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Murraya Koeingii a Protector Against Insecticide Wastes Leachates Induced Oxidative Stress in *Drosophila Melanogaster* Third Instar Larvae

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Abstract:- **The current study was assessed to know whether an aqueous extract of the leaves of *Murraya Koenigii* (Curry leaves) has the ability to protect against insecticide wastes leachates induced oxidative stress in the third instar larvae of *Drosophila melanogaster* (Oregon *K*). The study shows that the curry leaves dose dependently protected the biomarkers of tissue damage, oxidative stress and antioxidant enzymes from getting altered in the larvae following treatment with leachates. The results specified that the aqueous curry leaf extract (CuLE) might have protected the larvae from leachates induced oxidative stress through antioxidant mechanism(s).**

Keywords: Oxidative stress; Biomarkers; Antioxidant; Leachates; Murraya Koenigii

Abbreviations: CuLE: Curry leaves; ROS: Reactive oxygen species; *M.koeingii*: Murraya koeingii; SOD: Superoxide dismutase ; CAT: Catalase ; LPO: Lipid peroxidation

I. INTRODUCTION

Free radicals or activated oxygen has been connected to environmental stresses in animals and appears to be a common participant in most of the degenerative conditions in eukaryotic cells. Oxidative stress is the redox state resulting from an imbalance between the generation and detoxification of reactive oxygen species (ROS) (Wang and Michaelis, 2010). Under carefully controlled situations, ROS function as important physiological regulators of intracellular signaling pathways (Finkel, 2011). However, the overloaded amount of ROS causes damage to cells by oxidizing cellular biomolecules, including nucleic acids, proteins and lipids (Lobo et al., 2010). Summarizing the earlier conducted large body of studies, on different environmental toxicants i.e. metals, pesticides, solvents, industrial and municipal runoffs, in different model systems revealed increased oxidative stress caused by these xenobiotics (Dorts et al., 2009b; Singh et al., 2009; Bhargav et al., 2008; Siddique et al., 2008; Avci et al., 2005; Pandey et al., 2003; Livingstone et al., 2000). From previous studies it was confirmed that industrial soil samples from few industrial sites in Rohtak contained heavy metal concentration (Pb, Zn, Cu, Ni, Cd) that exceed the calculated worldwide mean of unpolluted soil, indicating high level of pollution on the site of industries due to solid wastes dumped (Annu, 2016, 2015; Urmilla, 2015) but *in vivo* study has not been done yet.

Antioxidants are substances, when present in small quantities prevent the oxidation of cellular organelles by minimizing the damaging effects of ROS and RNS (Reactive Nitrogen species). Under normal healthy conditions, a balance is maintained between oxidative stress and antioxidant requirements. Antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), metal sequestering proteins forms the endogenous antioxidant defenses. However under pathological conditions or during radiation injury, stress and pollution etc. the balance is lost and excessive supplementation of antioxidants is necessary. During this study we used a very common plant *Murraya Koeingii* (Curry leaf) as an antioxidant to ameliorate the effect of leachates on Drosophila larvae. This plant has been reported to have anti-oxidative, cytotoxic, antimicrobial, antibacterial, antiulcer, positive inotropic and cholesterol reducing activities (Rahman MM et al., 2005; Kesari et al., 2005; Shrinivasan et al., 2005). In a study on pharmaceutical wastes leachates it was found that CuLE act as a potent antioxidant to enhance climbing ability affected by oxidative stress (Saroha et al., 2019).

The present study was designed using *Drosophila melanogaster* as an animal model. *Drosophila melanogaster* commonly known as fruit fly, is one of the well-studied model organism, has several advantages over other animal models, as it has shorter life cycle (10-12 days at 25^{o}C), high fecundity (females lay 600 eggs in a life time), easy to handle and fewer number of chromosomes (3 pairs of autosomes and 1 pair of sex chromosomes) with 75% similarity with human disease genes. Using fruit fly, we demonstrate the toxic potential of insecticides industrial

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leachates on third instar larvae of Drosophila and antioxidant property of *M. koenigii*.

II. MATERIAL AND METHODS

A. Collection and Extraction of *Murraya koeingii*

Murraya Koeingii leaves were collected dried and grinded into powder and a known 250 g of dried leaves were extracted with distilled water at 45°C for 3 h filtered through whattman No. 1 filter paper and evaporated to get a crude extract.

B. Collection of soil and leachate preparation

Randomized sampling technique was used for collection of industrial soil and solid wastes for the assessment of toxicity (Houk, 1992). Control soil samples were collected from the institute only. Five random samples were collected and made single. For the preparation of 10% leachates, leachates from soil and the industrial solid wastes at three different pH viz, 7.00 (in MilliQ water, neutral), 4.93 (5.7ml glacial acidic acid + 64.3ml IN NaOH + 930ml MilliQ water; low acidic) and 2.88 (5.7ml glacial acetic acid + 994.3ml MilliQ water; highly acidic) Toxicity Characteristics Leachate Procedure (TCLP) [Method-1310; USEPA, 1990] was used. The leachates (prepared at three different pHs) were referred to as N, M and H, respectively.

C. Biochemical markers study

Newly emerged larvae (22 ± 2 hr) collected from synchronized egg collections were transferred to food vials containing different concentrations of the leachates prepared from industrial waste and soil at different pHs and CuLE and allowed to grow for 96±2 hr. Study is divided in four groups as mentioned below:

Group 1 Control (Standard diet)
Group 2 Leachates treated
Group 3 Standard diet along with CuLE
Group 4 Leachates treated along with CuLE concentrations

C.1 Effects of CuLE on leachates induced oxidative stress

For the biochemical study, prepared plant extract were used to ameliorate the effects of industrial leachates on drosophila larvae. Newly hatched larvae were allowed to feed on industrial leachates (0.5%, 1%, 2 %) and plant extract (4 g/l and 6 g/l) till third instar larvae emerges. Third instar larvae were collected and washed and homogenized in PBS pH 7.4 and centrifuged for 10 minute supernatant thus obtained was used to determine, lipid peroxidation (LPO), the activity of antioxidant enzymes (SOD and Catalase).

C.2 Measurement of Lipid Peroxidation (Okhawa et al., 1979)

The amount of LPO was measured by measuring Thiobarbituric acid reactive substances in larvae. Reaction mixture consisted of tissue homogenate, 10% (w/v) sodium dodecyl sulfate, 0.8% thiobarbituric acid, 20% (v/v) acetic acid (pH 3.5). Absorbance was taken at 532 nm and expressed as malondialdehyde equivalents.

C.3 Measurement of SOD activity (Marklund 1974)

It was determined by measuring the inhibition of pyrogallol autoxidation. Reaction mixture consisted of 2 mM pyrogallol, tissue homogenate, 0.1 M tris-HCl buffer (pH 8.2). Absorbance was measured for 3 minutes at 420 nm. Activity was expressed as enzyme units required to inhibit 50% pyrogallol autoxidation.

C.4 Measurement of Catalase (Aebi 1974)

Catalase activity was analyzed by quantitating the rate of H_2O_2 decomposition by the enzyme by adding 1% (v/v) H_2O_2 to reaction mixture containing tissue homogenate and 0.05 M phosphate buffer (pH 7). Absorbance was observed for 3 minute at 240 nm and expressed as µmoles of H_2O_2 decomposed/min/mg protein.

C.5 Measurement of Total protein content (Lowry 1951)

Protein content was measured by Lowry method by taking BSA as standard.

C.6 Statistical analysis

The data are represented as Mean±S.E. Statistical differences at $P<0.05$ between groups were analyzed by Two-way analysis of variance (ANOVA) followed by Dunnett's multiple comparision test using Graphpad prism 8 software.

III. RESULT AND DISCUSSION

The present study aims to establish that aqueous CuLE has the potential to protect *D.melanogaster* against leachates induced oxidative stress. Industrial solid wastes leachates were found to have contrary effects on the exposed organisms as apparent by deviations in oxidative stress markers. *Murraya* extract has been characterized to be full of alkaloids, polyphenols, flavonoids and chlorophyll. Plants are used as a source of antioxidant and alternative medicines in various models against oxidative stress (Tachibana et al., 2001; Ningappa et al., 2008; Menezes et al., 2013).

A significant ROS generation and MDA was observed in the exposed larvae $P<0.05$.

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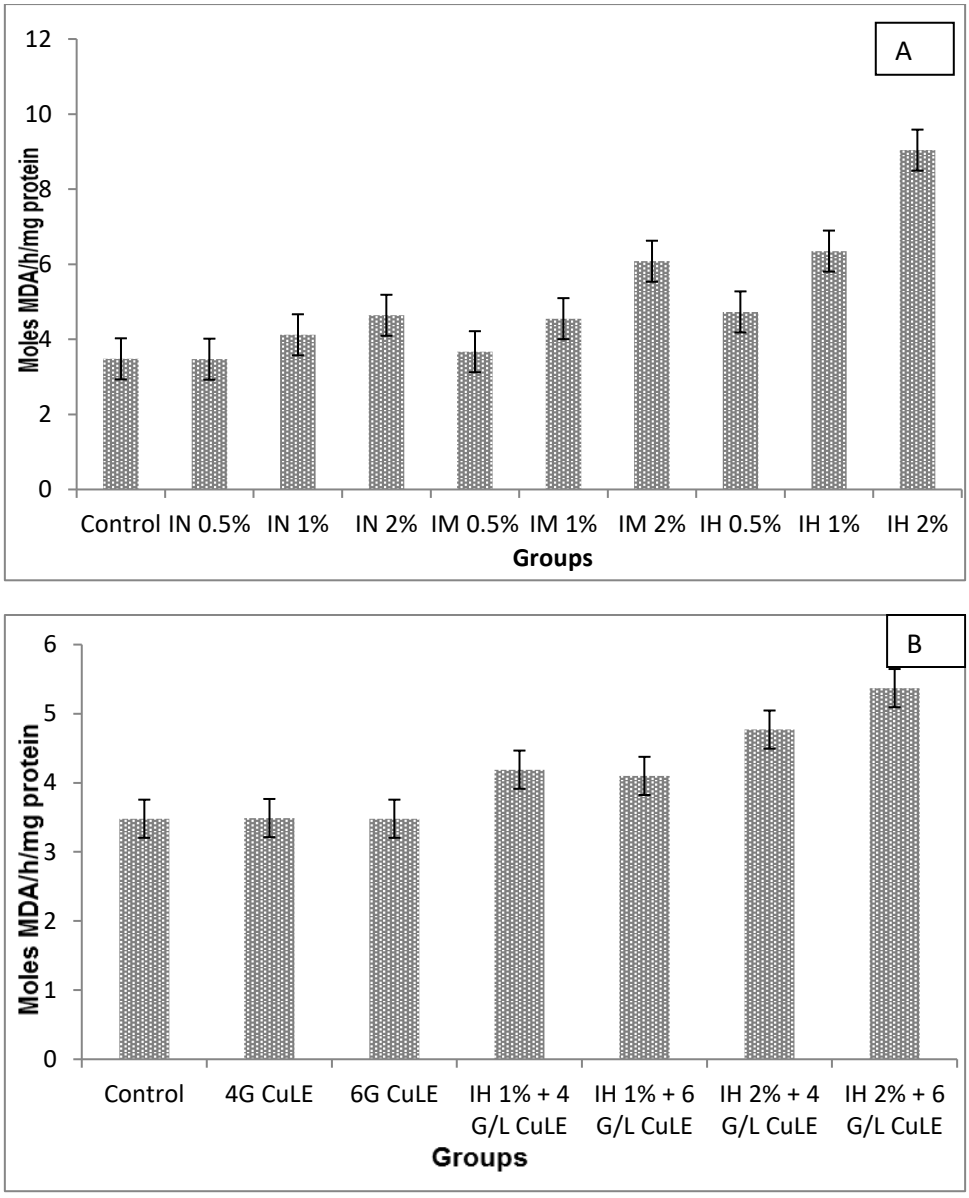

Figure 1 MDA level. Values are Mean ± S.E A) insecticide leachates B) Plants treated and insecticide leachates exposed

Malondialdehyde (MDA) oxidatively modified molecules is well-thought-out to be a marker of oxidative damage (Ali et al., 2004; Paragasam et al., 2006). Lipids are attacked by ROS by free radical chain reaction mechanism to produce lipid peroxidation products. MDA is the lipid peroxidation product produced mostly in oxidative stress conditions.

A significant increase in MDA level was observed in larvae exposed to leachates ($P<0.05$ vs. Control) Figure1 (A). When larvae were supplemented with CuLE they showed 40 % to 52% decrease in MDA level in larvae exposed to leachates (Figure 1(B). At low concentrations, of leachates (0.5%) larvae shows nonsignificant effects on MDA level with $P>0.05$.

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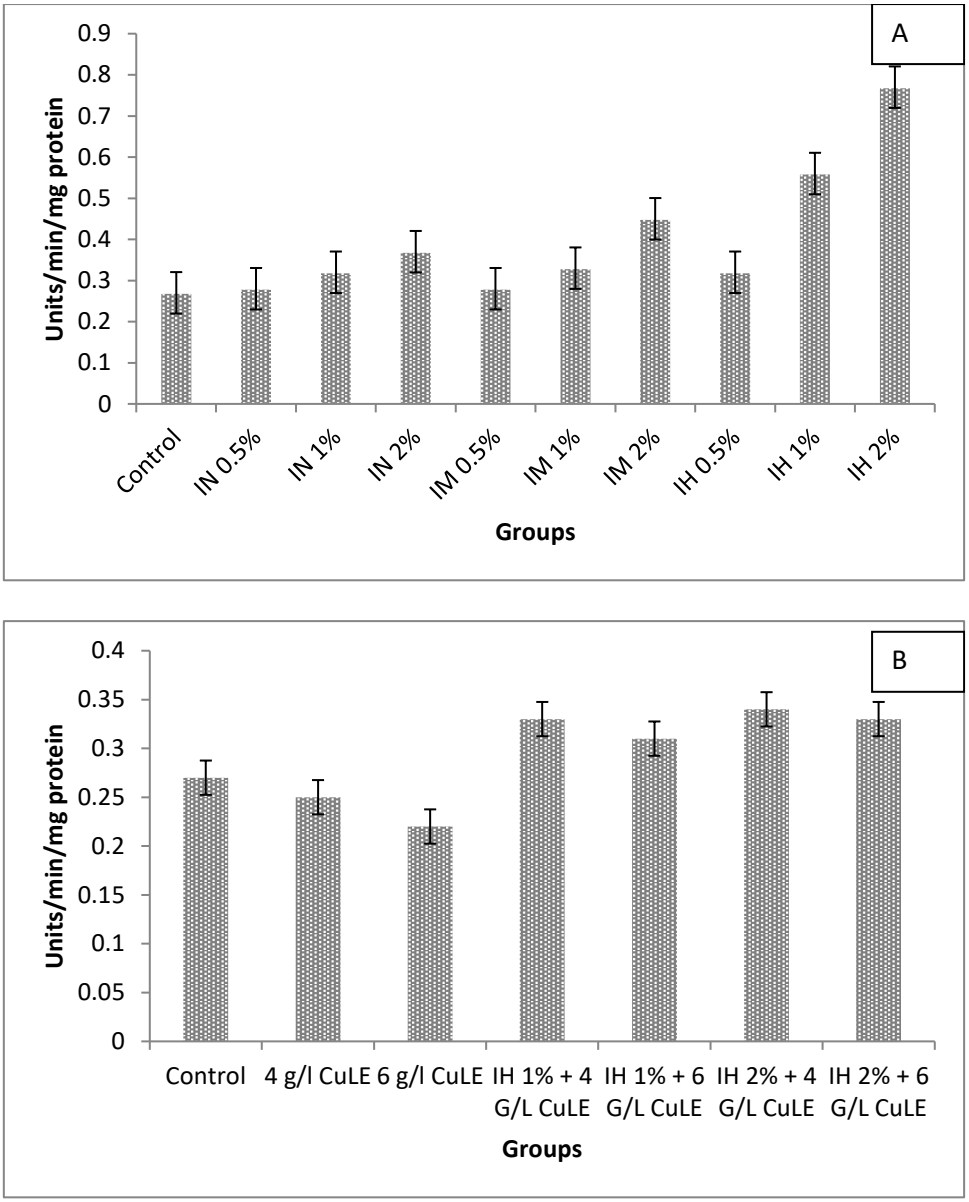

Figure 2 SOD level values are Mean ± S.E A) insecticide leachates B) Plants treated and industrial leachates exposed

At highest concentration and acidic pH leachates evoke significant (P<0.05) 1.07 and 1.85 fold increase in SOD activity exposed to insecticide leachates Figure 2 (A). When provided CuLE a significant P<0.001 upto 55% decrease in SOD enzyme activity was observed Figure 2 (B).

The major antioxidant enzyme is catalase consisting of heme as the prosthetic group. Molecular oxygen and water is formed in the presence of NADH which acts as cofactor for enzyme activation from H_2O_2.. Acidic industrial leachates exposure significantly increased the catalase activity by 1.31 and 1.46 fold in drosophila larvae P<0.001. About 31% decrease in catalase activity was observed in larvae supplemented with CuLE along with insecticide leachates. SOD and CAT are responsible to scavenge superoxide anion and hydrogen peroxide.

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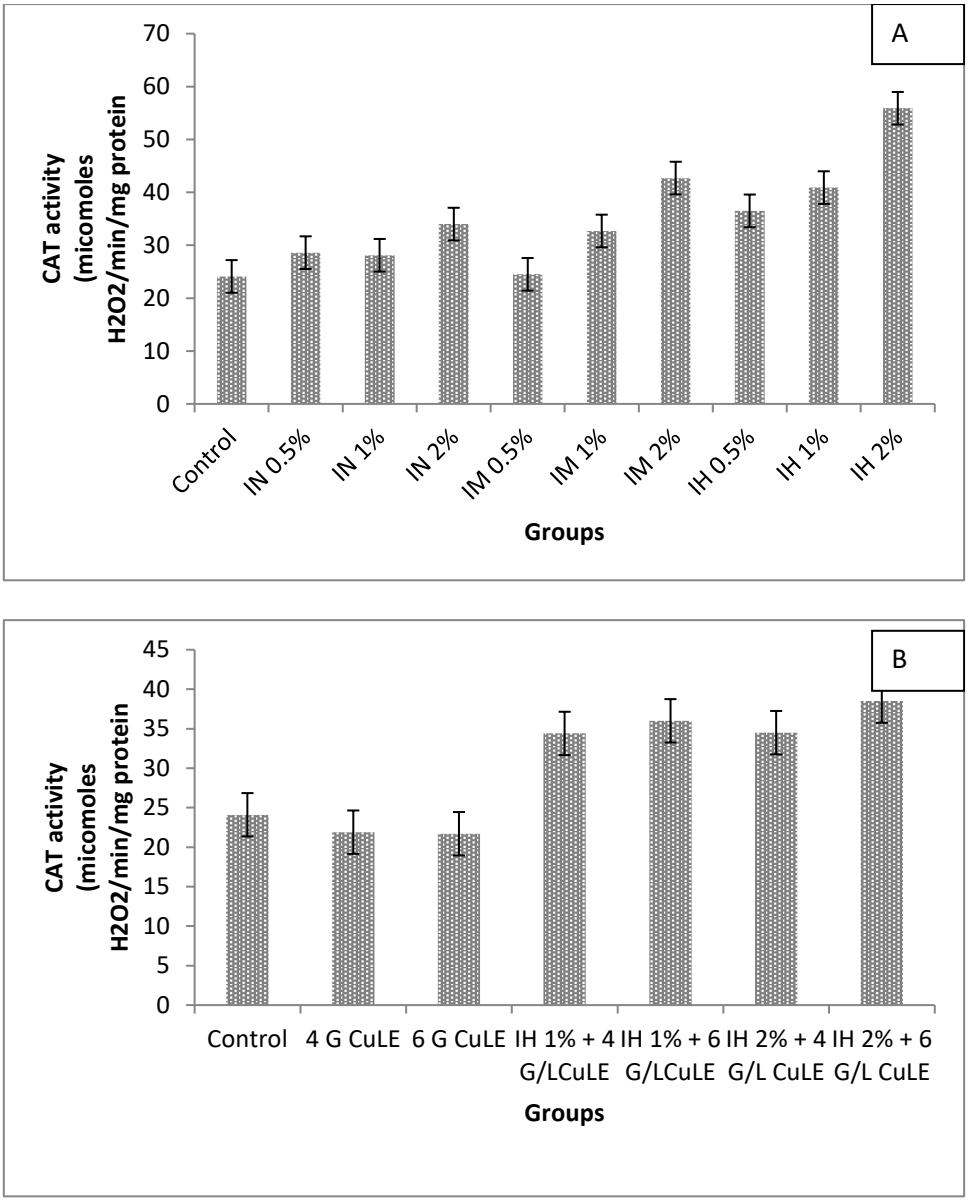

Figure 3 Catalase activity Values are Mean ± S.E A) insecticide leachates B) plants treated and industrial leachates exposed

A previous study on industrial leachates indicates induction of biochemical stress markers in D. melanogaster against complex mixture (Siddique et al., 2007). Increase in the activities of SOD and CAT in the exposed organisms is an effort to reduce the induced damage. SOD and CAT activities remain unaltered in the groups provided with CuLE and in leachates exposed group they protect from getting altered. The protection may be due to the ability of CuLE to reduce the accumulation of free radicals produced following industrial leachates. Figure 4 shows total protein content in control, leachates exposed and plant extract treated larvae. Proteins are found in large quantity in biological systems and its can be taken as indicator of toxicity (Davies, 2005). Leachates exposed reveals significant 53% to 55% decline in protein content $P<0.05$ as compared to control larvae. Change in protein content level in larvae and its inflection by CuLE was recorded.

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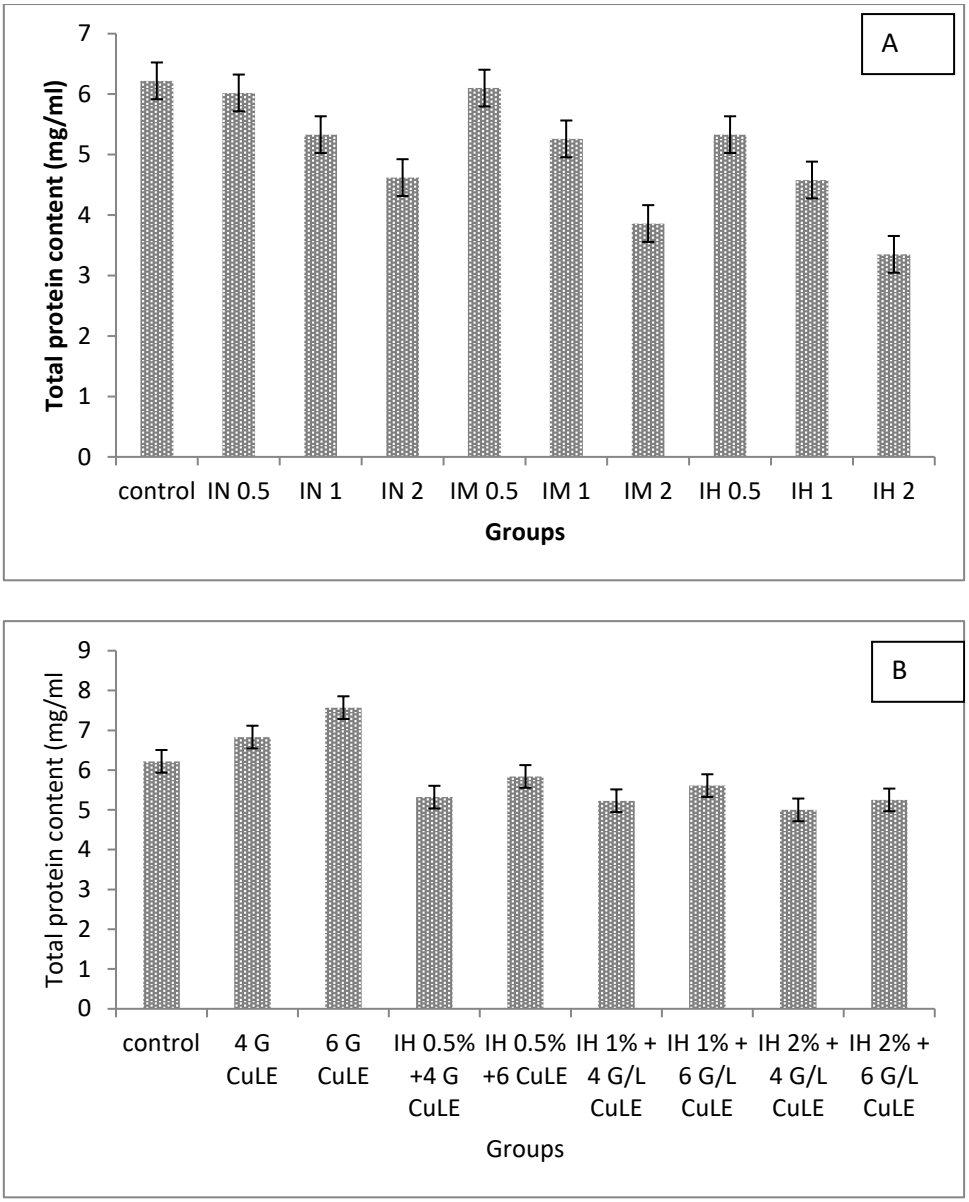

Figure 4: Total protein content, values are Mean ± S.E A) Protein content in Insecticide leachates B) Protein content in plant extract treated and leachates exposed

At highest concentration of CuLE protein content evoke to 56% and in larvae exposed to insecticide leachates. Statistically significance: $P<0.001$ and $P<0.05$ shows significant increase compared to groups exposed to leachates. However the aqueous CuLE seem to possess an ability to increase protein content probably by scavenging the reactive oxygen species or by chelating the heavy metal or by both. With increase in concentration of CuLE in food medium the increase in oxidative stress decreased until a point of concentration afterward no such effect was observed may be due to absorption threshold of CuLE, it could be because higher concentration may not get absorbed completely.

The content of total anti-oxidant activity of M. koenigii leaves was found highest (2691µmol of Ascorbic acid/ gm) amongst all green leafy vegetables (The Wealth of India) but there in vivo study in Drosophila has not been done so far. Experiments conducted on animals indicated that Pb is both genotoxic (Shaik et al., 2006) and carcinogenic (Fowler et al., 1994), Ni (Haugen et al., 1994) and Cd (Elinder and Jarup, 1996) are carcinogenic, Cu generated free radicals when present in free form and produces ROS that causes damage to biomolecules like DNA, protein, and lipid (Galaris and Evangelou, 2002).

We finally hypothesize that the changes in biochemical parameters and physiological parameters are somehow affected by leachates induction. Secondly we observed that M. koeingii protect against induced oxidative stress.

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