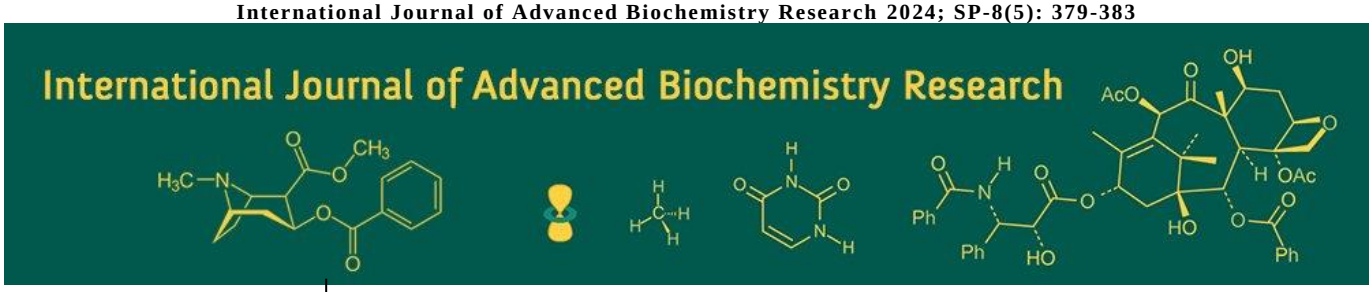

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Effect of lemon peel extract gold nanoparticals on neurotoxicity induced by lead and arsenic in wistar rats

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Abstract
In Indian system of Ayurveda gold is used in the form of ash (Swarnabhasma) since 800AD mainly as a rejuvenator and its medical indications can be found in all ancient medical classics. Considering therapeutic potential of gold the current study was carried out to evaluate the antioxidant activities of Lemon peel extract gold Nanoparticals (LPGNP) supplement against lead acetate and sodium arsenite – induced nephrotoxicity in wistar rats. In this study, adults male rats were treated for 14 days with lead acetate @ 116.4 mg/kg, Sodium Arsenite ($NaAsO_2$) @ 13.8mg/kg body weight p.o for 14days. Our study showed that supplementation with LPGNP brought improvement in oxidative stress–related parameters (SOD, CAT, Glutathione) and histopathology of brain showed corrective effect of LPGNP on Pb and As -induced brain neurotoxicity. Overall, LPGNP alleviated the toxic effects of these heavy metals on brain tissue, suggesting its role as a potential antioxidant and neuroprotective agent.

Keywords: Antioxidant, lead, Arsenic, neurotoxicity, rats, LPGNP

Introduction
The noble metal Gold has been used since time immemorial for medicinal purposes. Ayurveda, the ancient Indian medicine mentions about rejuvenating and therapeutic potential of gold as aphrodisiac, to enhance vigour, vitality, memory, immunity, and longevity. In Ayurveda it is used in the form of Swarnabhasma (gold ash). It is one of the few metals which can be used at nano scale due to its resistance to oxidation. Gold nanoparticles found to be effective against certain cancers [1]. In the decade of 1990 pharmacology of Gold compounds like Auranofin and Gold sodium theomalate was explored. Auranofin is known for its efficacy in rheumatoid arthritis, tuberculosis and as a nervine drug [2, 3]. The green synthesised gold nano particles prepared from peel extract of lemon (*Citrus lemona*) acts as reducing and capping agent in the synthesis of bioactive and stable gold nano particles from gold salt. Lead (Pb) is a toxic metal that induces a wide range of biochemical and physiological effects in humans. Oxidative damage has been proposed as a possible mechanism involved in Pb toxicity. Lead is a toxic metal that induces a broad range of physiological, biochemical and neurological dysfunctions in humans as well as in animal model. It is a well-known multi-organ toxicant which damages the liver, kidney, reproductive system and other physiological organs [4]. Oxidative damage has been proposed as a possible mechanism involved in Pb toxicity. Arsenic is an endocrine disrupting nonessential metal present ubiquitously in environment and biota as pollutant. Arsenic a major toxic metalloid, exists in air, water and soil. Exposure to higher-than-acceptable level of arsenic occurs either in workplace, e.g., in smelting industries, coal-fired power plants, cosmetic industries, agriculture, etc, or through arsenic-contaminated food or drinking water, Arsenic poisoning in the groundwater has caused severe health hazards in the exposed population [5]. Arsenic is known to be a potent carcinogen that can induce the generation of reactive oxygen species. In recent years, green synthesis of metal NPs has received tremendous attention due to the advantages of energy efficiency, safety, less toxicity and environmentally friendly.

Considering incredible therapeutic potential of gold nano particles, the present research study was undertaken to evaluate the effect of *lemon peels* extract mediated gold nano particles on lead and arsenic co-exposure induced neurotoxicity in wistar rats.

Materials and Methods

All reagents used in the present study were of analytical grade. Sodium Arsenite ($NaAsO_2$) and Gold salt ($HAuCl_4$) were obtained from Hi-Media India, Pb (in the acetate form) was obtained from SD Fine Chemicals, Boisar, Maharashtra, India.

Lemon peels extracts gold nano particles

In the present study the stable, encapsulated gold nanoparticles were prepared from gold salt ($HAuCl_4$) with the use lemon peel extract. The 2 mM of HAuCl4 solution is added to 20% lemon peel extract solution in the ratio of 2: 1 with stirring. The gold nanoparticles fabrication happened quickly within 10 minutes. The color of the aqueous solution of HAuCl4 changed from yellow to ruby red after adding the lemon peel extract indicating the formation of gold nano particles. Lemon peel extract acted as a powerful reducing and capping agent for nanoparticles [6].

Animals

The sixty healthy adult Wistar rats of 160 to 190g were acclimatized for a week before the start of the experiment. The experimental protocol was approved by Institutional Animal Ethics Committee (Approval No. IAEC/312/2000/02/2022). The experimental animals were housed in polypropylene cages. The animals were provided with rat pellet chow supplied by Nutrivet life Sciences, Pune and water ad-libitum and maintained under 12-12 h cycle of darkness and light. The standard temperature was maintained with a temperature of 25±2 ºC and a relative humidity of 40-55%. The subacute toxicity was induced with Sodium Arsenate and Lead acetate administered @ 13.8mg/kg and 116.4 mg/kg, p.o. respectively to wistar rats for 14 days followed by LPGNP supplementation @10 and 20 mg/kg, p.o. for 6 weeks. The neurological effect LPGNPs was studied with different estimations such as evaluation of antioxidant enzymes in brain and histopathological examination of brain tissue.

Table 1: Different groups of animal, treatment, dose and duration of experiment

Groups	Treatment	NO. of animals	Duration of experiment
I	Control – NS/DW p.o for 8 weeks	6	8 weeks
II	Sodium Arsenite ($NaAsO_2$) @ 13.8mg/kg body weight p.o for 14 days	6	8 weeks
III	Lead acetate ($C_4H_6O_4pb$) @ 116.4 mg/kg body weight p.o for 14 days	6	8 weeks
IV	Sodium Arsenite 13.8mg/kg body weight + Lead acetate @116 mg/kg body weight for 14 days	6	8 weeks
V	Sodium Arsenite dosed as per grp II + Lemon peel extracts AuNPs 10mg/kg from 15th day till last day of experiment	6	8 weeks
VI	Sodium Arsenite dosed as per grp II + Lemon peel extract AuNPs (20mg/kg) from 15th day till last day of experiment	6	8 weeks
VII	Lead Acetate dosed as per grp III + Lemon peel extract AuNPs (10mg/kg)	6	8 weeks
VIII	Lead Acetate dosed as per grp III + Lemon peel extracts AuNPs (20mg/kg) from 15th day till last day of experiment	6	8 weeks
IX	Sodium Arsenite dosed as per grp II + Lead Acetate dosed as per grp III + Lemon peel extract AuNPs (10mg/kg) from 15th day till last day of experiment	6	8 weeks
X	Soduim Arsenite dosed as per grp II + Lead Acetate dosed as per grp III + Lemon peel extracts AuNPs (20mg/kg) from 15th day till last day of experiment	6	8 weeks

Estimation of CAT, SOD, GR

Catalase activity was assayed by the method of Sinha [7] and is expressed as µmoles of H_2O_2 consumed/min/mg protein. Superoxide dismutase was assayed by the method of Marklund and Marklund [8] and the enzyme activity is expressed as Units/mg protein. Reduced glutathione was determined by the method of Ellman[9].

Statistical analysis: Data was analyzed using one way ANOVA followed by Tukey's post hoc test in IBM SPSS software, Version 22. Significance was observed at 5% level.

Results

The SOD values of rats brain decrease significantly (p<0.05) in all treatment groups as compared to control. The highest significant (p<0.05) decrement in SOD brain was recorded in toxic control group II and IV. The significant (p<0.05) improvement in SOD was observed in group V and VI as compared to respective control group II, similarly significant revival in SOD observed in group IX and X as compared to toxic control group IV. Thus the treatment with LPGNPs shows retrieval effect on alteration of brain SOD in arsenic induced toxicity and also in As +Pb combined exposure groups. However, no significant revival in SOD activity was observed in Pb exposure groups. The brain CAT differ significantly between control and different treatment groups, the lowest value of CAT was recorded in group number II and III. Treatment with LPGNPs showed significant (p<0.05) revival in CAT in group VIII as compared to toxic control group III. The brain (GR) decreased significantly between control and different treatment groups. The lowest value of GR was recorded in toxic control group number II, III and IV. Among the different LPGNPs treatment groups significant revival in GR observed in groups VI and VII as compared to respective toxic control group II and III.

Table 2: Brain SOD, CAT and Glutathione Reductase in control and different treatment group of rats

Group	Superoxide dismutase	Catalase	Glutathione Reductase
I	26.43 ± 1.64^{a}	29.11±5.90^{a}	0.72 ± 0.04^{a}
II	14.22 ± 0.61^{de}	18.20±1.55^{d}	0.32 ± 0.01^{c}
III	16.77 ± 0.94^{cd}	18.74±1.18^{cd}	0.33 ± 0.032^{c}
IV	12.63 ± 0.60^{e}	18.74±1.18^{cd}	0.39± 0.03^{bc}
V	18.16 ± 1.14^{c}	21.45±1.87^{bcd}	0.40 ± 0.05^{bc}
VI	17.41 ± 1.43^{c}	22.84±2.55^{bcd}	0.46 ± 0.04^{b}
VII	17.82 ± 1.68^{c}	24.26±2.25^{bc}	0.47 ±0.06^{b}
VIII	19.31 ± 0.58^{bc}	25.05±2.37^{b}	0.42 ± 0.03^{bc}
IX	17.15 ± 0.50^{cd}	18.70±2.04^{cd}	0.40 ± 0.046^{bc}
X	22.24 ± 0.80^{b}	17.86±1.81^{d}	0.48 ± 0.04^{b}

Histopathological findings: In the microscopic examination of brain the control group showed mild blood vessel congestion and normal brain parenchyma (Fig 1), group II rats showed gliosis, venous congestion and moderate neuronal degeneration (Fig 2). Brain tissue from group III showed mild to moderate congestion and neuronal degeneration (Fig 3). Sections of brain from group IV revealed mild neuronal degeneration (Fig 4). Sections of brain from group V revealed mild vacuolation, neuronal degeneration and atrophy of neurons (Fig 5). Group VI showing the milder focal necrosis and mild neurodegenerative changes. Group VII and VIII showing mild focal necrosis, mild neurodegenerative changes (Fig 6-8). Group IX and X brain showing mild degenerative changes (Fig 9-10).

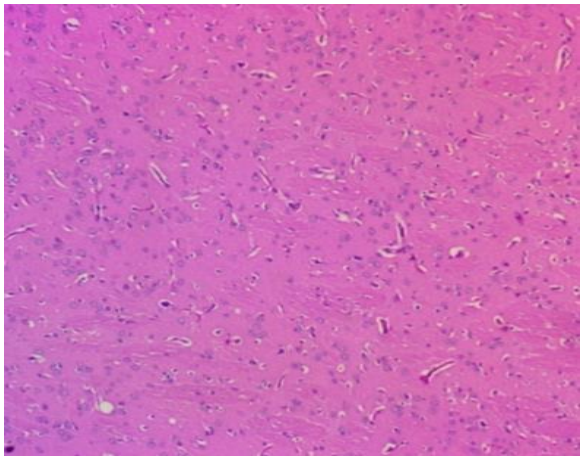

Fig 1: Control group I (H&E 100X)

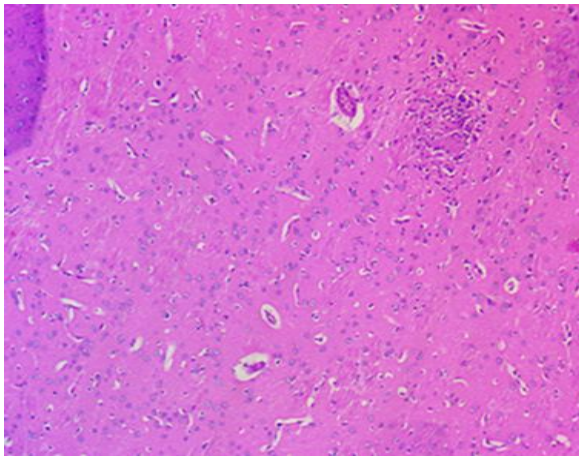

Fig 2: Arsenic Toxic group II (H & E 100X)

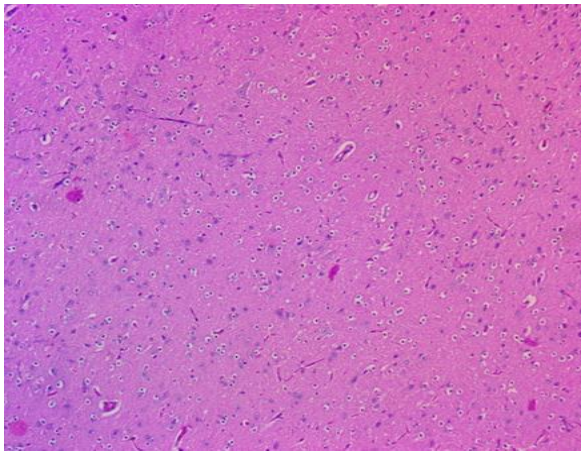

Fig 3: Lead control group III (H&E 100X)

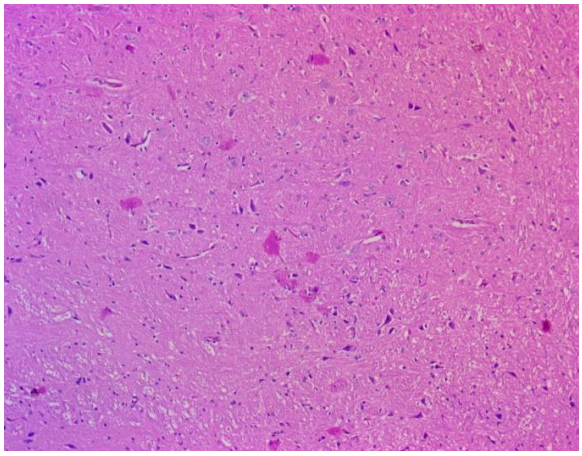

Fig 4: Lead + Arsenic group IV (H&E 100X)

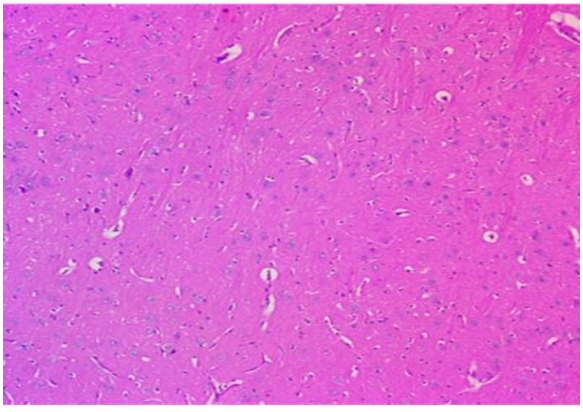

Fig 5: Arsenic+LPGNP group V (H&E 100X)

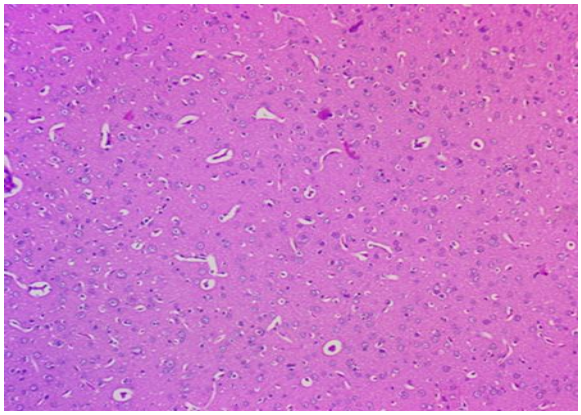

Fig 6: Arsenic+LPGNP group VI (H&E 100X)

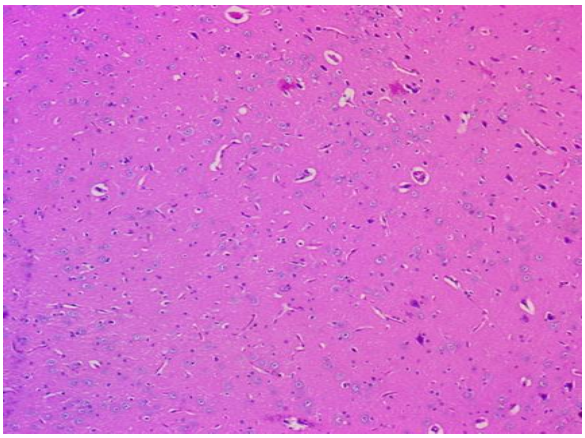

Fig 7: Lead + LPGNP group VII (H&E 100X)

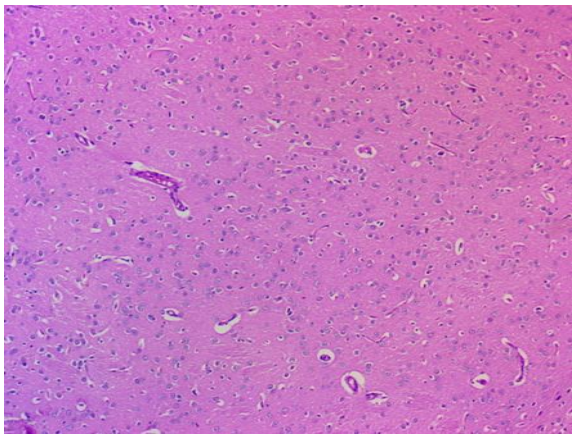

Fig 8: Lead +LPGNP group VIII (H&E 100X)

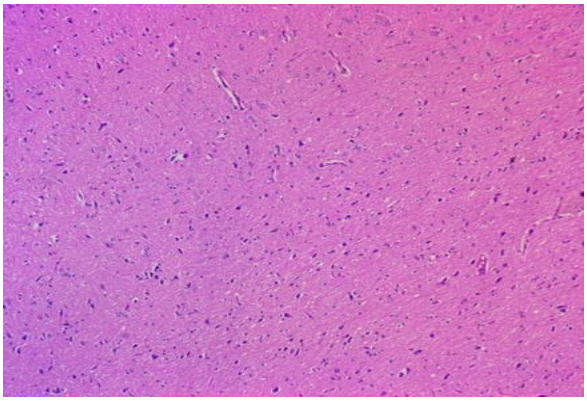

Fig 9: Lead +Arsenic+LPGNP group IX (H&E 100X)

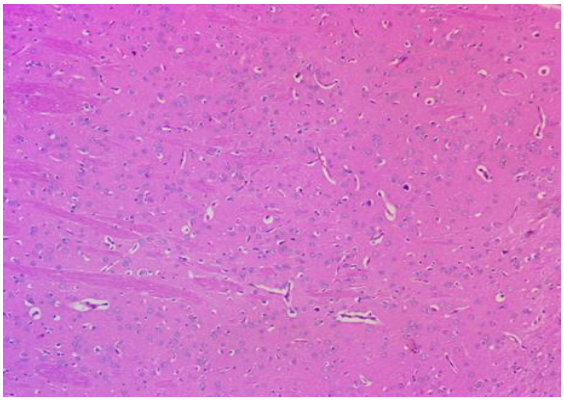

Fig 10: Lead+Arsenic+ LPGNP group X (H&E 100X)

Discussion

The arsenic, lead and their co-exposure caused significant decrease in the activity of CAT, SOD and GR in brain of wistar rats. These findings on antioxidant enzymes activity in present study align with the previous studies of Abdollahzade et al. [10] stated that arsenic administration significantly reduced the activity of antioxidant enzymes CAT, SOD, GPx, GR, and GST in the brain of rats exposed to arsenic and Fazal *et al*. (2020) observed that there was a marked decrease in antioxidants enzymes (SOD, CAT, GPx, GST, GR, GSH) in the brain of arsenic-lead induced toxicity group compared to control. The obtained data revealed that lead may induce significant inhibition in the activities of superoxide dismutase (SOD) and concentration of total antioxidant and reduced glutathione (GSH), in cerebrum, cerebellum and medulla oblongata of affected rats. In microscopic examination sections of brain from rats of toxic control groups of Pb and As showed gliosis, venous congestion, moderate neuronal degeneration and perivascular lymphoid aggregation with mild vacuolar changes. The histological changes in the brain in toxic groups coincide with the previous report of El-Shetry *et al*. [11] observed lead caused neuronal toxicity in brain tissue as lead treated rats showed brain oedema in the hippocampus area associated with focal gliosis in the cerebrum. The cerebellum showed vacuolization, while the medulla oblongata had neuronal degeneration and gliosis. Amal and Mona [12] mentioned that brain sections of rats received 100 mg/L lead acetate in drinking water for 3–6 weeks, showed pyknosis of neurons associated with focal gliosis. Also, focal cerebral haemorrhage was evident. In As, Pb and their co-exposure treatment groups receiving LPGNP, minimal aforesaid histological changes were observed.

Conclusion

From the results of the present investigation it is concluded that, oral administration of Pb, As and their co-exposure caused marked alterations in antioxidant enzymes and histoarchitecture of brain. Treatment with LPGNPs resulted in lesser alteration in the activity of antioxidant enzymes and brain histoarchitecture, suggesting its role as a potential antioxidant and neuroprotective agent. However, further detail studies are required to evaluate its neuroprotective role and safety of the gold nanoparticles.

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