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Modulatory Effect of Picralima Nitida on Oxidative Stress: Cobalt, Thiol, Total Protein in Drosophila Melanogaster Exposed to Lead.

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Abstract
Drosophila melanogaster, commonly known as fruit fly, vinegar fly, is a small model organism used in the study of various disciplines. D. melanogaster shares basic biological, biochemical, neurological and physiological similarities with human beings and has about 75% functional homologs of genes causing diseases in humans. Picralima nitida is a therapeutic herb used in ethnomedicine for the management of several disease conditions. The objective of this study is to induce oxidative stress in Drosophila melanogaster using lead and determine its effect on the levels of selected oxidative stress parameters. Determining the potential modulatory effect of Picralima nitida on selected oxidative stress parameters in lead-induced oxidative stress in Drosophila melanogaster was also known in this study. The phytochemicals of Picralima nitida, and survival rate in Drosophila melanogaster exposed to different concentrations of lead and/or Picralima nitida in meal were also studied. A total of 250 Drosophila melanogaster flies were seen in each of the 9 groups (A-I). Group A was used as the control group and was fed with normal standard meal with water only. Group B were fed with 0.25mg/dl lead acetate meal, group C were fed with 1mg/dl lead acetate meal, group D were fed with 10mg/kg Picralima nitida meal, group E were fed with 100mg/kg Picralima nitida diet, group F were fed with 0.25mg/dl lead acetate and 10mg/kg Picralima nitida diet, group G were fed with 1 mg/dl lead acetate and 10mg/kg Picralima nitida diet, group H were fed with 0.25mg/dl lead acetate 100mg/kg Picralima nitida meal and group I were fed with 1mg/dl lead acetate and 100mg/kg Picralima nitida diet. After about 5 days, flies were homogenized and the supernatant recovered were then used for the various biochemical and survival assay, Cobalt was determined with inductively coupled plasma - mass spectrometry, total protein was determined with Lowrys method and total thiols was determined with Ellman's method. The results showed that lead at various concentrations had a toxic effect on the level of oxidative stress parameters (cobalt, thiols, total protein) in Drosophila melanogaster. While Picralima nitida a medicinal plant had a modulatory effect on the level of oxidative stress parameters (Cobalt, total thiols, total protein) in lead- induced Drosophila melanogaster, although it can become toxic when taken in high concentration. All varying concentrations of lead acetate or/and Picralima nitida reduced the survival rate in the flies. From this study it could be deduced that at low concentration Picralima nitida has modulatory effect on oxidative stress

Keywords: Drosophila melanogaster, picralima nitida, oxidative stress, lead

Introduction: The fruit fly, *Drosophila melanogaster* has been widely used as a model organism to understand many molecular and biological processes in human. The use of *Drosophilia melanogaster* exclude the problem of obtaining ethical clearance for the animals. It also have many other advantages such as; it is 3-4mm long in size, smaller reagent required for assays and 75% of the human genes implicated in diseases are conversed in *D. melanogaster*, with about 90% nucleotide sequence identified in

some of its species (Reiter *et al.,* 2001). Flies can easily be handled, bred and genetically manipulated in large numbers. There exist now some number of genetic tools in Drosophila that is allowing researchers to attend to some of the outstanding issues that concerns the basic processes behind human diseases. Its other benefits include being much less costly and time consuming to use than a mouse model for example (Chan *et al*., 2000). Due to their rapid reproduction time and short lifespan, giving a higher throughput in experiments. The ability to introduce human genes into *D. melanogaster* has enabled scientists to recapitulate both the symptoms and the progression of human diseases in the fruit flies. *Drosophila melanogaster* is a small and friendly invertebrate used to study human diseases. This is quite remarkable considering that the genome of this animal is separated from ours by 795 million years. But what makes this organism so significant for the study of human diseases is that the entire Drosophila genome has been sequenced making it very simple to study and manipulate a particular gene (Adams *et al.*, 2000). The genome of this fly is about 60% identical to humans; also, about 75% of the genes that are responsible for human diseases have a homolog in the fruit flies (Pandey *et al*., 2011). In addition, their small size (2–3 mm), short generation time, the easy and inexpensive way to culture them in the laboratory, and their powerful genetic tools have established Drosophila as one of the leading animal models for education and biomedical research. Indeed, Drosophila can be used anywhere from teaching basic genetics to primary school, to understanding the more complicated metabolic pathways controlling fundamental physiological and pathological conditions (Millburn *et al.,* 2016).

Oxidative stress is a general term used to describe a serious imbalance in organisms between the production of reactive oxygen species (ROS) and the antioxidant defence mechanism in favor of the former, leading to a situation of potential risk (Sies, 1997). Under these conditions, ROS may damage membrane lipids and DNA, and affect the function of cellular proteins (Fiers *et al.*, 1998). ROS is a common name for diverse chemical species, including superoxide anions, hydrogen peroxide, hypochlorous acid, nitric oxide, peroxynitrite, singlet oxygen, hydroperoxyl, and the hydroxyl radicals, that are produced in cells as by-products of normal cellular metabolism. However, each of these molecules has its own unique characteristics, such as way of inactivation, substrate preferences, reactivity, kinetics, diffusion properties (Tonelli *et al.*, 2018).

For these reasons, oxidative stress contributes to the general decline in optimum bodily functions and may be involved in the pathogenesis of several disorders, whether as a cause or as an effect.

Superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen, are commonly defined reactive oxygen species (ROS); they are generated as metabolic by-products by biological systems (Sato *et al.*, 2013). Processes, like protein phosphorylation, activation of several transcriptional factors, apoptosis, immunity, and differentiation, are all dependent on a proper ROS production and presence inside cells that need to be kept at a low level (Rajendran *et al.,* 2014). When ROS production increases, they start showing harmful effects on important cellular structures like proteins, lipids, and nucleic acids (Wu *et al.,* 2013).A large body of evidences shows that oxidative stress can be responsible, with different degrees of importance, in the onset and/or progression of several diseases (i.e. cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular diseases) (Taniyama *et al.,* 2003).

Cobalt is a naturally occurring element. In humans, a single cobalt atom is the central metal component of vitamin B12, a cofactor and activator of several essential enzymes that is present in most tissues, chiefly in the liver (Siegel *et al.*, 2015). Although vitamin B12 is essential for erythrocyte formation, protein metabolism, and central nervous system function, cobalt and its related compounds can induce oxidative stress (Battaglia *et al*., 2009). Cobalt ions have been observed to generate reactive oxygen species (ROS) in vivo and in vitro and has been shown to induce the formation of hydroxyl radicals (OH) from hydrogen peroxide (H2O2) (Beyersmann *et al.,* 2008).

Proteins have long been considered a principal target for oxidants as a result of their abundance in biological systems. However, Halliwell *et al.* (2007) observed that there is increasing evidence that significant antioxidant activity exists some in proteins such as albumin.

Thus, Albumin is considered as a major circulating antioxidant in plasma. It is known to be exposed to continuous oxidative stress (Halliwell, 1996).

Thiols are organic compounds that contain sulfhydryl group (-SH). Thiols are made up of a hydrogen atom and a sulfur atom that is attached to a carbon atom (Rossi *et al*., 2009). In an organism, in the oxidation created by ROS, excess electrons pass to thiols and disulphide bonds are formed. Due to the balance in the oxidative state, electrons in these reversible bonds can easily return to thiols. The antioxidant ability of thiol-disulphide homeostasis is important in enzymatic reactions, signal transduction, detoxification, transcription, regulation of enzymatic activation, cellular signaling mechanisms and apoptosis reaction (Erel *et al.*, 2014).

Picralima nitida (*P. nitida*) is the only species of the genus *Picralima* and it is related to Hunteria and Pleiocarpa. *P. nitida* is commonly called *Picralima*, Akuamma or Pile plant, it belongs to the hunterieae tribe of the apocynaceae family (NNMDA, 2008). Preparations from different parts of the plant are employed as crude drug or crude herbal extract as remedy for various kinds of human diseases. Based on the claimed ethnomedicinal uses, scientists have investigated several pharmacological parameters such as antimalarial, anti-inflammatory, analgesic, antidiabetic, antimicrobial, antioxidant, antiulcer, cytotoxic and toxicological profile of extracts as well as isolated compounds from *P. nitida* (Iwu *et al.*, 2002).

Materials and Methods Fruit Flies and Treatments

Drosophila melanogaster stock; Wild-type fruit fly (Harwich strain) stock culture was used. The flies were allowed to mate in vials monitored under a regulated temperature until the eggs metamorphosed into young adult fruit flies under a natural photoperiod of about 12 hours light and 12 hours dark daily for the period of administration of the chemical compound under investigation. Flies were collected and separated into six experimental groups with five vials of 50 flies in each group and the flies were then treated as stated below in the experimental design.

***Drosophila Melanogaster* Feed Formulation and Its Handling**

The flies were fed with the standard formulated corn meal diet.

Plant Collection and Extraction

Picralima nitida fresh leaves were sourced from Upper Siluko, Egor, Benin-City, South -South, Nigeria and were identified by was validated at the Department of Plant Biology and Biotechnology (PBB), University of Benin. Aqueous extract of *P. nitida* leaves were processed by drying, pulverization, filtration and dehydration.

Experimental Design

Survival Assay

For the survival assay, flies (both genders) of 1-3 days were divided into nine groups, with each group having 3 vials each. Each vial contained 50 flies each with varied concentration of lead acetate and *Picralima nitida.*

The survival assay was carried out in three replicates of each concentration. The diet was changed every 7 days, during the period of the experiment. The survival rate was determined with all the concentrations and both the live and dead flies were recorded daily for 10 days. By the end of the experiment (14 days), the data obtained was accumulated and plotted as percentage of live and dead flies. The result was then compared with that of the control.

Tissue Homogenate Preparation for Biochemical Assay

For the determination of biochemical assays, a second group experiment was carried. In this experiment, flies (both gender) were divided into nine groups, with each group having 5 vials each. Each vial contained 50 flies with vary concentration of lead acetate and/or *Picralima nitida* in each treatment vial for a period of 5 days.

Homogenization
At the end of the treatment period, flies were transferred into an empty treatment vial and immobilized using a refrigerator and then kept in an empty Eppendorf tube. It was weighed, homogenized. Then the supernatant separated into labelled Eppendorf tubes, and used for the various biochemical assays. All the assays were carried out in five replicates for the nine groups and relative absorbance read using Jenway spectrophotometer 7315, by Bibi Scientific Ltd, UK.

Biochemical Assays
Biochemical parameters that were assessed to understand the possible effect of *Picralima nitida* on lead induced Drosophila melanogaster.

Protein Determination
The protein concentrations of the various samples were determined using Lowry method as described by Lowry (1951) with some few modifications.

Determination of the Levels of Total Thiols
The total thiols level was assayed for according to the method of Ellman (1951).

Cobalt determination
Cobalt in the homogenized sample was determined using the inductively coupled plasma mass spectrometer (ICP-MS) (Thermo Elemental, X series I, Germany), based on standard methods described by Fong *et al*. (2007).

Results
Figure 1 shows the survival rate of flies at different *Picralima nitida* and lead meal concentrations incorporated into the diet in *drosophila melanogaster*. In the survival curve produced, all flies fed with different *P. nitida* and lead meal concentration in all the treatment groups (group B-I) showed a decrease in survival rate by 6%, 35%, 7%, 13%, 7%, 29%, 24% and 29% respectively at day 14 when compared to that of the control group (group A).

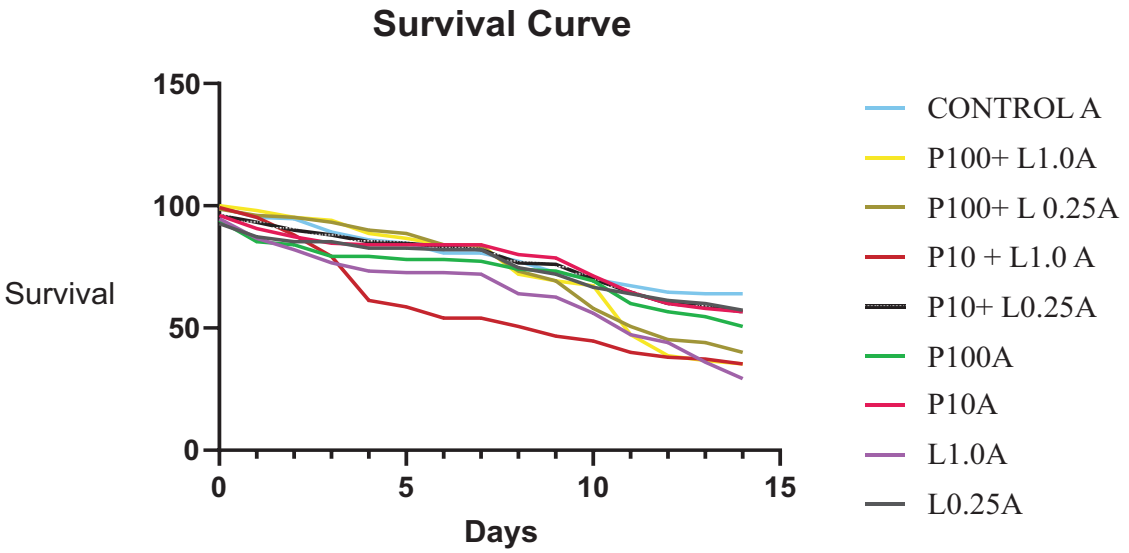

Figure 1: Survival curve showing the effects of *Picralima nitida* and lead incorporated into the diet in *Drosophila melanogaster*.

Table 1: shows the comparison of the levels of Total Protein, Total thiols and Cobalt at different concentrations of lead acetate and *P. nitida* meal fed to *D. melanogaster* with control group. The *D. melanogaster* flies fed with 0.25mg/dL lead acetate showed no significant different activity of Total Protein 0.04±0.01 vs 0.04±0.01, p>0.05), The *D. melanogaster* flies fed with 0.25mg/dL lead acetate showed lower significant activity of TSH (0.99±0.59 vs 0.38±0.09, p<0.039). The *D. melanogaster* flies fed with 0.25mg/dL lead acetate showed no significant activity of Cobalt (0.26±0.09 vs 0.20±0.05, p>0.05).

Also, the flies fed with 10mg/kg *P.nitida* had insignificantly lower activity levels of protein, Thiol and a significant lower level of Cobalt (0.26±0.09 vs 0.13±0.08) p=0.0228. Similarly, flies fed with

100mg/kg *P.nitida* had insignificantly lower activity levels of Protein, Thiol and Cobalt. However, flies fed with 0.25mg/dL lead acetate +10mg *P.nitida* had insignificantly lower levels of Protein (0.04±0.01 vs 0.04±0.04 p<0.001), TSH (0.68±0.21 vs 0.99±0.59) whereas Cobalt (1.11±0.14 vs 2.06±0.09) was significantly lower (p=0.0049). Also flies fed with 0.25mg/dL lead acetate+ 100mg/kg *P. nitida* had no significant difference across the group p>0.05.

Table 1: comparison of tested Protein, Thiol and Cobalt in different concentrations of Lead and *P. Nitida* meal with control (n=250)

Treatment	Measured parameter		
	PROTEIN	**Thiol**	**Cobalt**
Group A Control	0.04±0.01*	0.99±0.59*	0.26±0.09*
Group B 0.25mg/dl lead Acetate	0.04±0.01*	0.38±0.09[a]	0.20±0.05*
Group D10mg/kg PN	0.04±0.01*	0.65±0.09*	0.13±0.08[b]
Group E 100mg/kg PN	0.04±0.00*	1.19±1.07*	0.31±0.03*
Group F 0.25mg/dl lead Acetate+10mg/kg PN	0.04±0.00*	0.68±0.21*	0.11±0.02[c]
Group H 0.25mg/dl lead Acetate+100mg/kg PN	0.04±0.01*	0.55±0.11*	0.34±0.03*

Values are shown as Mean ± SD; a=0.0399; b=0.0228; c=0.0049*=p>0.05

KEY:
PN: *Picralima nitida*
TSH: THIOL

Table 2 shows the comparison of the activity levels of Protein, TSH and Cobalt at different concentrations of lead acetate and *P. nitida* meal fed to *D. melanogaster* with control group. The *D. melanogaster* flies fed with 1mg/dL lead acetate showed no significant lower activity of Protein 0.04±0.01 vs. 0.04±0.0 p>0.05), The *D. melanogaster* flies fed with 1mg/dL lead acetate showed no lower significant activity of TSH (0.99±0.59 vs 0.42±0.31, p>0.05) The *D. melanogaster* flies fed with 1mg/dL lead acetate showed no significant activity of Cobalt (0.26±0.09 vs 0.18±006 p>0.005).

However, flies fed with 1mg/dL lead acetate +10mg *P.nitida* had insignificant lower activity levels of Protein (0.04±0.01 vs 0.04±0.04 p>0.05), TSH (0.99±0.59 vs 0.0.54±0.18), Cobalt showed no significant Decrease (0.26±0.09 vs 0.17±0.05) p=. In the same vein, flies fed with 1mg/dL lead acetate+ 100mg/kg *P. nitida* had no significant difference across the group.

The activity levels of Protein (r=-0.59; p<0.001), TSH (r=-0.31; p<0.03) and Cobalt (r=-.0.97; p<0.003) correlated with lead acetate concentrations (table 3).

Table 2: comparison of tested Protein, TSH and Cobalt in different concentrations of Lead and *P. Nitida* meal with control (n=250)

Treatment	Measured parameter		
	PROTEIN	**TSH**	**Cobalt**
Group A Control	0.04±0.01*	0.99±0.59*	0.26±0.09*
Group C 1mg/dl lead Acetate	0.04±0.00*	0.42±0.31*	0.18±0.06*
Group D10mg/kg PN	0.04±0.01*	0.65±0.09*	0.13±0.08[b]
Group E 100mg/kg PN	0.04±0.00*	1.19±1.07*	0.31±0.03*
Group H 1mg/dl lead Acetate+10mg/kg PN	0.04±0.01*	0.54±0.18*	0.17±0.05*
Group I 1mg/dl lead Acetate+100mg/kg PN	0.04±0.01*	0.60±0.10*	0.21±0.13*

Values are shown as Mean ± SD; *=p>0.05

KEY:
PN: *Picralima nitida*
TSH: THIOL

Table 3: correlation of tested parameters with concentrations of Lead Acetate

Measured parameters	R-values	P-values
Protein	-0.5853	0.001
TSH	-0.3074	0.03
Cobalt	-0.8743	0.003

P>0.05

KEY:
TSH: THIOL

Discussion

There was a significant (P<0.05) decrease in Cobalt after the addition of 10mg/kg of just *Picralima nitida* when compared with control group. On the addition of 100mg/kg *Picralima nitida* to the flies, there was no significant (P>0.05) effect on the level of the fruit flies, *Drosophila melanogaster* is one of the alternative invertebrate models useful for genetics, biochemistry, cell biology and developmental biology (Abiolaji *et al*., 2013). It has been used to elucidate human diseases and recently adopted for toxicological testing (Wangler *et al*., 2015). It meets the standard of the European centre for the validation of alternative methods (ECVAM), Reduction, Refinement, and Replacement (3RS) of the usage of laboratory animals (Festing *et al*.,1998) *Picralima nitida* does not only serve as an indispensable constituent of human diet but also one of the most important medicinal species (Ghilsan *et al*., 2020). It can be used for the treatment of several disease, malaria, diabetes, jaundice, and pain (Erharuyi *et al*., 2014). Lead toxicity is one of the major environmental hazards in the world and can also induce oxidative stress (Jangid *et al*.,2016).

Oxidative stress is one of the commonest pieces of evidence of toxicity. All types of agents (chemical, physical, and microbial) can lead to oxidative-mediated stress in tissues and cells (Halliwell, 2007).

In this study incorporation of varying concentration of *Picralima nitida* or/and lead acetate into diet was used to evaluate the survival rate and modulatory effects of *Picralima nitida* on selected oxidative stress parameters (total protein, thiol and Cobalt) in lead-induced oxidative stress in *drosophila melanogaster***.**

From the survival assay it was observed that flies fed with varying concentration of lead or/and *Picralima nitida* resulted in higher death than the control group. This means that there was a lower survival of flies fed with lead or/ and *Picralima nitida* than the regular meal used to feed flies in the control group.

As observed both concentrations of *Picralima nitida* (10mg/kg and 100mg/kg) had a modulatory effect on the *Drosophila melanogaster* fed with 1mg/dl of lead acetate.

In *Drosophila melanogaster* fed with 0.25mg/dl lead acetate, concentration of 10mg/kg *Picralima nitida* had a better modulatory effect in those fed with 100mg/kg. The finding in this study agrees with previous report (Compos *et al.,* 2020), which states that despite the medicinal benefit of *Picralima nitida*, it also has toxic effect in high concentration in *Drosophila melanogaster.*

In this study, it was observed that the varying concentrations (0.25mg/dl and 1mg/dl) of lead acetate laden meal had no significant (P>0.05) effect on the level of protein when compared with the control group. There was no significant (P>0.05) effect on the level of protein after the addition of varying concentrations of only *Picralima nitida* (10mg/kg and 100mg/kg) when compared with the control group. After infusing 10mg/kg and 100mg/kg *Picralima nitida* to the flies that were previously fed with 0.25mg/dl lead acetate, no significant (P>0.05) effect was observed on the protein level. There was still no significant (P>0.05) effect after infusing 10mg/kg and 100mg/kg *Picralima nitida* to the flies that were previously fed with lmg/dl lead acetate.

There was a significant (P<0.05) decrease in the level of thiol after the addition of 0.25mg/dl lead acetate when compared with control group. There was no significant (P>0.05) effect on the level of thiol after the addition of 1mg/dl lead acetate when compared with the control group. There was no significant (P>0.05) effect on the level of thiol after the addition of varying concentrations of just *Picralima nitida* (10mg/kg and 100mg/kg) in *D. melanogaster* infused with 1mg/dl lead acetate. After infusing 10mg/kg and 100mg/kg *Picralima nitida* to the flies previously fed with 0.25mg/dl lead acetate, there was a significant increase in level of thiol when compared with flies that were exposed to only 0.25mg/dl lead acetate, this implies that *Picralima nitida* in varying concentrations (10mg/kg and 100mg/kg) had a modulatory effect on thiol in *Drosophila melanogaster* fed with 0.25mg/dl lead acetate. In flies fed with 0.25mg/dl lead acetate and varying concentration of *Picralima nitida* (10mg/kg and 100mg/kg) there was no significant (P>0.05) effect on the level of thiol when compared with the control group.

There was also no significant (P>0.05) effect on the level of thiol after infusing 10mg/kg and 100mg/kg to flies previously fed with 1mg/dl lead acetate.

There was no significant (P>0.05) effect on the level of Cobalt following the addition of varying concentration (0.25mg/dl and 1mg/dl) of lead acetate when compared with the control group.

Cobalt when compared with the control group. After infusing 10mg/kg *Picralima nitida* to the flies that were previously fed with 0.25mg/dl lead acetate, a significant (P<0.05) decrease on the level of Cobalt was observed when compared with the control group. After infusing 100mg/kg *Picralima nitida* to flies previously fed with 0.25mg/dl lead acetate there was no significant effect in the level of Cobalt when compared with the control group. After infusing varying concentration of Picralima nitida (10mg/kg and 100mg/kg) to flies previously fed with 1mg/dl lead acetate there was no significant (P>0.05) effect in the level of Cobalt when compared with the control group.

Cobalt in its active biological form is called cobalamin (vitamin B12) (Neve, (1991). The nutritional importance and pharmacologic effects of cobalt and vitamin B 12 in man.

As a component of vitamin B12 it contributes to megaloblastic anaemia, homocysteinuria, impaired DNA synthesis. The common symptoms due to acute cobalt deficiency are paleness, weakness, fatigue, loss of appetite, weight loss, and subsequent poor growth, shortness of breath, dizziness, scaly ears and watery discharge from the eyes. Furthermore, a marked deficiency can cause tingling or loss of sensation in the hands and feet, muscle weakness, muscle cramps, diminished reflexes, difficulty in walking, confusion, dementia and decreased thyroid function.

The reduction of total thiol level in *Drosophila melanogaster* on exposure to 0.25mg/dl lead acetate indicates the inability of the flies to combat free radical damage. However, the result revealed that varying Concentrations of *Picralima nitida* (10mg/kg and 100mg/kg) ameliorated the lead-induced depletion of total thiols confirming its antioxidant potential.

Conclusion

From the study, it could be deduced that the survival rate of *Drosophila melanogaster* was decreased with increasing concentration of lead acetate administered. The induction of oxidative stress was also higher with increasing concentrations of lead acetate consumption. The incorporation of 10mg/kg and 100mg/kg *Picralima nitida* into flies fed with 0.25mg/dl lead acetate was able to restore the activity level of thiol to the level observed in the control group. The activity of cobalt in flies fed with only 10mg/kg *Picralima nitida* was reduced, in flies fed with 100mg/kg *Picralima nitida* the activity of Cobalt was similar to that observed in the control group, this implies that the activity of Cobalt decrease with lower concentration of *Picralima nitida.*

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