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Ameliorative Effect of Ethanolic Extract of *Oxalis corniculata* L. on the Blood Biochemistry of $HgCl_2$ Intoxicated Male Albino Rats

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Abstract: Heavy metal mercury chloride ($HgCl_2$), a strong toxic agent for the liver and kidney, induces severe oxidative stress and cellular damage to various organs. This study investigated the ameliorative potential of the ethanolic extract of *Oxalis corniculata* L. (EEOC) against $HgCl_2$ induced hepatotoxicity in male albino rats. Ninety six rats were divided into four groups (each group n=24): control, $HgCl_2$ (6 mg/kg body weight) treated, and two co-treatment groups receiving mercury chloride and EEOC (200 mg/kg body weight and 400 mg/kg body weight) simultaneously for 28 days regularly. $HgCl_2$ administration significantly elevated serum levels of hepatic enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and glucose along with a reduction in cholesterol indicating severe hepatic damage. Conversely, co-treatment with EEOC significantly attenuated $HgCl_2$-induced hepatic damage in a dose and time-dependent manner. EEOC administration significantly reduced serum levels of AST, ALT, ALP, and glucose along with elevation in cholesterol as compared to $HgCl_2$ treated rats indicating a restoration of hepatic function. The results of this study demonstrate that the ethanolic extract of plant *Oxalis corniculata* exhibit hepatoprotective activity against $HgCl_2$ induced hepatic damage.

Keywords: *Oxalis corniculata*, Blood biochemistry, Mercury chloride, Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase, Glucose, Cholesterol, Hepatic enzymes

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Introduction

Mercury is one of the most hazardous heavy metal contaminants (Kayani and Mohammed, 2025) that commonly exists in forms of $HgCl_2$ (Rice *et al*., 2014; Gworek *et al*., 2020). Inorganic mercury compounds, including mercury chloride ($HgCl_2$), are dangerous to living organisms and results in significant environmental and health risks due to their wide range of applications and release into ecosystems (Clarkson and Magos, 2006; Wu *et al*., 2024). The National Institute for Minamata Disease (NIMD) in Japan and the World Health Organization has been concerned about people

eating seafood intoxicated with mercury. Mercury exposure has been shown to induce a variety of adverse biological effects, including neurological, renal, and hepatic damage. Malfunctioning of several physiological systems, oxidative stress and inflammation leading to alterations in liver biomarkers can be attributed to exposure to $HgCl_2$ (Wu *et al*., 2024). Mercury exposure has been associated with significant alterations in blood biochemical parameters, including elevated levels of serum alanine amino transferase (ALT), serum aspartate amino transferase (AST), and alkaline phosphatase (ALP), disruptions in glucose and lipid metabolism, leading to change in blood glucose and cholesterol levels (Waddan, 2009). These biochemical alterations are indicative of mercury-induced hepatotoxicity and metabolic disturbances**.** These disruptions may further exacerbate the toxic effects of mercury by impairing the body's ability to respond to and recover from other forms of stress (El-Demerdash, 2001). In view of the severity of mercury-induced toxicity, there is a growing interest in exploring potential therapeutic interventions that may help to mitigate these harmful effects.

Oxalis corniculata L. is a little perennial herb generally known as creeping woodsorrel with cosmopolitan distribution and abundant in gardens, lawns, agricultural forms. It is medicinally very important due to its extensive ethno-medicinal applications. It is a rich source of polyphenols, glycosides, flavonoids, oxalic acid, potassium oxalate, vitamin C, and other essential components for an individual's good health. This plant is reported to protect against an extensive range of infections and exhibit several biological actions including anti-inflammatory (Dutta *et al*., 2015), antioxidant (Gudasi *et al*., 2023), anti-bacterial (Mohan *et al*., 2015; Rehman *et al*., 2015), antimicrobial (Mohan and Pandey, 2016), antidiabetic (Al-Qalhati *et al*., 2021), hypoglycemic (Kaushik *et al*., 2023), antifungal (Rehman *et al*., 2015; Mohan and Pandey, 2016; Kumar *et al*., 2024), anticancer (Ansari *et al*., 2024; Kumar *et al*., 2024), cardioprotective (Abhilash *et al*., 2011), gastro protective (Juvekar *et al*., 2012), wound healing (Tamta *et al*, 2023), nephro-protective (Khan and Zehra, 2013), and hepato-protective (Sreejith *et al*., 2014). Considering the biological effects exhibited by the *Oxalis corniculata*, an attempt has been made in the present study to investigate the ameliorative effect of ethanolic extract of this plant (entire plant except root) on the liver biomarkers, glucose, and cholesterol of male albino rats.

Materials and Methods

Preparation of plant extract:

The plant was collected from the campus of DDU Gorakhpur University, Gorakhpur, India. The plant was identified at the Department of Botany, DDU Gorakhpur University. After collection of the plants, roots were cut off and then the remaining portion was dried in shade at room temperature for a week. After drying, fine powder was prepared with the help of mortar and pestle. 100 g of the powdered plant sample was extracted continuously with 1 L of 90% ethanol using a Soxhlet apparatus for 24 h. The resulting dark brown sample was evaporated to dryness in a rotary evaporator at 40℃ and residue was designated as ethanolic extract of *Oxalis corniculata* L. (EEOC) that was kept in sterile tubes in the refrigerator at -4°C for further use. The residues were further dissolved in distilled water whenever needed for the experiment.

Animals and experimental protocol:

Ninety-six male albino rats were procured from Asia Scientific Emporium, Varanasi, India. Rats were maintained at temperature (23±3℃), humidity (60±7%) and a light and dark cycle (12 h: 12 h) for 2 weeks prior to the start of the experiment. They were provided healthy pellet food and drinking water *ad libitum*. The protocol of the experiment was approved by the Institutional Animal Ethical Committee (IAEC).

Rats were distributed in four groups having 24 rats in each and different treatments were provided as follow:

Group I: Control; received normal food and water

for 28 days.

Group II: 6 mg/kg body weight of mercury chloride orally with distilled water for 28 days.

Group III: Rats were exposed to mercury chloride (6 mg/kg body weight) and EEOC (200 mg/kg body weight) orally with distilled water for 28 days.

Group IV: Rats were exposed to mercury chloride (6 mg/kg body weight) for 28 days and EEOC (400 mg/kg body weight) orally with distilled water for 28 days.

The doses for mercury chloride and plant extract were decided on the basis of previous studies (Das *et al.*, 2011; Oriquat *et al.*, 2012; Sheikh *et al.*, 2013).

Biochemical analysis:

Blood samples were collected through the cardiac puncture on the 7th, 14th, 21st, and 28th days of the experiment and ALT, AST, ALP, glucose and cholesterol were analyzed by using a blood biochemical analyzer (Beacon Diagnostics Private Ltd, India).

Statistical Analysis:

Data are presented as mean ± standard error (SE) (n=6). A comparison between two groups is conducted using Student's *t*-test, while a comparison among multiple groups is performed using two-way analysis of variance (ANOVA). A p-value less than 0.05 were considered statistically significant.

Results

Serum alanine aminotransferase (unit/L) significantly increased ($p<0.05$) in the rats exposed to mercury chloride (Group II) with respect to the control (Group I) on day 7. This increase was highly significant ($p<0.01$) on 14th, 21st and 28th day. Rats treated with a low dose of EEOC (200 mg/kg body weight) and mercury chloride (Group III) showed insignificant alteration in ALT on 7th day of the experiment, significant reduction on 14th and 21th day, respectively and highly significant reduction on 28th day with respect to the rats treated with mercury chloride (Group II) (Table 1). A significant fall in ALT level on 7th and 14th day of the experiment was observed in rats of group IV exposed to mercury chloride and higher dose of EEOC (400 mg/kg body weight) while this alteration was more significant on the day 21 and 28 as compared to group II. Two-way ANOVA indicated a significant ($p<0.001$) interaction for different variations.

A significant ($p<0.05$) elevation was observed in aspartate aminotransferase (unit/L) levels in the rats exposed to mercury chloride with respect to the control (Group I) on 7th day. The alterations in AST were more significant ($p<0.01$) on 14th, 21st and 28th day (Table 2). In rats treated with low dose of EEOC (200 mg/kg body weight) and mercury chloride (Group III) insignificant change in AST was observed at 7th day. However, a significant reduction was observed on 14th and 21st day while a highly significant ($p<0.01$) reduction was observed on 28th day of the experiment with respect to group II. Rats of group IV administered with a high dose of EEOC (400 mg/kg body weight) and mercury chloride showed a significant fall in AST level on 7th day and this alteration was more significant on 14th, 21st and 28th day (Table 2). Data analysis using two-way ANOVA showed a significant ($p<0.001$) interaction for different variations.

There was a significant ($p<0.05$) increase in serum alkaline phosphatase (unit/L) level in the rats exposed to mercury chloride (Group II) with respect to the control (Group I) on 7th day (Table 3). This increase was observed to be highly significant ($p<0.01$) on 14th, 21st and 28th day. Rats treated with low dose of EEOC (200 mg/kg body weight) and mercury chloride (Group III) indicated insignificant alterations in ALP on 7th day while a significant reduction on 14th day and a highly significant ($p<0.01$) reduction was observed on 21st and 28th day of the experiment with respect to the rats treated with mercury chloride (Group II) (Table 3). A significant decrease in ALP level on 7th and 14th days and a

Table 1: Effect of $HgCl_2$ and ethanolic extract of *O. corniculata* on the variations of serum ALT (units/L) in male albino rats

Group	Days			
	Day 7	Day 14	Day 21	Day 28
Group I: Control	22.340±0.006	22.330±0.014	22.322±0.006	22.328±0.124
Group II: $HgCl_2$	25.545±0.938*	27.107±0.808**	29.577±0.565**	34.397±1.223**
Group III: $HgCl_2$+EEOC (200 mg/kg b.wt.)	24.990±0.495	26.865±0.666#	27.548±0.428#	29.068±0.603##
Group IV: $HgCl_2$+EEOC (400 mg/kg b.wt.)	21.700±0.887#	24.710±0.157#	26.490±0.115##	27.280±0.246##

Interaction p <0.001, F = 6.146; Treatments p <0.001, F = 74.85; Days interval p <0.001, F =32.36; *Indicates significant (p<0.05) difference between control and $HgCl_2$ treated rats; ** indicates highly significant (p<0.01) difference between control and $HgCl_2$ treated rats; # indicates significant (p<0.05) difference between $HgCl_2$+ EEOC and $HgCl_2$ treated groups; ## indicates highly significant (p<0.01) difference between $HgCl_2$+ EEOC and $HgCl_2$ treated groups.

Table 2: Effect of $HgCl_2$ and ethanolic extract of *O. corniculata* on the variations of serum AST (units/L) in male albino rats

Group	Days			
	7	14	21	28
Group I: Control	90.487±0.2346	89.648±0.3728	88.632±0.7238	89.790±0.5697
Group II: $HgCl_2$	96.562±0.8786*	104.212±0.8827**	107.690±1.4161**	108.810±1.1386**
Group III: $HgCl_2$+EEOC (200 mg/kg b.wt.)	94.677±0.3267	97.527±0.6075#	101.433±1.3343#	102.433±1.0898##
Group IV: $HgCl_2$+EEOC (400 mg/kg b.wt.)	92.308±0.4254#	94.677±0.3267##	96.860±0.6005##	97.933±0.2743##

Interaction p <0.001 F = 8.395; Treatments p <0.001 F = 179.5; Days interval p <0.001 F =32.86; *Indicates significant (p<0.05) difference between control and $HgCl_2$ treated rats; ** indicates highly significant (p<0.01) difference between control and $HgCl_2$ treated rats; # indicates significant (p<0.05) difference between $HgCl_2$+ EEOC and $HgCl_2$ treated groups; ## indicates highly significant (p<0.01) difference between $HgCl_2$+ EEOC and $HgCl_2$ treated groups.

highly significant decrease on 21st and 28th day were observed in rats treated with high dose of EEOC (400 mg/kg body weight) and mercury chloride (Group IV) with respect to the group II (Table 3). Two-way ANOVA shows a significant (p<0.001) interaction for different variations.

Level of cholesterol (mg/dL) significantly (p<0.05) reduced in rats exposed to mercury chloride (Group II) on 7th, 14th, 21stand 28th day with respect to the control (Group I) (Table 4). Rats treated with a low dose of EEOC (200 mg/kg body weight) and mercury chloride (Group III) exhibited insignificant alterations in cholesterol on the 7th day of the experiment while a significant increase was observed on the 14th, 21st and 28th day of the experiment in comparison to group II.

Table 3: Effect of $HgCl_2$ and ethanolic extract of *O. corniculata* on the variations of serum ALP (units/L) in male albino rats

Group	Days			
	7	14	21	28
Group I: Control	260.785±0.369	261.427±0.515	262.235±0.457	261.052±0.597
Group II: $HgCl_2$	266.043±0.669*	269.573±0.870**	271.948±1.502**	280.807±0.995**
Group III: $HgCl_2$+EEOC (200 mg/kg b.wt.)	265.043±0.360	266.573±0.517#	268.782±1.027##	271.085±0.508##
Group IV: $HgCl_2$+EEOC (400 mg/kg b.wt.)	262.363±0.736#	264.405±0.972#	267.615±1.716##	268.948±0.590##

Interaction $p<0.001$, $F = 6.589$; Treatments $p<0.001$, $F = 76.68$; Days interval $p<0.001$, $F = 33.63$;
*Indicates significant ($p<0.05$) difference between control and $HgCl_2$ treated rats; **indicates highly significant ($p<0.01$) difference between control and $HgCl_2$ treated rats; # indicates significant ($p<0.05$) difference between $HgCl_2$+ EEOC and $HgCl_2$ treated groups; ## indicates highly significant ($p<0.01$) difference between $HgCl_2$+ EEOC and $HgCl_2$ treated groups.

Table 4: Effect of $HgCl_2$ and ethanolic extract of *O. corniculata* on the variations of serum cholesterol (mg/dL) in male albino rats

Group	Days			
	7	14	21	28
Group I: Control	30.669±0.084	30.855±0.067	31.010±0.169	31.398±0.178
Group II: $HgCl_2$	25.122±0.677**	22.058±0.759**	20.713±0.835**	19.016±1.203**
Group III: $HgCl_2$+EEOC (200 mg/kg b.wt.)	25.552±0.645	26.180±0.721#	27.800±0.645#	28.778±0.488##
Group IV: $HgCl_2$+EEOC (400 mg/kg b.wt.)	26.798±0.410#	27.725±0.264#	27.907±0.140##	28.555±0.764##

Interaction $p<0.01$, $F = 6.016$; Treatments $p<0.01$, $F = 74.12$; Days interval $p<0.01$, $F = 32.02$;
*Indicates significant ($p<0.05$) difference between control and $HgCl_2$ treated rats; **indicates highly significant ($p<0.01$) difference between control and $HgCl_2$ treated rats; # indicates significant ($p<0.05$) difference between $HgCl_2$+ EEOC and $HgCl_2$ treated groups; ## indicates highly significant ($p<0.01$) difference between $HgCl_2$+ EEOC and $HgCl_2$ treated groups.

A significant elevation in cholesterol levels on 7th and 14th days of experiment, and highly significant elevation on 21st and 28th days was observed in rats treated with a high dose of EEOC (400 mg/kg body weight) with mercury chloride (Group IV) in comparison to the group II (Table 4). Two-way ANOVA demonstrated a significant ($p<0.01$) interaction for different variations.

A significant ($p<0.05$) increase was observed in serum glucose (mg/dL) level of rats exposed to mercury chloride (Group II) with respect to the control (Group I) on day 7 while these alterations were highly significant ($p<0.01$) on 14th, 21st and 28th day. In rats treated with low dose of EEOC (200 mg/kg body weight) and mercury chloride (Group III) insignificant change in glucose level

Table 5: Effect of $HgCl_2$ and ethanolic extract of *O. corniculata* on the variations of serum glucose (mg/dL) in male albino rats

Group	Days			
	7	14	21	28
Group I: Control	131.140±0.085	130.995±0.188	130.875±0.145	131.064±0.238
Group II: $HgCl_2$	137.924±0.782*	141.610±1.147**	147.371±0.848**	152.141±1.348**
Group III: $HgCl_2$+EEOC (200 mg/kg b.wt.)	136.949±0.390	137.597±0.331#	142.677±0.230##	144.717±0.647##
Group IV: $HgCl_2$+EEOC (400 mg/kg b.wt.)	134.078±0.777#	136.287±1.302##	140.683±0.130##	141.167±0.338##

Interaction p <0.001, F =10.27; Treatments p <0.001,F = 194.4; Days interval p <0.001 F =65.98
*Indicates significant (p<0.05) difference between control and $HgCl_2$ treated rats; **indicates highly significant (p<0.01) difference between control and $HgCl_2$ treated rats; # indicates significant (p<0.05) difference between $HgCl_2$+ EEOC and $HgCl_2$ treated groups; ## indicates highly significant (p<0.01) difference between $HgCl_2$+ EEOC and $HgCl_2$ treated groups.

was observed on the 7thday while a significant reduction on 14th and highly significant reduction was observed on 21st and 28th day of the experiment in comparison to group II. Rats of group IV administered with a high dose of EEOC (400 mg/kg body weight) and mercury chloride demonstrated a significant fall in glucose levels on day 7 and a highly significant fall on 14th, 21stand 28th day of experiment (Table 5). Two-way ANOVA indicated a significant (p<0.001) interaction for different variations.

Discussion

Exposure to mercury has been reported to significantly alter key biochemical markers of hepatic and metabolic function. Heavy metal like mercury chloride is well documented to cause lipid abnormalities by interfering with fatty acid metabolism, facilitated lipid peroxidation by restraining antioxidant pathways and damaging cellular structure via peroxidation of cell membrane (Koyama *et al.*, 2024). These metabolic disturbances, along with hepatic enzyme leakage, indicate the systemic toxicity of mercury and its capacity to impair multiple physiological pathways. These findings are consistent with previous researches conducted on rats exposed to inorganic and organic mercury compounds, which reported similar trends in altered biochemical profiles (El-Demerdash, 2001; Mahboob *et al*., 2001). A significant elevation in the serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) and alkaline phosphatase (ALP) indicates hepatic cell membrane damage and compromised liver integrity. Serum ALT, a cytoplasmic enzyme found in the hepatocytes; generally, works within the cells; on the other hand ALP is a membrane bound enzyme and perform the function of transportation of metabolites (Ozer *et al*., 2008; Thakur *et al*., 2024). The elevated levels of ALT, AST and ALP in serum suggest that these enzymes are released into circulation following hepato-cellular injury and their increase serves as a reliable indicator of mercury-induced hepatotoxicity due to organ damage, necrosis or cell lysis, increased permeability and loss of functional integrity of the hepatocyte's membrane leading to leakage of enzymes from cells to the circulation (Merzoug *et al*., 2009; Jagadeesan and Bharathi, 2014; Sreejith *et al*., 2014;). The present study derives support from the observations of Yadav *et al*. (2024) who have also noticed elevated level of ALT, AST and

ALP in serum of albino rats treated with a heavy metal cadmium.

In addition to these liver-specific enzymes, mercury exposure has also been correlated with significant disruptions in glucose and lipid metabolism. Elevated serum glucose has been observed in experimental animal models, suggesting that mercury interferes with insulin signaling. Increase in glucose level may also be attributed to increased requirement of energy due to the toxicity of ingested heavy metal in rats (Maqbool *et al*., 2019).

The decrease in serum cholesterol level as observed in the present study after mercury exposure is in agreement with the reports of Waddan (2009) and Merzoug *et al*. (2009) who have also noticed a significant reduction in serum cholesterol level in rats exposed to mercury chloride. Leila *et al*. (2022) found increased level of cholesterol in male rats treated with mercury chloride while there was no alteration in female rats. However, impact of mercury on lipid level is not consistent across all studies-- some researchers indicate decreased level of cholesterol, triglycerides and VLDL cholesterol in some tissues while other indicates increase in total cholesterol with decrease in HDL cholesterol. Exposure to heavy metals is known to induce cholesterol redistribution by modulating the expression of cholesterol efflux proteins (Wang *et al*., 2018). A decrease in serum cholesterol can be attributed to the interference of mercury with lipid regulatory pathways, potentially through oxidative stress and mitochondrial dysfunction (Uzunhisarcikli *et al*., 2016) and to the bio-accumulation of metal in the liver and kidney resulting in inhibition of the conversion of esterified cholesterol to free cholesterol.

The administration of EEOC exhibited a significant protective effect against mercury-induced toxicity. Co-treatment with EEOC significantly attenuated the levels of serum liver enzymes (ALT, AST, and ALP), glucose, and cholesterol induced by $HgCl_2$ exposure, suggesting a restoration of hepatic and metabolic function. This protective effect can be attributed to the phytoconstituents present in *Oxalis corniculata* L., particularly flavonoids, phenolic compounds, and essential oils, which possess strong antioxidant and anti-inflammatory properties (Badwaik *et al*., 2011; Srikanth *et al*., 2012; Sarkar *et al*., 2020). These bioactive compounds may help in scavenging reactive oxygen species generated by mercury, elevating antioxidant defense system and by stabilizing the cellular membranes, thereby preventing organ damage (Bhattacharya, 2018; Bharti *et al*., 2024). *Oxalis corniculata* L. is reported to possess hypoglycemic action via a positive influence on glycogen metabolism and insulin-releasing action (Kaushik *et al*., 2023). Plant products play a very important role in health care. Natural phyto therapeutics exhibit a range of biological impacts and have been gaining importance in the health field. The present study indicates that the ethanolic extract of *Oxalis corniculata* L. has the potential to ameliorate the adverse effect caused by mercury chloride.

Conclusion

The findings of the present study suggest that extract of *Oxalis corniculata* L. offers significant protection against mercury-induced liver and metabolic toxicity, likely by enhancing antioxidant defenses and influencing the metabolism of toxic substances. Further investigation is needed to characterize the active constituents and delineate their mechanistic pathways.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Institutional Animal Ethical Committee (IAEC).

Author Contributions

Begam R.: Carried out experiments, data collection, preparation of the original manuscript; *Kushwaha V. B.*: Experimental planning, supervision, data validation, manuscript editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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