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Renal Protective Effect Of Ginger And Garlic Extract On Rats Exposed To Lead Poisoning.

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Abstract: *The present study investigated the ameliorating effect of ginger (Zingiberofficinale) and garlic (Allium sativium) aqueous extract on some renal function parameters in lead-induced nephrotoxicity in adult albino rats. A total of fifty rats were used for this study. Of these, twenty were used for the LD50 determination. The remaining thirty rats were divided randomly into five groups of six rats each. Group A: Served as negative control and were administered with 10ml/kg/b/wtdistilled water. Group B: Served as positive control and received 100mg/kg b wt of lead acetate. Group C: Received 100mg/kg b wt lead acetate and 100mg/kg b wt garlic aqueous extract. Group D: Received 100mg/kg b wt lead acetate and 100mg/kg b wt ginger aqueous extract. Group E: Received 100mg/kg b wt lead acetate and 100mg/Kg b wt ginger and 100mg/Kg b wt garlic extract. All treatments were by oral gavage and lasted for a period of six weeks. After the last day of treatment, the animals were sacrificed and blood samples were collected for determination of biochemical parameters. The result showed no significant (P>0.05) reduction in the levels of serum uric acid and creatinine and a significant (p< 0.05) decrease in urea, sodium and chloride and a significant (p< 0.05) increase in the level of potassium in the garlic treated group compared with the control group B. There was a no significant reduction (P>0.05) in serum urea and creatinine levels; and a significant elevation in serum potassium and also significant reduction serum urea, sodium and chloride in the ginger treated group compared with the control group B. There was also no significant (P>0.05) decrease in serum creatinine and a significant (P<0.05) decrease in uric acid, sodium, potassium, chloride and urea levels in the rats treated with a 50-50 mixture of ginger and garlic extracts when compared with the control group B. Arising from the findings of this study, it appears that combined effect of ginger and garlic extracts has reno-protective capacity on lead-induced renal system of rats.*

Keywords*: Ginger, garlic, nephrotoxicity, lead, poison.*

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I. Introduction

Lead is an abundant, ubiquitous and important but toxic environmental contaminant (Karamalaet al., 2011) and the most common environmental pollutant naturally occurring in the earth's crust in small concentrations. Lead is one of the most toxic heavy metals (Laraque&Trasande, 2005) and causes a variety of behavioural, biochemical and physiological dysfunctions in both humans and experimental animals. The persistent environmental and or occupational exposure to this metal is associated with renal (Vargas et al.,2003; Damek-Poprawaet al., 2004;Rastogi, 2008; Sharma et al., 2011), hepatic(Patraet al., 2001; Flora et al., 2008; Sharma et al., 2011), haematological (Lanphearet al., 2000;&Adeniyiet al., 2008), reproductive (Flora et al., 2008), cardiovascular (Adeniyi et al., 2008), Immunological (Bunn et al., 2001; Rosenberg et al., 2007) and nervous (Flora et al., 2006; El-Sayed and El-Neweshy, 2009 and Ashryet al., 2010) disorders in man and animals . The kidney is the major excretory organ of lead from the body and higher content of lead has been estimated in renal tissue than in liver and brain of the lead intoxicated animals(Karamalaet al.,2011). Kidney autopsies studies have demonstrated that the kidney is the second largest repository of lead among soft tissues (Adikwuet al., 2015). It is described as an environmental nephrotoxic heavy metal (Prementeret al., 2011) as environmental exposure could cause nephrotoxicity in humans and animals (Diamond, 2005; Patel et al., 2012).
The precise biochemical and molecular mechanisms of lead toxicity is not fully understood (Shalanet al., 2005). However, Oxidative stress has been reported as one possible molecular mechanism involved in toxicity of lead in biological systems (Pandeet al., 2002; Flora et al., 2009; Khalafet al., 2012). Oxidative stress is a consequence of an imbalance between oxidants and antioxidant defence systems (Flora et al., 2009). Lead is reported to cause oxidative stress by inducing the over production of Reactive Oxygen Species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals and lipid peroxides (Xu et al., 2005; El-Nekeetyet al., 2009;Ibrahim et al., 2012). Consequently, lipid peroxidation is enhanced, the saturated fatty acids are

decreased and the unsaturated fatty acid contents of membranes increased (Ibrahim et al., 2012). This becomes a hindrance in membrane transport.

Previous studies have shown the separate effects of Allium sativumand Zingiberofficinaleon biomarkers of renal function such as urea, uric acid and creatinine and oxidative stress markers(malondialdehyde, superoxide dismutase, catalase, glutathione peroxides) in lead exposed rats. Separate administration of either of these plants extracts have been demonstrated to have ameliorating effects on the biochemical alterations and histological damage of kidney induced by lead in exposed rats (Ajithet al, 2007; Ashouret al, 2007; Adeniyiet al, 2012; Jarad, 2012; Tugboboet al, 2012; Pratap, 2014) but record of their combined effects in lead-induced nephrotoxic rats is scarce. Also, oxidative stress has been implicated in lead toxicity. Several studies have reported the ameliorating effect of either ginger or garlic on oxidative stress injury in lead-induced nephrotoxic rats (Adegbesanet al., 2007). There is however paucity of literature on the nephron-protective effect of aqueous mixture of ginger and garlic on the oxidative stress injury in lead-induced nephrotoxic rats

II. Materials And Methods

2.1. Management of Experimental Animals

Fifty (50) adult albino rats (Rattusnorvegicus) of both sexes and weighing between 150-270g were procured from the animal house and housed in well aerated laboratory cages in a room under standard conditions, temperature range of 25 ± 3^{O}C and a 12/12 hours of light and dark cycle. The rats were fed with commercial rat feeds supplied by the animal house and were given drinking water ad libitum during the experimental period. They were allowed to acclimatize to the laboratory environment for a period of two weeks before the commencement of the experimental protocol.

2.2 Preparation of Ginger Aqueous Extract

The fresh ginger rhizome was washed to remove dirt. Excess water was allowed to drain off and five-hundred (500g) gram was weighed. These were peeled on crushed ice and cut into small pieces. These cut pieces were homogenized in 750ml of cold sterile 0.9% sodium chloride solution and 250ml of ice cold distilled water in a blender for 12miutes. The homogenate was filtered three times through a cheese cloth. The filtrate was then centrifuged for at 2000 rpm for 10miutes. The clear supernatant was made up to1000ml with the 0.9% normal saline and stored at -20^{o}C until use. The concentration of this aqueous ginger preparation was considered to have 500mg/ml on the basis of the weight of the starting material (Majeed, et al, 2003).

2.3 Preparation of Garlic Aqueous Extract

The garlic aqueous extract was prepared according to the method of Ghiasi, (2014).Thirty (30g) gram of garlic was crushed and added to 100 ml distilled water. The juice was obtained using a fruit juice extracting machine. The resultant homogenized mixture was filtered three times using a cheese cloth, and then centrifuged at 2000 rpm for 10 minutes. The clear supernatant was quickly collected and kept in dark bottles. It was stored at 2 – 8^{o}C in a refrigerator until used. Based on weight of the starting material (30 g per 100 ml), concentration of prepared garlic is considered to be 500 mg per ml. (Asadpouret al., 2013&Ghiasi, 2014).

2.4 Treatment of Animals

Group A: Control. This group of rats received rat feeds and water ad libitum

Group B: lead acetate (Pb). This group of rats received rat feeds and was gavaged with lead acetate (100mg/kg body weight/day in drinking water).

Group C: Pb + Garlic. The rats received rat feeds and were gavaged with lead acetate (100mg/kg body weight/day in drinking water) and ginger aqueous extract (100mg/kg body weight/day).

Group D: Pb + Ginger. The rats standard rat feeds and were gavaged with lead acetate (100mg/kg body weight/day in drinking water) and garlic extract (100mg/Kg body weight/day)

Group E: Pb + Ginger + Garlic. The rats received rat feeds and were gavaged with lead acetate (100mg/kg body weight/day in drinking water) and a mixture of ginger (100mg/Kg body weight/day) and garlic extract (100mg/Kg body weight/day). All treatment was for duration of six (6) weeks. The ginger and garlic doses were selected based on a pilot study.

2.5 Collection, Preparation and Preservation of Blood Samples for Biochemical Assays

Blood samples were collected at the end of the experiment via cardiac puncture from each anaesthetized rat after completion of six (6) weeks of treatment.All other biochemical tests were determined using an auto-analyzer spectrophotometer.

2.6 Statistical Analysis of Data

The data from this study was analyzed using GraphPadPrism version 5.0 and Microsoft Excel, 2007. The normality of the data was determined using D'Agostino and Pearson Omnibus testing. The independent t – test was used to determine differences between two groups, whereas ANOVA was used for multiple groups. Data was considered significant at $p < 0.05$.

III. Results

Biochemical parameters of adult albino rats exposed to lead are shown in table 4.1. There was an increase in serum uric acid level in group B (lead induced group) compared with group A (control), and was statistically not significant at $f^2= 1.48$ and $p>0.05$. Also there was a slight increase in serum creatinine level in group B compared with control, but statisticallynotsignificant at $f=0.07$, $P>0.05$. Urea showed an increase in serum level and statistically significant.

Furthermore serum electrolytes showed upward trend in group B compared with control (group A). Potassium, Sodium andchlorideions were all statistically significant at f =5.09, 4.79andf=3.47, respectively for potassium, sodium and chloride (<0.05).

Table 4.1 Toxicological Assessment of Oral Lead Poisoning on Some Biochemical Parameters of Adult Albino Rats.

Parameters (Units)	GP A	GP B	t statistic	p value	Remark
Uric Acid (µmol/l)	267.80 ± 73.50	308.70± 40.21	1.48	.1577	NS
Creatinine (µmol/l)	78.70± 7.78	78.89± 3.41	0.07	.9472	NS
Urea (mmol/l)	6.84± 0.20	7.86± 0.77	4.04	.0008	S
Potassium (mmol/l)	5.47± 0.37	7.02± 0.88	5.09	.0001	S
Sodium (mmol/l)	148.20± 2.62	160.20± 7.46	4.79	.0002	S
Chloride (mmol/l)	102.60± 2.55	114.70± 10.71	3.47	.003	S

Key: values expressed as Mean ±SD, S = $p < 0.05$, NS = $p > 0.05$

GP A = Normal (10mgkg^{-1} of water), GPB = Lead Administered (100mgkg^{-1} of Lead Acetate)

The effect of garlic extract administered to(group C) lead exposed Albino Rats are depicted in table 4.2. There was reduction in uric acid level compared with group B when compare with group A (control) at($F=1.56$ and $P> 0.005$) and post hoc analysis showed difference within group. Garlic extract showed an increase in creatinine level when group C was compared with group B and a return to control level.

Furthermore, urea showed a decrease in serum level in group C compared with group B at $P< 0.008$. Potassium was increased significantly compared to group A post garlic treatment.Sodium and chloride showed reduction in serum level in group C when compared with groups B and were both statistically significant at $P<0.002$ and $p < 0.003$respectively.

Table 4.2: Effect of Aqueous Garlic Extract on Some Biochemical Parameters of Oral Lead Induced Poisoning on Adult Albino Rats Studied

Parameters (Units)	GP A	GP B	GP C	F statistic	p value	Remark
Uric Acid (µmol/l)	267.80 ± 73.50	308.70 ± 40.21	301.70 ± 38.66	1.56	.2292	NS
Creatinine (µmol/l)	78.70 ± 7.78	78.89 ± 3.41	77.89 ± 3.18	0.09	.9150	NS
Urea (mmol/l)	6.84 ± 0.20	7.86 ± 0.77	7.09 ± 0.67$^{\alpha}$	7.54	.0027	S
Potassium (mmol/l)	5.47 ± 0.37$^{\beta}$	7.02 ± 0.88	7.83 ± 0.30	42.17	.0001	S
Sodium (mmol/l)	148.20 ± 2.62$^{\gamma}$	160.20 ± 7.46	156.00 ± 4.50	13.29	.0001	S
Chloride (mmol/l)	102.60 ± 2.55	114.70 ± 10.71	105.00 ± 3.35δ	8.87	.0012	S

Key: values expressed as Mean ±SD, S = $p < 0.05$, NS = $p > 0.05$ Using ANOVA (F)

GP A = Normal (10mgkg^{-1} of water), GPB = Lead Administered (100mgkg^{-1} of Lead Acetate) and GPC= received 100mgkg^{-1} of lead acetate + garlic extract

All post hoc testing were done using Bonferroni multiple comparison. $^{\alpha}$Significant difference observed in the urea concentration between GP B and GP C, $p < .05$. $^{\beta}$Significant difference observed in the potassium concentration between GP A and GP C, $p < .005$. $^{\gamma}$Significant difference in the sodium concentrationbetween Group A and Group C, $p < .01$. $^{\delta}$Significant difference observed in the chloride concentrationbetween Group B and Group C, $p < .05$.

The effect of Aqueous ginger extract on some biochemical parameter in lead exposed adult albino rat are shown in table 4.3. Uric acid level in group D ginger administered group post lead acetate poisoning showed a decreased compared with group B, although not significant at P < 0.005. Creatinine was non-significantly decreased. Also Urea, sodium and chloride showed a decreased in serum levels and was statistically significant at P <0.005. Potassium was significantly elevated compared with group B.

Table 4.3: Effect of AqueousGinger Extract on Some Biochemical Parameters of Oral Lead Induced Poisoning on Adult Albino Rats Studied

Parameters (Units)	GP A	GP B	GP D	F statistic	P value	Remark
Uric Acid (µmol/l)	267.80 ± 73.50	308.70 ± 40.21	239.40 ± 54.03	3.04	.0666	NS
Creatinine (µmol/l)	78.70 ± 7.78	78.89 ± 3.41	75.88 ± 2.03	0.86	.4338	NS
Urea (mmol/l)	6.84 ± 0.20	7.86 ± 0.77	6.99 ± 1.11	4.85	.0170	S
Potassium (mmol/l)	5.47 ± 0.37$^{\alpha}$	7.02 ± 0.88	7.09 ± 0.36	22.66	.0001	S
Sodium (mmol/l)	148.20 ± 2.62	160.20 ± 7.46	151.60 ± 2.00$^{\beta}$	16.01	.0001	S
Chloride (mmol/l)	102.60 ± 2.55	114.70 ± 10.71	104.10 ± 1.81$^{\gamma}$	9.47	.0009	S

Key: values expressed as Mean ±SD, S = p < 0.05, NS = p > 0.05

GP A = Normal (10mgkg^{-1} of water), GPB = Lead Administered (100mgkg^{-1} of Lead Acetate) and GP D= group received 100mgkg^{-1} of lead + ginger extract

All post hoc testing were done using Bonferroni multiple comparison. $^{\alpha}$Significant difference observed in the potassium concentration between GP A and GP D, p < .005. $^{\beta}$Significant difference observed in the sodium concentration between GP B and GP D, p < .01. $^{\gamma}$Significant difference in the chloride concentrationbetween Group B and Group D, p < .01.

The effect of aqueous ginger and garlic extracts combined on some biochemical parameters in lead exposed adult albino rats are displayed in table 4.4. There was decrease in all the biochemical parameters studied. Uric acid, urea, potassium, sodium and chloride of group E (ginger – garlic combined extract on post – oral lead poisoning) all showed statistically significant reduction at p <0.05 while creatinine was not statistically reduced (p > 0.05).

Table 4.4: Effect of Ginger and GarlicAqueous Extract on Some Biochemical Parameters in Lead Exposed Adult Albino Rats Studied

Parameters (Units)	GP A	GP B	GP E	F statistic	P value	Remark
Uric Acid (µmol/l)	267.80 ± 73.50	308.70 ± 40.21	230.40± 56.31$^{\alpha}$	3.96	.0320	S
Creatinine (µmol/l)	78.70 ± 7.78	78.89 ± 3.41	76.56± 7.04	0.37	.6939	NS
Urea (mmol/l)	6.84 ± 0.20	7.86 ± 0.77	6.30± 0.39$^{\beta}$	22.23	.0001	S
Potassium (mmol/l)	5.47 ± 0.37	7.02 ± 0.88	6.66± 0.30	19.32	.0001	S
Sodium (mmol/l)	148.20 ± 2.62$^{\gamma}$	160.20 ± 7.46	152.40± 2.65	15.48	.0001	S
Chloride (mmol/l)	102.60 ± 2.55	114.70 ± 10.71	103.20 ± 3.07$^{\delta}$	10.08	.0006	S

Key: Values expressed as Mean ±SD, S = p < 0.05, NS = p > 0.05 Using ANOVA (F)

GP A = Normal (10mgkg^{-1} of water), GP B = Lead Administered (100mgkg^{-1} of Lead Acetate) and GP E= group received 100mgkg^{-1} with garlic + ginger combine extracts

All post hoc testing were done using Bonferroni multiple comparison. $^{\alpha}$Significant difference observed in the uric acid concentration between GP B and GP E, p < .005. $^{\beta}$Significant difference observed in the urea concentration between GP B and GP E, p < .005. $^{\gamma}$Significant difference in the sodium concentrationbetween Group A and Group E, p < .005. $^{\delta}$Significant difference observedin the chloride concentrationbetween Group B and Group E, p < .01.

IV. Discussion

Some studies have shown the ameliorating effect of ginger and garlic extracts on blood-lead concentrations (Adeniyi et al., 2012). In the current study, post treatment with the garlic aqueous extract reduced the blood lead concentration non-significantly in the intoxicated rats compared with the lead group B. This result was in consonance with the reports of the study by Sharma et al., (2010).The blood lead concentration in the ginger treated rats was significantly increased compared with the lead group B. This result was inconsistent with a previous study (Raddyet al., 2011).The mixture of garlic and ginger also reduced the blood-lead concentration significantly compared with the lead group B. This reduction may be due to the protective role of garlic and garlic been an antioxidant rich plants.

It has been reported that elevated levels of serum uric acid, urea and creatinine are very reliable for investigating drug-induced nephrotoxicity in animal and man (Ghalibkandiet al., 2012) and that in lead toxicity the constant findings are elevated uric acid and creatinine (Adeiniyiet al., 2012; Missounet al., 2010).This is in

agreement with the result of the present study**.** The serum uric acid and creatinine levels were not significantly elevated in group B compared with the control group A.

Post treatment of the rats with the garlic aqueous extract resulted in a non-significant reduction in the level of serum uric acid ad creatinine while urea, potassium, sodium and chloride were significantly reduced compared with the control group B. The finding of decreased creatinine and urea in this study was in consonance with the reports by Ghabehkandiet al, (2010). However, the observed decrease in uric acid in the present study was contrary to the observations of Ghabehkandiet al, (2010) who reported increase uric acid level. The observed decrease in sodium and chloride levels in the current study is in agreement with the study by Abubakaret al., (2014). Abubakar and co-workers reported a significant decrease in the level of sodium and chloride ions. The significant increase in potassium is in conformity with the study of Tendeet al.,(2012).This result suggests that garlic aqueous extract may be useful in the management of electrolyte related disorders. The decreased in the level of sodium ion observed in this study may be due to a change in glomerular filtration and/or renal blood flow. It may also be due to interference with aldosterone secretion and/or aldosterone action on the distal tubules or interference with adrenergic sodium handling caused by garlic administration. (Asdaq&Inamdar, 2010). Tendeet al., (2012)suggested that the increase in potassium may be due to the alteration in potassium transport produced by garlic. Post treatment of the lead exposed rats with ginger extract resulted in a none significant reduction in uric acid and creatinine and a significant reduction in urea, sodium and chloride. Potassium was significantly increased. The decrease in the level of serum electrolytes(Na^+ and Cl^-) in the ginger treated group in the present study is conformity with the study by Abubakaret al., (2014). The decrease uric acid, urea and creatinine level in the ginger treated rats werenotinconsonance with the study by Ghabehkandiet al., (2010). Increase in potassium level was in agreement with Tendeet al., 2012. The effect of ginger on the biochemical parameters in this study is indicative of a nephroprotective role. It also suggests that ginger will be useful in the management of electrolyte related disorders as with garlic. Also, in the present study, post treatment with the 50-50 mixture of garlic and ginger extract significantly reduced the concentration of uric acid, urea, sodium, potassium and chloride and none significantly reducedcreatinine level. The decreased in the level of sodium and chloride in this study was in agreement with the observation by Tende et al., 2012. The observed decrease in the level of potassium, following post-administration with the 50-50 mixture of the extract is not agreement with the observation by Tendeet al., (2012). These results imply that the mixture of ginger and garlic mixture in the present study also have a nephroprotective effect. The possible explanation could be as a result of their antioxidant potentials.

V. Conclusion

The 50-50 mixture of ginger and garlic extracts reduced significantly chloride, sodium and urea levels but increased potassium level in the lead intoxicated rats, depicts that the combination of the extract may proffer some level of nephroprotection, which may not be seen in using the plant extract singly.

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