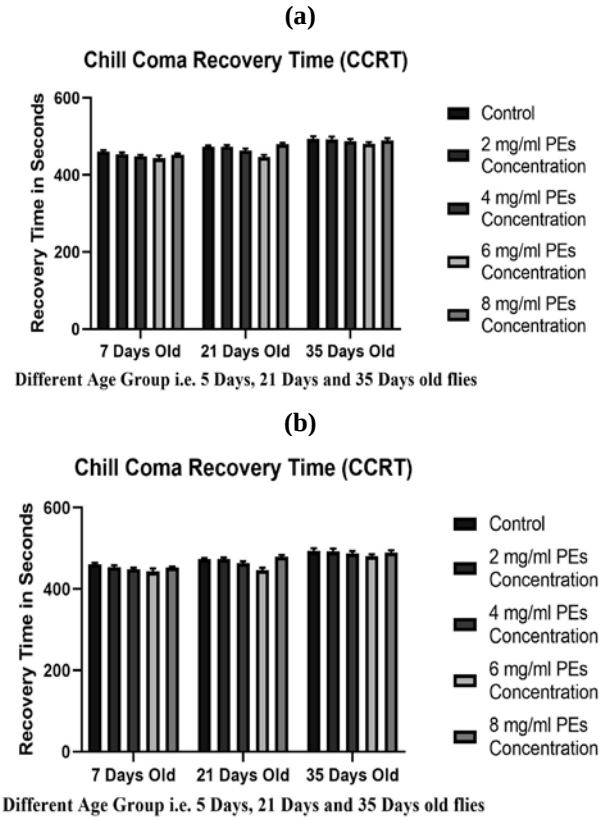

Fig 9: The representation of the Chill Coma Recovery Time (CCRT) of Control (a) male and (b) female flies of *Drosophila melanogaster*, Shimla 21°C of different age groups i.e. (7, 21, and 35) days against the different PEs concentration of *Tinospora crispa* i.e. (2, 4, 6, and 8) mg/ml

Negative Geotactic Movement

The Negative Geotactic Movement of fly is observed as the activeness and physical health against the gravity of Earth. The 10 cm tall glass vial was used to observe this physical fitness. As the aging going to increase in the body the body's movement especially flight muscles suffers from oxidative stress that is a consequence of free radicals result to the protein carbonyls, muscle sarcopenia and aging. The PEs was used to show the therapeutic effect on these above said aging related effects. In the present study only flies was recorded which attained the maximum height in the vial in 10 s. These experimental flies were devoid of anesthesia. The 50 number of flies was used for each PEs concentration and age group.

The results from the Negative Geotactic Movement of male and female flies of *Drosophila melanogaster* using the PEs gradient concentration of *Tinospora crispa* were analyzed by One Sample t Test processed in software GraphPad Prism 8.3. The mean number of flies reached up to top of vial in 10 s was observed. The significant result was observedfrom male and female flies by the PEs concentration of 4 mg/ml and 6 mg/ml of *Tinospora crispa*. All values are analyzed by one sample t- Test (P-Value ≥ 0.0001) (Fig 10). Thus, the results show that the PEs concentration of 4 mg/ml and 6 mg/ml was successfully improved the physical health and the flies from oxidative stress of aging in all three age groups of male and female flies.

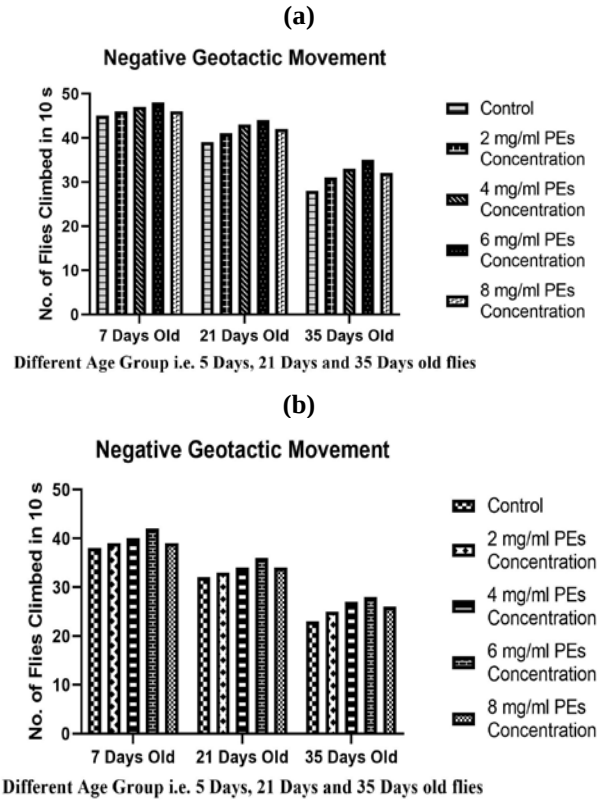

Fig 10: The representation of the Negative Geotactic Movement of Control (a) male and (b) female flies of *Drosophila melanogaster*, Shimla 21°C of different age groups i.e. (7, 21, and 35) days against the different PEs concentration of *Tinospora crispa* i.e. (2, 4, 6, and 8) mg/ml

DISCUSSION AND CONCLUSION

It is also observed that the fecundity of fly was observed for 21 days continuously and when the age of fly rose, the number of egg laid by the female fly was reduced. As we know, the reproduction in the individuals takes huge amount of energy and production of oxidative stress result to the retard in the body and physical illness. Thus, the increasing age of fly couldn't arrange sufficient energy

and metabolites for reproduction successfully result to the decrease in per day fecundity.

As we observed, the PEs concentration of plant of 2 mg/ml showed very significant result in respect of aging more than control flies and other PEs concentration treated flies in the case of *Tinospora crispa*. Also when PEs concentration raised consistently the decrease in per day fecundity of fly was observed. The PEs concentration of 2 mg/ml might successfully scavenge the free radicals generated oxidative stress. Although other than 2 mg/ml concentration i.e. 4 mg/ml, 6 mg/ml and 8 mg/ml didn't showed significant result in respect of enhancement in per day fecundity. The possible reason for it may be that PEs concentration other than 2 mg/ml of plant *Tinospora crispa* could be toxic or could be hurdle in the developing phase of eggs.

The developmental time in days showed the regression by the treatment of the PEs diet of *Tinospora crispa* in the development developmental behavior. As we were going to increase the concentration of PEs the time to develop was also increased. Although the body size and body mass was increased in respect of PEs concentration in the diet of *Drosophila*. Due to increase in the PEs concentration the vitelline membrane of eggs was become thickened, just because the larvae and pupae were taken more time to develop.

In this study, it was successfully evaluated the antioxidant capacity of *Tinospora crispa* in the *Drosophila melanogaster*. The oxidative stress markers SOD, Catalase Lipid Peroxidation, Phenoloxidase and Protein carbonylation activity showed the significant result towards the suppression in the formation of free radicals. These results indicate that the hydro-ethanolic extract of *Tinospora crispa* is a valuable source of antioxidant compound that can potentially inhibits the damage by oxidative stress. The successful scavenging of free radicals appears to be very interesting and have important biological activities that can be exploited for the treatment of aging related illness. Hence, the presence of active antioxidant compounds in *Tinospora crispa* could lead to discovery of active anti-aging agents.

Acknowledgements

The author expresses the thanks to Department of Biotechnology, UIET, MDU, Rohtak, India for providing all the facilities to conduct work. Authors also express thanks Dr. Vikram Munday for the moral support.

Conflicts Of Interest

The authors declare that there are no conflicts of interest regarding publishing of this research article.

Funding Sources

This research was funded and supported by the *Drosophila* Genetics Lab, Department of Biotechnology, UIET, MDU, Rohtak, India.

REFERENCES

1. AEBI H.E., (1984) – Catalase. In: Bergmeyer, H. U., editor. *Methods of Enzymatic Analysis, vol. 3, Wiley, New York*, pp. 273-286.
2. Choudhary, M. I., Ismail, M., Ali, Z., Shaari, K., Lajis, N. H., & Atta-ur-Rahman. (2010). Alkaloidal constituents of *Tinospora crispa*. *Natural product communications*, *5*(11), 1934578X1000501109.
3. Harman, D. (1956). Aging; a theory based on free radical and radiation chemistry. */. Geront.*, 77:298-300,.
4. Harman, D. (1972). The biologic clock: the mitochondria?. *Journal of the American Geriatrics Society*, *20*(4), 145-147.
5. Levine RL (2002) Carbonyl modified proteins in cellular regulation, aging, and disease. *Free Radic Biol Med* 32: 790–796
6. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., (1951). Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
7. McCord, Joe M., and Irwin Fridovich. (1969). "Superoxide dismutase an enzymic function for erythrocuprein (hemocuprein)." *Journal of Biological chemistry* 244.22: 6049-6055.
8. Wills, E. D. (1969). Lipid peroxide formation in microsomes. Relationship of hydroxylation to lipid peroxide formation. *Biochemical journal*, *113*(2), 333-341.
9. Zulkhairi A, Hasnah B, Sakinah I, Nur Amalina I, Zamree MS, Mohd Shahidan A. Nutritional composition, antioxidant ability and flavonoid content of *Tinospora crispa* stem. . *Advances in Natural and Applied Sciences*, 2009; 3(1): 88-94.