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Ameliorative effect of Tinospora cordifolia stem aqueous extract on Hematological parameters of wistar rat against $HgCl_2$ induced toxicity

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ABSTRACT

Blood is crucial for the human body's health and survival. It carries out a variety of functions, including as delivering nutrients and oxygen. RBCs, WBCs, plasma, and platelets are the blood's major four constituents. It serves as a pathological sign for the condition of animals under different situations and toxicant exposure. This work aimed to explore the in vivo therapeutic mechanism of *Tinospora cordifolia* aqueous extracts against the haematotoxic effects of $HgCl_2$. Ameliorative effect of *T.cordifolia* aqueous extract was evaluated in two groups of rats, including a treated group and a control group. The treated group was divided into following subgroups, I: $HgCl_2$ treated, subgroup II: $HgCl_2$ + *T. cordifolia treated,* subgroup III: *T. cordifolia treated.* These treated subgroups were further divided into 5 set of rats, one for acute (1d), three for sub-acute (7, 14 and 28ds), and one for sub-chronic (60 ds) study. The controls were run simultaneously. Male Wistar rats were given aqueous extracts of T. cordifolia (400 mg/kg body weight, orally, once daily) along with $HgCl_2$ treatments. Dose of $HgCl_2$ administered to animals after calculation of LD_{50} (25 mg/kg body weight). Animals used in acute, sub-acute, and sub-chronic studies of mercuric chloride received 1/10, 1/50, and 1/100 of the calculated LD_{50}. This corresponds to 2.5, 0.5 and 0.25 mg/kg respectively. In mercury-treated mice, significant reductions in WBC, RBC, Hb, PCV and platelet count were also observed. These $HgCl_2$-related effects were circumvented by regular intake of *T. cordifolia aqueous* extract. These results show that *T. cordifolia* protects against $HgCl_2$ poisoning.

Keywords: Hematology, Mercuric Chloride ($HgCl_2$), wistar rat, Tinospora cordifolia.

1. INTRODUCTION

Mercury is a widespread environmental toxin that has a wide range of detrimental effects on human health. It may be found in three different forms: organic, inorganic, and elemental. They each have a distinct toxicity profile. Mercury originates from both natural and man-made sources. Volcanoes, forest fires, cinnabar (ore), and fossil fuels like coal and petroleum are all natural sources of mercury. Mining,

industrial operations, fossil fuel combustion (especially charcoal combustion), cement manufacture, and municipal, chemical, and medical waste incineration are all significant anthropogenic sources of mercury in the environment. People are exposed to mercury mostly by inhalation or ingestion while oral exposure is the most common route for inorganic mercury salts [1]. The compound mercuric chloride ($HgCl_2$) is most toxic form of mercury because of its affinity with proteins. [2]. Mercury has been reported to have an impact on the liver [3], kidney [4], brain, blood and testis [5].

Mercuric chloride induces changes in hematological parameters through suppress the hematopoiesis tissue activity [6]. Hematological markers are good predictors of an animal's physiological state. Hematological parameters are those that are related to blood and blood-forming organs. [8]. $HgCl_2$ gets absorbed into the bloodstream and either interacts with proteins in the plasma or penetrates RBCs. [9].Variations in hematological markers are frequently used to evaluate various body states and pressures induced by environmental, nutritional, and/or infectious factors. Values beyond typical boundaries are diagnostic for situations such cancer, immunological diseases, and cardiovascular problems. [8].

Overall the effects of $HgCl_2$ poisoning seriously damage the body's metabolic processes. Few antitoxic medications are available to address the harmful effects of $HgCl_2$. In order to combat $HgCl_2$ mediated toxicity, medicinal plants can be used; if effective, they can even be given as an antidote [10].Thus, certain therapeutic plants have been demonstrated in animal models to be particularly effective against metal poisoning caused by mercury, arsenic, lead, and cadmium [11-13]. A vital medicinal plant and drug in the Indian medical system is *Tinospora cordifolia*, sometimes referred to as “giloy” and a member of the Menispermiaceae family. Its antioxidant, antibacterial, antifungal and free radical scavenging capabilities are due to the abundance of phytochemicals in it. This plant is traditionally used to treat oxidative stress-related illnesses and conditions such as cancer, diabetes, arthritis, inflammation, pain, diarrhoea, asthma, and respiratory infections [14].

Moreover, *T. cordifolia* extract has the ability to scavenge free radicals, as demonstrated by a recent study [15]. There is an urgent need to establish a relationship between extract concentration and free radical scavenging activity. Because there are just a few dispersed studies available, ongoing work is necessary. This knowledge gap prompted us to propose the current investigation. As a result, the current study aimed to determine the beneficial effects of *T. cordifolia* stem aqueous extract on $HgCl_2$-induced hematotoxicity in Wistar rats.

2. MATERIALS AND METHODS

2.1 Experimental chemical

$HgCl_2$ was purchased from Hi-Media laboratories Ltd. Mumbai, India. Inorganic mercury ($HgCl_2$) was employed in the current investigation. It was dissolved in distilled water to determine the concentrations utilized in the experiment.

2.2 Preparation of plant extracts material

T. cordifolia was carefully separated, rinsed, and dried in the shade after collecting the appropriate quantity. The dried stem of the plant was pulverized into a powder via an electric blender. The powder was

kept at 4°C in a dry, clean, airtight glass container. 100 g of the resulting powder was macerated and immersed in 500 ml of distilled water for 24 hours. It was subsequently passed through a 1mm mesh screen, and the resulting filtrate was concentrated into a dark green residue by heating at 40°C until all of the water had evaporated.100 mg of this concentrated extract was dissolved in 1ml of distilled water and the resultant solution was given to rats.

2.3 Experimental animal

Adult healthy male Wistar rats were chosen for the experiment and obtained from the animal house's inbred colony. Rats were fed pellets supplied from M/s Lipton India Ltd., Kolkata, and water was provided *ad libitum*. The mean room temperature of the animal house was maintained at 22 ± 2 ^{0}C with 12 h light/dark cycle. These rats were kept in standard polypropylene cages. All the groups of rats were acclimatized under the laboratory housing conditions for 15 days prior to the beginning of the treatment.

2.4 Selection of dose

The Dixon's up and down approach was used to establish the LD_{50} [16], which allowed us to determine $HgCl_2$ dosages for experimental application. The animals used for the acute, sub-acute and sub-chronic study for $HgCl_2$ received $1/10^{th,}$ $1/50^{th}$, and $1/100^{th}$ of the calculated LD_{50} which were equivalent to 2.5, 0.5, and 0.25 mg /kg respectively. *T. cordifolia* dosage was 400 mg/kg calculated based on animal model body weight and study of literature (Tabe-1)

Table-1: Oral administration of $HgCl_2$ and *T. cordifolia* as per treatment schedule

Groups	Days of treatment	Dose / mg kg-1 day-1	
		HgCl$_2$	***T. cordifolia***
Acute	**1**	LD_{50}/10= **2.5**	
Sub-acute	**7**	LD_{50}/ 50=**0.5**	**400 *mg /kg***
	14		
	28		
Sub-chronic	**60**	LD_{50}/100= **0.25**	

2.5 Experimental protocol

Rats were properly separated into control and treatment groups. Treated groups were divided further in to three sub groups:

- Subgroup I: $HgCl_2$ treated
- Subgroup II: $HgCl_2$+ *T. cordifolia* treated
- Subgroup III: *T. cordifolia* treated

These subgroups were further divided in to following sets: Acute (1 day), Sub-acute (7, 14 and 28 days) and Sub-chronic (60 days) [17]. Each set had 5 rats.

We employed the OECD (Organization for Economic Cooperation and Development) test guidelines to investigate the acute, sub-acute, and sub-chronic oral toxicity of $HgCl_2$:

- OECD TG 425[18] up-and-down procedure: for acute oral toxicity.
- OECD TG 407 [19] for sub-acute oral toxicity (repeated dosage 28-day oral toxicity study in animals).
- OECD TG 408 [20] for sub-chronic oral toxicity.

2.6 Sample collection:

Diethyl ether was used to anaesthetize and sacrifice every group and subgroup of rats when the entire period of treatment was finished. In order to measure hematological parameters, blood samples were taken straight from heart punctures using sterile needles and placed in tubes containing EDTA.

2.7 Determination of hematological parameters:

The entire blood samples were taken in EDTA-coated vials and tested for hematological parameters. The counts of red blood cells (RBCs), white blood cells (WBCs), haemoglobin (Hb), haematocrit (HCT), and platelets (PLT) were determined using a fully automated haematological analyzer (BC-2800, Mindray, China).

2.8 Statistical analysis

The findings are displayed as the mean ± standard error of mean (S.Em.) for each group of five rats. ANOVA was used to analyse the total variation in the data set, and Tukey's test (a post hoc test) with multiple comparisons was carried out after that. The thresholds of significance were set at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ [21]. $P \leq 0.001$ was deemed statistically very significant, $P \leq 0.01$ was judged very significant, and $p \leq 0.05$ was considered significant.

3. RESULTS:

Hematological measurements revealed a highly substantial ($P<0.01$) drop in WBCs count, Hb concentration, and RBCs when compared to control group. The present study found the significant ($p < 0.01$) decreased in RBC, WBC, Hb, Platelet count and PCV (HCT) parameters of rat after acute, sub-acute and sub-chronic treatment when treated with $HgCl_2$. However, when rats were treated with $HgCl_2$ + *T. cordifolia*, the LFT parameters showed a significant decrease (p/0.05) but less than the $HgCl_2$ treated sub-groups during acute, sub-acute, and sub-chronic treatment in comparison to the control group. While only *T. cordifolia* treated rats showed significant (p/0.05) increment in blood parameters when compared to the $HgCl_2$ and $HgCl_2$ + *T. cordifolia* treated subgroups, they did not show significant changes when compared to the control groups of acute, sub-acute, and sub-chronic treatment (Tables- 2, 3, 4, 5 and 6).

Effect on haematological parameters:

Table-2: Hb (gm/dl) following acute, sub-acute and sub-chronic $HgCl_2$ and *T. cordifolia* treatment

Type of dose	Days of treatment	Type of treatment	Dose mg /kg b.wt.	Hb Mean±S.EM.	F-Value
Acute	1	Control	----------	13.4 ± 0.266	31.81
		$HgCl_2$	2.5	9.56 ± 0.426[a]	
		$HgCl_2$ + *T.c.*	2.5+400	11.76 ± 0.246[a]	
		T.c.	400	12.56 ± 0.169[ns]	

Sub-acute	7	Control	----------	13.12 ± 0.198	78.576
		$HgCl_2$	0.5	7.82 ± 0.333[a]	
		$HgCl_2$ + *T.c.*	0.5+400	9.08 ± 0.233[a]	
		T.c.	400	12.26 ± 0.345[ns]	
	14	Control	----------	13.26 ± 0.447	62.691
		$HgCl_2$	0.5	7.5 ± 0.430[a]	
		$HgCl_2$ + *T.c.*	0.5+400	8.98 ± 0.177[a]	
		T.c.	400	11.28 ± 0.208[a]	
	28	Control	----------	13.24 ± 0.354	101.048
		$HgCl_2$	0.5	6.42 ± 0.310 [a]	
		$HgCl_2$ + *T.c.*	0.5+400	9.06 ± 0.342 [a]	
		T.c.	400	12.9 ± 0.286 [ns]	
Sub-chronic	60	Control	----------	12.76 ± 0.48	62.833
		$HgCl_2$	0.25	6 ± 0.294 [a]	
		$HgCl_2$ + *T.c.*	0.25+400	9.22 ± 0.406 [a]	
		T.c.	400	12.12 ± 0.345[ns]	

Mean ± S.Em. (n=5) are used to express the values. The one-way ANOVA test is used to analyse the data, and for multiple comparisons, Tukey's post hoc test is performed next.

[a]$P < 0.01$, significant difference with the control group.

[b]$P < 0.05$, significant difference with the $HgCl_2$ group.

[ns] represent, non-significant difference with the control group.

Table-3: RBCs (Millions/mm^3) following acute, sub-acute and sub-chronic $HgCl_2$ and *T. cordifolia* treatment

Type of dose	**Days of treatment**	**Type of treatment**	**Dose mg /kg b.wt.**	**RBCs Mean±S.EM.**	**F-Value**
Acute	1	Control	----------	8.222 ± 0.206	23.373
		$HgCl_2$	2.5	4.902 ± 0.201 [a]	
		$HgCl_2$ + *T.c.*	2.5+400	6.256 ± 0.149 [a]	
		T.c.	400	7.706 ± 0.528 [ns]	
	7	Control	----------	7.57 ± 0.226	26.864
		$HgCl_2$	0.5	4.66 ± 0.273 [a]	
		$HgCl_2$ + *T.c.*	0.5+400	5.806 ± 0.187 [a]	
		T.c.	400	7.63 ± 0.386 [ns]	

Sub-acute	14	Control	----------	7.948 ± 0.262	48.625
		$HgCl_2$	0.5	4.002 ± 0.312 [a]	
		$HgCl_2$ + *T.c.*	0.5+400	6.446 ± 0.220 [a]	
		T.c.	400	7.702 ± 0.229 [ns]	
	28	Control	----------	7.982 ± 0.184	71.903
		$HgCl_2$	0.5	3.874 ± 0.131 [a]	
		$HgCl_2$ + *T.c.*	0.5+400	5.604 ± 0.198 [a]	
		T.c.	400	7.508 ± 0.326 [ns]	
Sub-chronic	60	Control	----------	7.768 ± 0.256	79.33
		$HgCl_2$	0.25	3.548 ± 0.239 [a]	
		$HgCl_2$ + *T.c.*	0.25+400	6.394 ± 0.193 [a]	
		T.c.	400	7.784 ± 0.199 [ns]	

Mean ± S.Em. (n=5) are used to express the values. The one-way ANOVA test is used to analyse the data, and for multiple comparisons, Tukey's post hoc test is performed next.

[a]$P < 0.01$, significant difference with the control group.

[b]$P < 0.05$, significant difference with the $HgCl_2$ group.

[ns] represent, non-significant difference with the control group.

Table-4: TLC ($\times 10^3/mm^3$) following acute, sub-acute and sub-chronic $HgCl_2$ and *T. cordifolia* treatment

Type of dose	Days of treatment	Type of treatment	Dose mg /kg b.wt.	TLC Mean ±S.EM.	F-Value
Acute	1	Control	----------	5.48 ± 0.165	24.448
		$HgCl_2$	2.5	3.286 ± 0.178 [a]	
		$HgCl_2$ + *T.c.*	2.5+400	4.208 ± 0.198 [a]	
		T.c.	400	5.406 ± 0.285 [ns]	
Sub-acute	7	Control	----------	5.56 ± 0.222	32.315
		$HgCl_2$	0.5	2.794 ± 0.195 [a]	
		$HgCl_2$ + *T.c.*	0.5+400	4.242 ± 0.275 [a]	
		T.c.	400	5.576 ± 0.229 [ns]	
	14	Control	----------	5.542 ± 0.224	43.557
		$HgCl_2$	0.5	2.65 ± 0.218 [a]	
		$HgCl_2$ + *T.c.*	0.5+400	4.076 ± 0.243 [a]	
		T.c.	400	5.69 ± 0.171 [ns]	

	28	Control	----------	5.704 ± 0.175	61.196
		$HgCl_2$	0.5	2.128 ± 0.185 [a]	
		$HgCl_2$ + *T.c.*	0.5+400	3.898 ± 0.341 [a]	
		T.c.	400	6.186 ± 0.205 [ns]	
Sub-chronic	60	Control	----------	5.528 ± 0.208	65.943
		$HgCl_2$	0.25	2.454 ± 0.233 [a]	
		$HgCl_2$ + *T.c.*	0.25+400	3.902 ± 0.112 [a]	
		T.c.	400	5.722 ± 0.179 [ns]	

Mean ± S.Em. (n=5) are used to express the values. The one-way ANOVA test is used to analyse the data, and for multiple comparisons, Tukey's post hoc test is performed next.

[a]$P < 0.01$, significant difference with the control group.

[b]$P < 0.05$, significant difference with the $HgCl_2$ group.

[ns] represent, non-significant difference with the control group.

Table-5: PLATELETS COUNT ($\times 10^3/mm^3$) **following acute, sub-acute and sub-chronic $HgCl_2$ and *T. cordifolia* treatment**

Type of dose	**Days of treatment**	**Type of treatment**	**Dose mg /kg b.wt.**	**PLATELETS COUNT Mean±S.EM.**	**F-Value**
Acute	1	Control	----------	501.8 ± 2.727	1585.608
		$HgCl_2$	2.5	290 ± 3.860[a]	
		$HgCl_2$ + *T.c.*	2.5+400	311.8 ± 2.267[a]	
		T.c.	400	516.4 ± 3.043[b]	
Sub-acute	7	Control	----------	498 ± 2.626	1409.696
		$HgCl_2$	0.5	284.2 ± 4.608[a]	
		$HgCl_2$ + *T.c.*	0.5+400	305.2 ± 1.743[a]	
		T.c.	400	504.2 ± 3.056[ns]	
	14	Control	----------	494.8 ± 3.120	1395.022
		$HgCl_2$	0.5	281.2 ± 3.397[a]	
		$HgCl_2$ + *T.c.*	0.5+400	293.2 ± 3.839[a]	
		T.c.	400	501.2 ± 2.557[ns]	
	28	Control	----------	493.2 ± 3.839	946.673
		$HgCl_2$	0.5	268.6 ± 4.178[a]	
		$HgCl_2$ + *T.c.*	0.5+400	291 ± 3.478[a]	
		T.c.	400	499.6 ± 4.707[ns]	

Sub-chronic	60	Control	----------	489 ± 4.301	894.058
		$HgCl_2$	0.25	260.8 ± 3.839[a]	
		$HgCl_2$ + *T.c.*	0.25+400	289.8 ± 3.397[a]	
		T.c.	400	494.2 ± 5.063[ns]	

Mean ± S.Em. (n=5) are used to express the values. The one-way ANOVA test is used to analyse the data, and for multiple comparisons, Tukey's post hoc test is performed next.

[a]P < 0.01, significant difference with the control group.

[b]P < 0.05, significant difference with the $HgCl_2$ group.

[ns] represent, non-significant difference with the control group.

Table-6: HCT (PCV) % following acute, sub-acute and sub-chronic $HgCl_2$ and *T. cordifolia* treatment

Type of dose	Days of treatment	Type of treatment	Dose mg /kg b.wt.	HCT (PCV) Mean±S.EM.	F-Value
Acute	1	Control	----------	40.536 ± 1.667	36.566
		$HgCl_2$	2.5	27.24 ± 0.486[a]	
		$HgCl_2$ + *T.c.*	2.5+400	31.348 ± 0.556[b]	
		T.c.	400	38 ± 0.853[ns]	
Sub-acute	7	Control	----------	42.952 ± 0.714	130.657
		$HgCl_2$	0.5	24.616 ± 0.497[a]	
		$HgCl_2$ + *T.c.*	0.5+400	32.208 ± 0.609[a]	
		T.c.	400	43.74 ± 1.201[ns]	
	14	Control	----------	41.28 ± 0.579	180.2
		$HgCl_2$	0.5	23.17 ± 0.578[a]	
		$HgCl_2$ + *T.c.*	0.5+400	30.49 ± 0.575[a]	
		T.c.	400	43.18 ± 0.982[ns]	
	28	Control	----------	42.18 ± 0.829	183.296
		$HgCl_2$	0.5	22.596 ± 0.638[a]	
		$HgCl_2$ + *T.c.*	0.5+400	29.4 ± 0.502[a]	
		T.c.	400	42.646 ± 0.883[ns]	
Sub-chronic	60	Control	----------	40.678 ± 0.398	229.679
		$HgCl_2$	0.25	20.488 ± 0.642[a]	
		$HgCl_2$ + *T.c.*	0.25+400	27.8 ± 0.940[a]	
		T.c.	400	44.938 ± 0.564[a]	

Mean ± S.Em. (n=5) are used to express the values. The one-way ANOVA test is used to analyse the data, and for multiple comparisons, Tukey's post hoc test is performed next.

[a]P < 0.01, significant difference with the control group.

[b]$P < 0.05$, significant difference with the $HgCl_2$ group.

[ns] represent, non-significant difference with the control group.

4. DISCUSSION:

$HgCl_2$ includes inorganic mercury, which is very toxic, corrosive, and used as a disinfectant. This type of mercury has the potential to cause severe acute poisoning. Mercury exposure can change the hematology of wistar rat blood by accumulating in many organs, notably the kidneys and liver, where it can impair the function of hematopoiesis tissues [22]. $HgCl_2$ exposure, whether acute, sub-acute, or sub-chronic, may have a deleterious influence on hematology.

RBCs (erythrocytes) are responsible for the transport of hemoglobin in general. RBCs were observed to be significantly reduced following acute, sub-acute, and sub-chronic exposure to $HgCl_2$. During breathing, hemoglobin combines with oxygen in the blood to generate oxy-hemoglobin. A reduction in both the quantity of carbon dioxide exhaled back into the lungs and the amount of oxygen carried to tissues is indicated by this drop in RBCs. Anemia may result from a high concentration of mercury in red blood cells [23]. When blood is exposed to Hg, the life duration of RBCs is reduced or their disintegration increases, causing the vast majority of blood changes. The current study indicated that a drop in RBCs may be related to a decrease in iron within erythrocytes or their hemoglobin content, which causes a decrease in the carrying capacity of oxygen by blood.

The hematological test in the current study also demonstrated a decrease in total hemoglobin levels if compared with the control group. The lysis and reduced generation of RBCs caused by decreased bone marrow formation might explain this drop, which would result in anemia and poor hemoglobin production [24]. Mercury exposure resulted in a drop in RBCs count [25]. The decrease in Hb might be attributed to the generation of reactive oxygen species (ROS) under the action of mercuric chloride [26]. The observed drop in Hb concentration might be attributed to either an increase in the rate of Hb destruction or a decrease in the rate of Hb synthesis [27].

White blood cells (WBCs), also known as leukocytes, protect the body against disease. White blood cells' principal functions are to fight infections, to protect the body from invading organisms through phagocytosis, and to manufacture or, at the very least, transport and spread antibodies in response to an immune response. Animals with low white blood cell numbers are therefore more susceptible to disease infection. The fall in WBC count in the treatment groups might be attributed to the production of adrenaline during stress, which can cause spleen constriction and a decrease in leucocyte count, both of which can impair the immune system. Mercury exposure reduced the number of leukocytes in mice [25]. The reduced quantity of WBCs (leucopenia) might be attributed to the measured metal's bio-concentration in the kidney and liver [29].

Blood platelets have a role in clotting. Low platelet concentration suggests that clot formation (blood clotting) may take a longer time, resulting in considerable blood loss in the case of an injury [30].

PCV is the percentage (%) of red blood cells in the blood, also known as erythrocyte volume fraction (EVF) or hematocrit (Hct) [31]. PCV has a function in the movement of nutrients and oxygen inside the

body [32]. Observed depression in hematocrit and hemoglobin values coupled with decreased and deformed erythrocytes are obvious signs of anemia [33]. In many investigations, decreases or increases in particular blood parameters have been linked to the characteristics of species and toxicants. The mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration represent blood level conditions. A low level is indicative of anaemia [34].

The hematological system's extreme sensitivity to harmful effects causes aberrant blood cell synthesis or blood cell inhibition [35]. The drop in hematocrit, haemoglobin, and erythrocytes that was reported is in line with earlier research [36]. Anaemia may result from erythrocyte breakdown in addition to a reduction in erythrocyte production and release in the bloodstream [37, 38]. Additionally, decreases in RBC, WBC, and PCV were documented in rats exposed to cadmium [26] and [39].The development of oxidative stress is one of the primary mechanisms causing $HgCl_2$ poisoning [40]. Exposure to $HgCl_2$ encourages the growth of reactive oxygen species (ROS), including superoxide, hydrogen peroxide (H_2O_2), and hydroxyl radicals. Free radicals are created during metabolic processes as a result of ROS formation in cells [41]. By scavenging free radicals, the *T. cordifolia* treatment reduced oxidative stress. *T. cordifolia's* phenolic content has free radical scavenging properties [42]. Previous studies reported that Mercury intoxication showed a significant decrease in hematological parameters. These Hg-induced changes in the bone marrow of those cells were accountable for a decrease in the number of blood cells. Hg accumulated in the bone rather than calcium, altering its structure and reducing its ability to synthesise blood cells [43].

In the current investigation, Tables 2, 3, 4, 5 and 6 demonstrate the ameliorative effects of *T. cordifolia* on $HgCl_2$-induced hemato-toxicity in wistar rats. $HgCl_2$ administration in rats resulted in severe hematotoxicity after acute (1 d), sub-acute (7, 14, and 28 ds), and sub-chronic (60 ds) studies, as evidenced by a very high significant decrease ($P \leq 0.001$), high significant decrease ($p < 0.01$) and significant decrease ($p < 0.05$) in the haematological parameters. Thus $HgCl_2$ exposure resulted in considerable depletion of various blood parameters, including RBCs, PCV, Hb content, WBCs and platelet count. These findings are consistent with prior findings [44]. In contrast to acute and sub-acute $HgCl_2$ exposure, the current study revealed that these haematological markers significantly more decreased during the sub-chronic (60 ds) studies. These results demonstrated the repeated dose-dependent toxicity of $HgCl_2$. As a result, $HgCl_2$ induces the development of ROS, which leads to a variety of injuries and undesirable changes in the blood parameters. The anti-oxidative activity of *T. cordifolia* can counteract or avoid the harmful effects of ROS, hence minimizing the risks.

As opposed to the $HgCl_2$-treated sub-group, the rats administered *T. cordifolia* after an hour of $HgCl_2$ intoxication exhibited a substantial improvement in the declined RBC, WBC, Hb, Platelet count and PCV parameters; however, this beneficial effect of *T. cordifolia* was more apparent throughout the sub-chronic trial as shown in Tables 2, 3, 4, 5 and 6 because in the $HgCl_2$ + *T. cordifolia* treated subgroups, the decreased level of blood parameters were dramatically increased during the sub-chronic investigation as compared to acute and sub-acute. On the other hand, oral administration of *T. cordifolia* only resulted in normal levels of RBC, WBC, Hb, Platelet count and PCV, indicating that this plant has antioxidant activity.

The hemato-protective effect of *T. cordifolia* was shown to be greater in sub-chronic studies than in sub-acute studies, and less in acute trials. Thus, hematological investigation revealed that *T. cordifolia* exhibited time-dependent protection (tables 2, 3, 4, 5 and 6). However, the *T. cordifolia* treatment ameliorated the oxidative stress as it significantly increased the blood parameters levels in $HgCl_2$ exposed rats. The anti-oxidative effects of *T. cordifolia* extracts are related to the excellent free radical scavenging activity, restoration of oxidative equilibrium, and enhancement of anti-oxidative enzyme activity. According to the findings of this investigation, *T. cordifolia* extract is a strong hemato-protective agent.

5. CONCLUSION

Mercury is a hazardous heavy metal. Hematological studies aided in tracking the body's responses to mercury stress. *T. cordifolia* has been shown to be useful in decreasing $HgCl_2$ triggered hematotoxicity. The current study investigated the rehabilitative impact of *T. cordifolia* in three sub-groups: $HgCl_2$ treated, *T. cordifolia* treated, and $HgCl_2$+ *T. cordifolia* treated, during acute (1 d), sub-acute (7, 14, and 28 ds), and sub-chronic (60 ds) $HgCl_2$ exposure. Blood parameters significantly dropped in the $HgCl_2$ treated groups, but rats treated with $HgCl_2$+ *T. cordifolia* showed a little decrease in blood parameters compared to the $HgCl_2$ treated sub-groups. *T. cordifolia*, on the other hand, preserved the blood parameter decline and kept the level of blood parameters at the normal level. Our hematological indicators show that *T. cordifolia* enhances blood marker function and boosts bio-methylation effectiveness as well as $HgCl_2$ clearance rate.

According to the current research, *T. cordifolia's* aqueous extract has potent hemato-protective properties. *T. cordifolia* stem aqueous extract efficiently managed the blood parameter values in this study. The study thus offers scientific support for the use of this plant in conventional medicine to treat disorders associated with blood parameters.

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