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Gastro-protective effect of Natural Chelators in Lead induced gastrointestinal toxicity in rats.

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ABSTRACT: Chronic lead exposure poses serious complications causing deterioration in anatomy and physiology in the body. In a view to observe the deleterious effect of lead poisoning on the gastrointestinal system and assess the amelioration by natural chelators, the parameters concerning gastric pH, the concentration of mucosal content, mucosal nitric oxide, and gastric ulcer index alongside histopathology of gastric mucosa were estimated. The main aim of the study is to determine the chelating effect of natural chelators on long-term exposure (24 weeks) of lead acetate concerning gastro-protective activity and to relate the chelating potency of natural and synthetic forms to include the one in day to day diet as a nutraceutical agent. Forty-two male albino Wistar rats (200-250g) were divided into seven groups (n=6). All the groups received lead acetate 0.4 mg/kg body wt. peroral (p.o) except the first-group (control) which receives sodium-acetate 1,000 mg/L in drinking water. Second-group is the toxic group, the third and fourth received Chitosan and Chitosamine 0.2 g/kg (p.o) respectively. Fifth, sixth & seventh group received ethylenediaminetetraacetic acid (EDTA) 495 mg/kg (p.o) whereas, sixth and seventh group received Chitosan and Chitosamine [0.2 g/kg (p.o)] respectively in addition. The findings revealed a decrease in stomach and body weights of animals alongside gastric pH, Mucin content, mucosal nitrite content, and increased oxidative stress by a reduction in SOD, CAT, GSH, and increase in MDA levels in the toxic group concerning the control group. These results were found to be ameliorated with a statistically significant increase in groups treated with chelators concerning the toxic group. Histopathological findings revealed congested blood vessels with cellular swelling in gastric mucosa along with cell infiltration in a toxic group with negligible change in gastric mucosal architecture in the control group. The reports of treatment groups of chelators showed normal gastric mucosal glands tubular architecture and related findings compared with the control group. Thus, it can be concluded that natural chelators have a gastro-protective role in lead-induced poisoning and are equipotent as synthetic ones to be used as a prophylactic heavy metal detoxifier nutraceutical agent.

KEYWORDS: Chitosan, Chitosamine, Lead Poisoning, Chelation, Gastro-Protective Effect.

I. INTRODUCTION

Saturnism is a common term used for lead toxicity. In spite being the number of heavy metals to have existed in the earth's crust, lead has proved to be a nuisance metal in disturbing the ecological balance. The unique physical and chemical property of lead ion has caused to increase its use and enhance its concentration making it a harmful environmental pollutant. [1] There is not a single system in the physiology of the body that is not being deteriorated by lead. Several previous studies revealed the distribution of lead ions in the blood, lung, liver, heart, brain, and kidney. The continuous use of lead made it accumulate in nature being non-biodegradable. Moreover, its lipophilic nature made it accumulate in adipose tissues, bones, and cartilages which are the least clearance region in the body. [2] The toxic consequences of lead are distinct not only in adults but also have major developmental side effects in children[3]. The lead is found to disrupt the proteins, enzymes, electron transport system as well as interferes in ATP formation. The major mode of the entrance of lead in the body is mainly inhalation and ingestion, where ingestion directly targets the gastrointestinal system.[4] The findings from previous studies revealed that lead toxicity decreases erythrocytic concentration leading to cellular ischemia.[5] It has also reported that lead ions enhances the pro-oxidant ability of ferrous ion and potentiates generation of reactive oxygen species (ROS), which might be one of the reasons of incidence of gastric ulcers[6].

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Gastric ulcer refers to the long penetration of inflammatory lesions in the gastric mucosa which due to hypersensitivity appears like a tumor. Ulcer healing is a rapid process that causes the marginal re-epithelisation of tissues at the lesion area and also enhances the growth factors responsible for the protective action of the mucosal wall against hyperacidity[7]. Alongside, gastric pH, Mucin, mucosal nitrite content, pepsin concentration also plays a vital role in the maintenance of the normal architecture of gastric mucosa [8]. This study enables us to determine the distortion created by chronic exposure of lead on gastric mucosa and its amelioration by chelators by assessing variation in morphological and histopathological characteristics in gastrointestinal function. To overcome heavy metal toxicity, the simple approaches are its prevention and treatment. Prevention refers to the identification and avoidance of contact with the sources of lead ions whereas; treatment refers to the removal of heavy metals from the body by the process called 'chelation'. [9] Chelation can be practiced by synthetic as well as natural chelators. Synthetic chelators viz BAL, DMSO, Desferroxamine, EDTA, etc. are used clinically in hospitalized patients as a specific medical treatment with strict and careful supervision of health care professionals [10]. These drugs are targeted through parenteral routes hence comes with several serious side effects. Our study thus made an effort to identify chelators present naturally which can be used as a diet meaning a non-xenobiotic supplement that not only detoxes the body due to heavy metals but also has no side effects, biodegradable in nature, has no bio-accumulation in the body, is innocuously excreted outside the body without costing the harm to the excretory system of the body [11]. Our study focused on marine sources to expose the agents having the ability to claw heavy metals and remove them out of the body harmlessly. Chitosan a polymer and chitosamine its monomer is used in our study as natural chelators against lead-induced toxicity [12]. These polysaccharides have several uses other than chelation such as extensively used pharmaceutical aid, anti-oxidant, cholesterol-lowering property, gastric acid ulcer protective agent due to its physical property of imbibition, anti-aging, etc. [13] Thus, in a view of studying the role of natural chelators along with their comparison with synthetic ones by determining their potency, we can further replace the synthetic world with natural ones in the future.

II. MATERIAL AND METHODS:

2.1 Animals

In this study male albino Wistar rats weighing between 200-250 g, were used. The animals were maintained at 23 ± 2°C temperature with open access to standard rat feed and water. A (12-12 h) light cycle was maintained in the animal house. The care and use of experimental animals were performed following the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, New Delhi, India, and was permitted by the Institutional Animal Ethical Committee of Y.B. Chavan College of Pharmacy, Aurangabad having the approval number (CPCSEA/IAEC/ P'col-58/2017-18/139).

2.2 Experimental design: Animals were randomly divided into seven groups (n=6). The treatment was expanded for 24 weeks (6 Months).

Table 1: Shows the experimental design protocol of the study dividing the animals (n=6) into seven groups.

Groups -	I	II	III	IV	V	VI	VII
Control (sodium acetate; 1000mg/L drinking water).	✔						
Lead Acetate (0.4 mg/kg body wt.)		✔	✔	✔	✔	✔	✔
Chitosan (0.2 g/ kg body wt.)			✔			✔	
Chitosamine (0.2 g/kg body wt.)				✔			✔
EDTA (495 mg /kg body wt.)					✔	✔	✔

At the end of the experimental period, animals fasted overnight; anesthetized using a carbon dioxide chamber and their respective samples were collected in test tubes. All respective specimens were separated and stored at 8 ^{0} C until they were analyzed.

2.3 Estimation of body and organs weight

In each group, the bodyweight of rats was taken before and after record treatment. The animals

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were sacrificed using a carbon dioxide chamber. Stomachs were first ligated at the pyloric sphincter and then isolated. These were then emptied for the gastric content by giving an incision on greater curvature. The stomachs were washed with distilled water, dried completely with filter papers, weighed, and recorded. [14]

2.4 Collection of various contents of the stomach:

The stomachs isolated were cut open from the greater curvature and the gastric acid was collected and centrifuged at 3500 rpm and used for further examinations.[15]

a) **Estimation of the gastric acid pH:**

The pH of the supernatant gastric acid obtained after centrifugation was measured using a digital pH meter. [16, 17]

b) **Estimation of pepsin activity :**

Pepsin obtained is a function of the proteolytic activity of gastric juices. Pepsin activity signifies the concentration of tyrosine in micromole/ml gastric juice /min using 0.01 ml gastric juice/100ml distilled water and 2% bovine albumin in 0.01 N HCl as substrate. Each sample of the gastric juice was diluted first by 1:100 with 0.01N HCl. 1 ml of the diluted mixture was added with 5ml of 2% bovine serum albumin solution and incubated at 370 C for 10 min using a water bath. 0.3 M trichloroacetic acid (10 ml) was added and boiled for 5 min. The mixture was centrifuged at 3000 rpm for about 5 min and filtered. To 1 ml of the filtrate, 2 ml of NaOH (0.5N) and 2-3 drops of Folin reagent were added. After 20 min of the interval, the color develops that is measured colorimetrically at 680 nm. A blank was estimated without gastric juice with the same procedure applies. Alongside a standard solution of 0.2 ml working tyrosine, the standard was also involved. [18]

c) **Estimation of Mucin content**: Mucin is composed of hexose and proteins. This method determines the concentration of hexoses in mucin in mg of hexoses /dL. It depends on the reaction of carbohydrate with concentrated Sulfuric acid using Orcinol (5-methyl resorcinol) giving a colored product which is measured using a colorimeter. The procedure includes 0.25 ml of diluted gastric juice in the ratio of 1:20 with an equal volume of 1.6% Orcinol and 60% sulfuric acid (2 ml). The solution was boiled in a water bath for 10 min and cooled on ice. The optical density was determined at 425 nm. [19]

d) **Estimation of Gastric Ulcer Index**: Subsequently the gastric contents of the stomach of each animal were emptied and rinsed thoroughly with saline water and the tissue was mounted on a corkboard. The ulcers were identified and scored using a stereoscopic microscope using an eyepiece with square grids and grading of 0-5 scale. Ulcers were scored according to the severity of lesions or vascular ischaemic congestion or hemorrhages as follows: [20]

Lesions	Grade
No lesions	0
Vascular congestions	1
1-2 lesions	2
Severe lesions	3
Very severe lesions	4
The mucosa is full of lesions of noticeable size.	5

Table2. Represents the severity of scores and grading of gastric ulcers in experimental rats. The ulcer index is calculated as: [ulcerated area (mm) / total stomach area (mm)] X 100.

e) **Estimation of Mean Ulcer Score and % Ulcer inhibition**

Mean ulcer score refers to the sum of all scores of animals in a group.

[U.I.in control − U.I. in test] × 100/U.I. in control.[21]

Where U.I = Ulcer Index.

f) **Estimation of Mucosal Nitric Oxide:**

It is the estimation of nitrite content in gastric mucosa. The cleaned stomach is carefully scraped off and contents were collected for determination of nitrite by Azotization reaction in acidic pH with Sulfanilic acid following its coupling with N-1-naphthyl-ethylenediamine to give a colored product that was measured by colorimeter using a wavelength of 548 nm. [22]

2.5 Preparation of Tissue homogenate:

The animals were sacrificed using a CO_2anesthetic chamber for rendering them unconscious (Euthanasia) followed by cutting the carotid artery. The stomachs were quickly removed, rinsed in ice-cold saline, dried on a filter paper, and weighed. A 10 % homogenate was prepared in 0.15 M Potassium Chloride (KCl) for the estimation of

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tissue malondialdehyde and the homogenate for the tissue glutathione was prepared in 0.02 M EDTA.

i) **EstimationofTissueGlutathione [23]**

A known weight of tissue ranging from (100-150 mg) was homogenized in 5 ml of EDTA (0.02 M) and then, 4 ml of cold distilled water was added to it. After mixing 1ml of TCA (50 %) was added and shaken intermittently for 10 min using a vortex mixer. After 10 min the content was transferred to centrifuge tubes (rinsed in EDTA) and centrifuged at 6,000 r.p.m for15 min at 4°C. After centrifugation, 2 ml of supernatant was mixed with 4 ml of tris buffer (0.4 M, pH 8.9). The whole solution was mixed and 0.1ml of DTNB (0.01 M) was added to it. The absorbance was read within 5 min of addition of DTNB at 412 nm against the appropriate blank.

ii) **Measurement of tissue MDA: [24]**

Measurement of lipid peroxidation by determination of stomach malondialdehyde content by the thiobarbituric acid (TBA) method was carried out. 10 % stomach homogenate was prepared in buffered 0.9 % KCl pH 7.4 for the estimation of tissue MDA. To 1 ml of homogenate, 0.5 ml of trichloroacetic acid (30 %) and 0.5 ml of thiobarbituric acid (0.8 %) were added and shaken for 5 min. The tubes were then subjected to heating on the water bath at 80°C for 30 min followed by cooling in ice-cold water for 10 min and centrifugation at 5,000 r.p.m for 15 min. The clear supernatant was separated and absorbance was measured at 540 nm using an appropriate blank.

Preparation of Post Mitochondrial Supernatant (PMS):

The tissues were homogenized in chilled potassium phosphate buffer (50mM, pH 7.4) using a Remi homogenizer. The homogenate was centrifuged in a refrigerated centrifuge at (10,500 rpm) for 20 minutes at 4 °C to obtain the PMS, which was used for various biochemical analyses. The post mitochondrial supernatant (PMS) was used for the estimation of antioxidant enzymes such as Catalase and Superoxide Dismutase.

iii) Assessment of Catalase (CAT) [25]

The cytosolic supernatant, (50µl) was added to the cuvette containing 2.95 ml of hydrogen peroxide (19 mM) solution prepared in potassium phosphate buffer (50 mM, pH 7.4). The change in absorbance was read at 240 nm on the Shimadzu spectrophotometer at 1 min interval for 3 minutes.

iv) Estimation of Superoxide Dismutase (SOD) Assay [26]

The supernatant was assayed for superoxide dismutase (SOD) activity by following the inhibition of pyrogallol auto-oxidation. 100 µl of cytosolic supernatant was added to TrisHCl buffer, pH 8.5. The final volume of 3 ml was adjusted with the same buffer. At last 25 µl of pyrogallol was added and changes in absorbance at 420 nm were recorded at the 1-minute interval for 3 minutes. The increase in absorbance at 420 nm after the addition of pyrogallol was inhibited by the presence of SOD.

2.6 Histopathologicalstudies [27]

The stomachs were fixed in 10% formalin. The specimens were then processed for the standard procedure and were embedded in paraffin wax. The blocks were then sectioned according to the hematoxylin and eosin methods. The sections were examined under the light microscope and photographs were taken under 10X.

Statistics Analysis

The mean ± SEM values were calculated for each group. One-way ANOVA followed by Tukey's tests was used for statistical analysis. Values of $p<0.05$ were considered statistically significant. The entire statistical analysis was performed using the statistical package, Graph Pad Instat Version 8.0 (Graph Pad Software Inc., USA) software at a level of significance of $P < 0.001$, 0.01, 0.05, and 0.1.

III. RESULTS:

3.1 Weight (organ and body):

Table 3.showed the results obtained from the body weight (g) of rats from each group along with their stomach weight (g). Each animal was weighed before and after the dosing period to signify the impact of treatment. The findings revealed that the bodyweight of the toxic group was in lower units as compared to the control ($p<0.01$) whereas, there was no significant reduction in body weight of the treatment group as compared to the toxic ones. The stomach weight of animals of the toxic group was found to be decreased as compared to the control group, whereas the difference between weights of treatment groups was found to be statistically increased ($p<0.05$)as compared to the toxic ones.

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Table 3: The effect of Chitosan, Chitosamine, and EDTA on Body and stomach weight in lead-induced toxicity in rats after 24 weeks of treatment.

PARAMETERS	GROUPS						
	Control	Lead	Chitosan	Chitosamine	EDTA	EDTA+ Chitosan	EDTA+ Chitosamine
Body weight (g)	381±7.34	205±14.6[a]	290± 5.63[e]	268±4.45e[e]	317±2.6[f]	344± 2.97[f]	277±3.4[e]
Stomach weight (g)	1.43±0.07	1.24±0.08[b]	1.34±0.06[f]	1.33±0.08[f]	1.45±0.02[f]	1.45±0.09[f]	1.38±0.03[f]

The data is indicated as Mean ± SEM. No. of samples (n) =6. [a] P<0.001, [b] P<0.01, [c] P<0.05 as compared to control group and [d] P<0.001, [e] P<0.01, [f] P<0.05 as compared to lead (toxic control) treated group while ns represents non-significant data.

3.2 Gastric contents

The findings obtained from various aspects concerning the gastrointestinal system are shown in Table 4. The findings revealed the decrease in gastric acid pH, pepsin, Mucin, gastric acid ulcer index, mucosal nitric oxide, and ulcer score in the toxic group as compared to the control group. These findings increased statistically in the treatment group as compared to the toxic ones.

Table 4: The effect of Chitosan, Chitosamine, and EDTA on gastric contents (gastric acid pH, pepsin, Mucin, gastric acid ulcer index, mucosal nitric oxide, and ulcer score) in lead-induced toxicity in rats after 24 weeks of treatment.

PARAMETERS	GROUPS						
	Control	Lead	Chitosan	Chitosamine	EDTA	EDTA+ Chitosan	EDTA+ Chitosamine
Gastric acid pH	6.8±0.5	4.1±0.5[a]	6.4±0.25[d]	6.6±0.4[d]	6.9±0.25[d]	6.4±0.5[d]	6.5±0.8[d]
Pepsin (µmol/ml/min)	9.6±0.9	3.4±1.1[a]	8.8±1.2[d]	7.6±0.8[d]	9.4±0.6[d]	8.9±0.8[d]	8.1±1.3[d]
Mucin (mg of hexose/dL)	454.2± 42.2	419.4±39.1[a]	463.4±44.4[d]	471.7±42.2[d]	466.1±43.3[d]	472.3±41.1[d]	488.3±44.3[d]
Gastric Ulcer Index	0.00±0.00	4.6±1.2[a]	1.24±0.56[d]	1.27±0.81[d]	1±0.5[d]	1.29±0.9[d]	1.36±1.21[d]
Mean Ulcer Score	0	3.4[a]	0.3[d]	0.6[d]	0.2[d]	0.4[d]	0.8[d]

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Mucosal Nitric Oxide (µg/g tissue)	23±3.1	11±2.5 [a]	19±3.5 [e]	16±2.5 [d]	24±2.2 [ns]	21±2.8 [f]	18±2.2 [e]

The data is indicated as Mean ± SEM. No. of samples (n) =6. [a] P<0.001, [b] P<0.01, [c] P<0.05 as compared to control group and [d] P<0.001, [e] P<0.01, [f] P<0.05 as compared to lead (toxic control) treated group while ns represents non-significant data.

Table 5 showed the calculations of ulcer index as mean ulcer score ± standard error mean values. The data showed the status of ulcers with their severity which is summed up to get the total value of ulcer and thus rated as follows.

Table 5: Findings of ulcer score or index (mm)

Groups	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Total Score	Ulcer Index
Control	0	0	0	0	0	0	0.00±0.00
Lead	4	4	4	4	1	17	3.4±0.59
CX	2	2	2	0	0	6	1.2±0.49
CN	1	0	1	1	0	3	0.6±0.24
EDTA	0	1	0	0	0	1	0.2±0.19
E+CX	0	1	0	0	1	2	0.6±0.24
E+CN	0	1	0	1	2	4	0.8±0.37

Key: 0 = Absence of Ulcer; 1= Haemorrhagic destructions < 5mm; 2= haemorrhagic destruction >5mm; 3= Many tiny linear ulcers > 2mm); 4= Numerous linear ulcers of mark size. 5= Mucosa is full of the lesion with noticeable sizes.

Mean ulcer score: it is the total score obtained by the individual animal in the group and presented as Mean ± SEM.

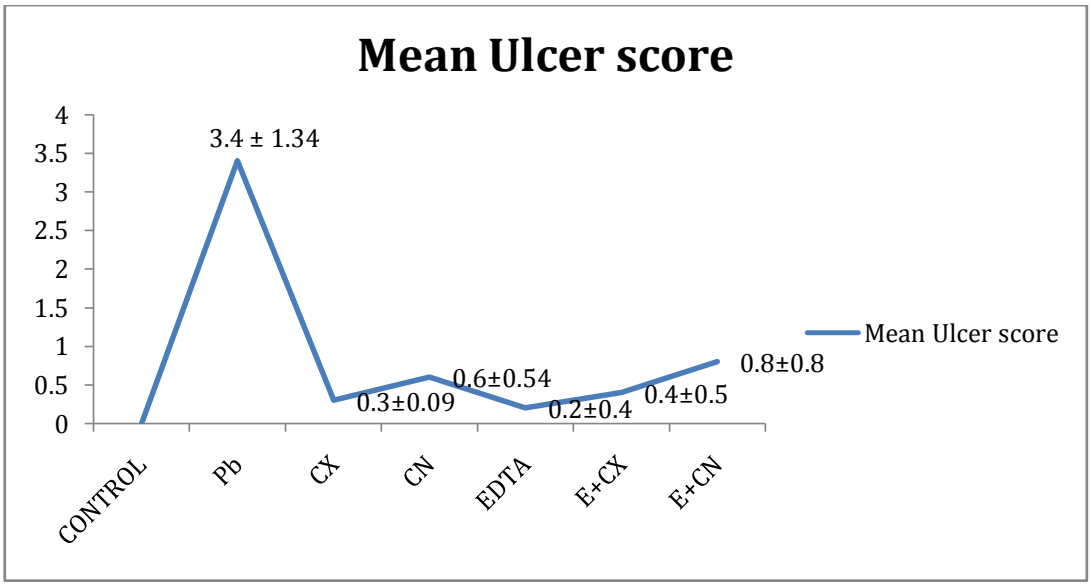

Figure 1. Result of the mean ulcer score in rats treated lead acetate (Pb), chitosan (CX), chitosamine (CN), Ethelenediamene tetra acetic acid (EDTA), combination of EDTA + chitosan(EDTA+CX) and edta+ chitosamine (EDTA+CN).

3.3 Oxidative stress

The data revealed in Figure 2 showed the results of oxidative stress in the toxic group and its comparison with control and treatment groups. The SOD, CAT, tissue GSH levels in stomach tissue were found to be decreased significantly in the toxic group as compared to the control group (P<0.001), the levels of which ameliorated in treatment groups

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in their comparison with the toxic group. The levels of MDA were increased as a result of the high lead blood burden in the toxic group as compared to the control group. The data obtained from the oxidative stress of the toxic group ameliorated due to the chelation effect of chelators of treatment groups. Figure 2. represents the comparative study of oxidative stress parameters in stomach tissue in rats treated with lead acetate and chelating agents, where lead acetate (Pb), chitosan (CX), chitosamine (CN), Ethelenediaminetetraacetic acid (EDTA), a combination of EDTA + chitosan(EDTA+CX) and EDTA+ chitosamine (EDTA+CN) are presented as respective groups.

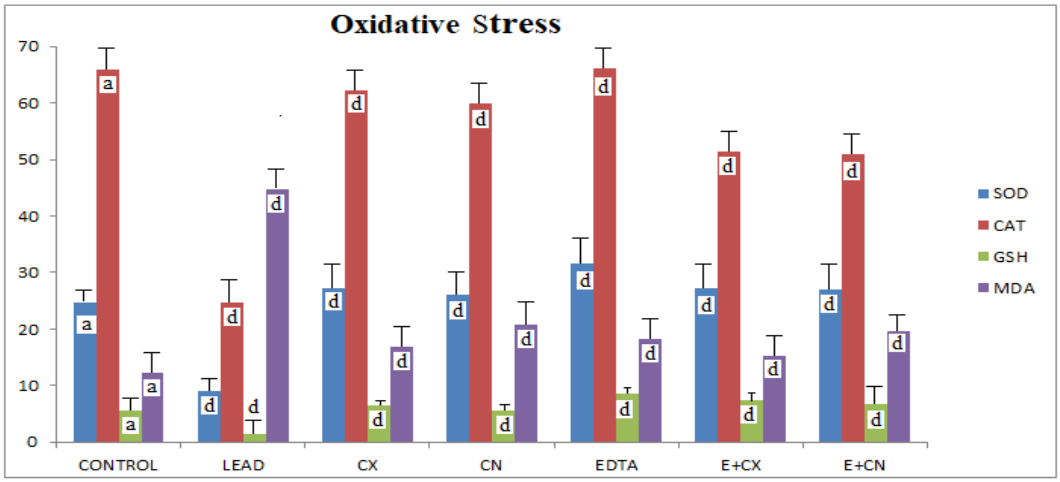

Fig 2.Represents the data of the oxidative stress in experimental animals treated with a chelating agent and lead as heavy metal.

The data is indicated as Mean ± SEM. No. of samples (n) =6. [a] P<0.001, [b] P<0.01, [c] P<0.05 as compared to control group and [d] P<0.001, [e] P<0.01, [f] P<0.05 as compared to lead (toxic control) treated group while ns represents non-significant data.

[SOD: Superoxide Dismutase (U/mg protein), CAT: Catalase (nmol of H_2O_2 consumed/min/mg protein), stomach tissue GSH: Glutathione (µmol/gm tissue) MDA: Malondialdehyde(µmol/gm tissue)]

3.4 Histopathological findings:

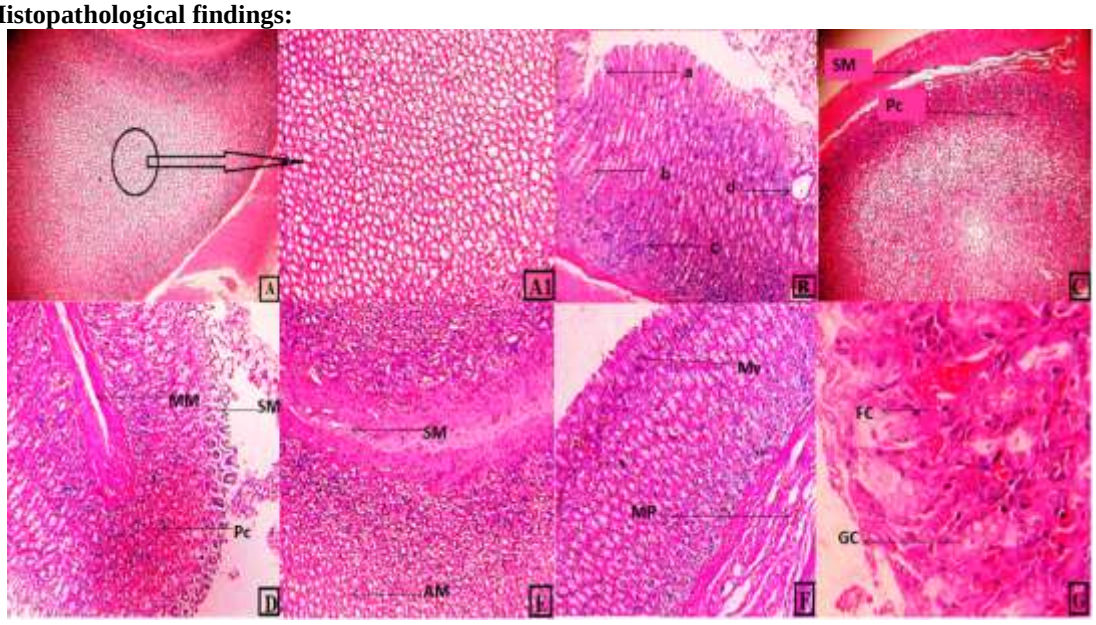

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Fig 3. Represents the H& E stained sections of experimental rats from each group. Section A represents the control group while A1 is the magnified image of the parietal cell of gastric mucosa with normal glandular stomach architecture having a uniform and compact arrangement of mucinous secreting cells. The build-up of the mucosa, sub-mucosa with MuscularisPropria seemed to be normal. Section-B refers to the toxic control group (a: microvilli of gastric mucosa, b: dilated glands with glandular distortion c: cellular swelling with lymphocytic infiltration, d: vacuolation with gastric cell erosion). Section-C refers to chitosan treated group (SM: submucosa, Pc: parietal cells), D-section is of rat treated with chitosamine (SM: submucosa, Pc: parietal cells, MM: mucosa muscularis,)and E refers to EDTA treatment group(AM: antral mucosa, SM: submucosa). The sections F and G represent the groups receiving combination doses of synthetic as well as natural chelators i.e section –F refers to EDTA+Chitosan treated group (mv: mucosal microvilli, MP: MuscularisPropria) and EDTA+Chitosamine treated group animal are showed in section-G (FC: Foveolar cells, Gc: goblet cells).

IV. DISCUSSION:

The present study was conducted to investigate the deleterious effects of heavy metal in general and lead in particular on the gastrointestinal system and to assess whether natural chelators prove to ameliorate the morphological and histopathological parameters comparable to synthetic chelators. Our findings revealed a considerable decrease in stomach and body weight of rats in the toxic group as compared to the control group. These results also showed a statistically significant increase in stomach and body weight (table.3) in treatment group animals as compared to the toxic group. Studies also showed the groups with lesser stomach weight showed a higher incidence of ulcer formation compared to normal stomach weight animals which is concordant with our findings.[28]. Tukeys test allowed us to signify the results obtained from the chelation of synthetic and natural ones to be equipotent and also showed chitosan to be more potent in heavy metal extraction compared to chitosamine. As stated in previous studies, ingestion is the common route of exposure of heavy metals to gastric mucosa, direct contact of which causes the generation of reactive oxygen species enhancing oxidative stress [29]. Lead absorption from the gut area causes a decline in nutrition absorption which in turn causes malnutrition triggering decreased body and stomach weight [30]. Studies also revealed chelators in general and chitosan, in particular, can absorb a considerable amount of heavy metal which can reverse the loss of body and stomach weight by again stabilizing nutrient and essential elements absorption from the diet. The gastric contents alteration can be observed in the lead toxic group viz. Mucin content, mucosal nitrite concentration, pepsin content, and gastric pH (table.4) concerning the control group, whereas these levels were found to be increased significantly in the treatment group compared to the toxic group. Studies showed heavy metals tend to increase aggressive mechanisms leading to an increase in ulcer formation. It is also confirmed from previous findings that heavy metals tend to decrease mucous production, Mucin content, mucosal thickness, reduce pepsin content, aggravate generation of ROS directly or indirectly which altogether triggers gastric mucosal eruption and ulcer formation [31]. Studies also revealed heavy metals have a role in tumorigenesis by causing gastric mucosal lesion at the DNA level which alters gene regulation, cell growth, and signal transduction ultimately inducing cancer. Heavy metals also prove to decrease and slow down the DNA lesion repair which further worsens the ulcer healing in gastric mucosa. Apart from this, heavy metals proved to induce and aggravate pro-inflammatory agents such as chemokines, interleukins, micro-RNAs, etc. which promotes tumor formation. Our study confirmed the findings of in-vitro studies where, chitosan and chitosamine chelated lead ions proved by IR studies, confirmatory tests of heavy metals, limit tests, etc. the findings revealed in this study highlighted amelioration concerning gastric content confirming chelation of lead by chelators. The mucosal nitrite content was also found to be decreased by lead exposure in a toxic group which increased statistically in treatment groups. Apart from oxidative stress, nitric oxide (NO) is responsible for the composition, constitution, and integrity of gastric mucosa [32]. The decrease in the levels of NO by depletion of NO synthase or by activation of NO inhibitors may worsen mucosal ulcers causing mucosal injury [33]. Moreover, NO has also proved to reduce lymphocytic infiltration which further enhances healing of a mucosal injury. These findings are in the correlation of our study showing the reduced concentration of mucosal NO in a toxic group which alleviated to normal limits in

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treatment groups compared to the control group [34]. The mean ulcer score (fig.1) was found to be on the higher side in the toxic group which may be due to increased oxidative stress that enhances the level of aggressive mechanism eventually forming ulcers. The oxidative stress parameters viz. SOD, CAT, stomach tissue GSH was found to be decreased in the toxic group as compared to the control ones. These findings were increased significantly in treatment groups as compared to the toxic ones. As stated by previous studies the ROS plays an important role in the aggravation of several inflammatory disorders in the gastrointestinal tract by activating pro-ulcerative agents viz. H.pylori in the gut, increasing lipid peroxidation as indicated by increased MDA levels, etc. [35,36]. Our study is in concordance with studies suggested that the depletion in endogenous antioxidants and antioxidizing enzymes viz. SOD and CAT and increased MDA levels worsen ulceration in the gastric mucosa (Fig.2). The superoxide radical ion which is highly reactive to the gastric mucosa causing its aging is excavated by dismutation reaction by dismutase enzyme (SOD) into less reactive hydrogen peroxide ion which eventually converts to water in presence of enzyme catalase and glutathione. The depletion of these antioxidant enzymes predisposes gastric mucosa to a higher degree of oxidation by ROS leading to high impact destruction and ulceration. The decrease in the concentration of antioxidizing enzymes is reflected in our findings in the toxic group and chelation ameliorated these levels in the treatment groups compared to the control groups.

Histopathological examination: The results obtained from anatomy, physiological and morphological changes are further confirmed by histopathological examinations. The sections highlighted in fig .3 showed H&E staining of the stomach of experimental animals from each group. Section A and A1 refer to the transverse section of the gastric mucosa of rats from untreated or control groups. The section shows the normal architecture of oxyntic gastric mucosa with eosinophilic parietal and basophilic chief cells compactly arranged which extends into the deep glandular compartments. The volume and thickness of mucosa far extend into the gastric pit mucosa. The toxicity in gastric mucosa in section B can be confirmed by distortion in gastric epithelium, erosion of antral mucosal region, and oedema with vacuolation which can be prominently observed in the toxic as compared to the control group where these results are correlated with previous studies [37]. The correlation between the mean ulcer score and disruption of the mucosal and sub-mucosal region can be certainly identified in the toxic group where the mucosal glandular components are visible with an oedematous appearance alongside bubbly eosinophilic cytoplasm (shown by arrows). The continuous layer of mucosal epithelium can be seen distorted and discontinued which can be related to inflammation or may be due to gastric ulcer which disturbed the normal gastric mucosal architecture. Our study is concordant with previous studies which showed gastric mucosal distortions occurred in genetically modified crops fed rats [38]. The groups treated with chelators showed repaired glandular pits along with refurbished mucous secreting cells observed in the submucosal layer as compared to the toxic group indicating achievement of chelation effect. The glandular swelling and lymphocytic infiltrations are obvious reasons to signify incidence of ulcer formation alongside inflammation and microvilli architecture distortion in the toxic group which was found to be alleviated in sections of rat stomach of treatment groups. Previous studies highlighted the lower the incidence of lymphocytic infiltration the hastening of ulcer healing takes place [39]. The goblet and foveolar cells i.e. mucous secreting cells found to be ameliorated in groups treated with chitosan, chitosamine, and EDTA concluding the effectiveness of natural chelators with synthetic ones. The normal gastric mucosal architecture between the treatments groups is found to be comparable with control group data indicating gastro-protective effect.

V. CONCLUSION

The heavy metal lead (Pb) was found to have a toxic effect on the gastrointestinal system as confirmed by morphological and histopathological alterations in the study. These deleterious effects were found to be ameliorated in treatment groups dosed by chelators (both synthetic and natural). Our study made an effort to investigate in vivo removal of lead ions from the body using chelators. To overcome the side effects of synthetic chelators, natural chelators should be used as a prophylactic detoxifier and should be included in day-to-day life as a nutraceutical agent. Hence, data obtained from our studies confirmed natural chelators have gastro-protective activity as the findings are comparable to the ones obtained from control group.

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SOME OF THE ADVANAGES FROM THE ABOVE RESULTS

a) Avoid lead metal exposure.
b) Include chelators and anti-oxidants containing diet in daily life
c) Use of an alternative metal other than heavy metals.
d) Natural chelators are economic, biodegradable, have several uses other than chelation and can increase quality of life.
e) Combination of synthetic and natural chelators are a good deal in getting rid of heavy metals as it reduces individual doses, gives synergistic action, reduces adverse effects if any, etc.
f)Chitosan and Chitosamine are from marine sources, hence already have several advantages as rich in calcium ions, and trace elements and can be used as a supplement in bone deformation diseases.

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