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Modulation of mercuric chloride nephrotoxicity in albino rat by flower extract of *Tagetes erecta*

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Abstract
Mercuric chloride, an inorganic form of mercury poses serious threat to all the living forms. In the present study, an effort has been made to assess the nephrotoxic potential of mercuric chloride against albino rats. In addition, the nephro-protective potential of *Tagetes erecta* (marigold) flower extract against mercuric chloride induced damage if any has also been assessed. Further, effort has also been extended to observe the effect if any, of introduction of *Tagetes erecta* flower extract prior or after the mercuric chloride intoxication. For this experimental, albino rats were divided firstly into four groups, then each group into five sets. Albino rats of group one received acute dose for one day only, whereas animals of group 2, 3 and 4 were administrated sub-acute doses for 7, 14 and 21 days respectively. All the doses were administrated orally. Set one of each group represented control animals and were neither administrated mercuric chloride nor *Tagetes erecta* flower extract. Albino rats of set 2 of each group were orally administrated different doses of *Tagetes erecta* flower extract as per experimental protocol. Rats corresponding to set 3 of each group were intoxicated with different doses of mercuric chloride only. Animals corresponding to the set 4, were pretreated with *Tagetes erecta* flower extract then after particular dose of mercuric chloride was given, whereas rats of set 5 of each group, post treated with *Tagetes erecta* flower extract following mercuric chloride intoxication. Acute dose for mercuric chloride came out to be 0.926mg/kg b.wt/day (1/10th of LD_{50}), whereas sub-acute for 7, 14 and 21 days as 0.132, 0.066 and 0.044 mg/kg/b.wt./day respectively. 10 mg/kg b.wt/day of *Tagetes erecta* flower extract was given to animals corresponding to acute set, whereas 1.43, 0.71 and 0.48 mg/kg b.wt for 7, 14 and 21 days (sub-acute sets). Nephrotoxicity of mercuric chloride as well as nephro-protective potential of *Tagetes erecta* flower extract has been ascertained on the basis of serum biochemical estimation of four electrolytes namely sodium, potassium, calcium and chloride, serum total protein content, albumin, globulin, A/G ratio as well as serum creatinine, urea, uric acid and Blood urea nitrogen. Among the selected parameters, sodium, chloride, calcium, total proteins, albumin and globulin were found to decrease in the serum of mercuric intoxicated rats, whereas potassium, urea, uric acid and BUN were increased. However, those supplemented with pre or post *Tagetes eretca* flower extract showed reduced deviation from the normal values.

Keywords: Mercuric chloride, *Tagetes erecta*, albino rats, serum, electrolytes, protein, urea

1. Introduction
Mercury is a naturally occurring metal of universal existence. However, it is also one of the hazardous heavy metal placed third in hierarchy among toxic heavy metals after arsenic and lead, thus provides a serious threat to the life of human and other living forms residing on this planet (Agha *et al*., 2014; Apaydin *et al*., 2016; Jalili *et al*., 2019) [1, 7, 14].
Mercury is present in the nature in numerous forms such as elemental mercury, inorganic mercury and organic mercury. Natural exposure of mercury occurs mainly through volcanic eruptions, gas exposures from rocks, etc., whereas anthropogenic exposure sources primarily includes rapid industrialisation, thermal plants, related products and wastes indiscriminately thrown here and there. The mercury or its compounds can find access inside the human body or within the body of any other organism either through direct exposure i.e. oral, respiratory and dermal route or through bio-magnification and thus a part of food chain (Sharma *et al*., 2007; Necib *et al*., 2013; Jha *et al*., 2019; Ajibade *et al*., 2019) [25, 19, 15, 2].
Mercuric chloride, an inorganic form of mercury is a white crystalline sublimate, highly toxic, present in variety of consumer products such as insecticides, batteries, antiseptic, preservative, photographic fixative, etc. Once mercuric chloride is introduced into the body of an organism, it initially is distributed to the liver through the blood, however its highest level is generally reported inside the kidney (Saxena *et al*., 2006; Agha *et al*., 2014, Saxena *et al*., 2017; Ajibade *et al*., 2019; Jalili *et al*., 2019) [23, 1, 22, 2, 14].
It is with the reason that nephrotoxicity assessment of mercuric chloride has been selected in the present study through estimation of the levels of some electrolytes namely Calcium,

Sodium, Potassium and Chloride in the serum, macromolecules namely serum total proteins, albumin, globulin, A/G ration. In addition to this, basic kidney functional tests namely Blood Urea Nitrogen, Serum Creatinine, Uric acid and Urea has been selected for estimation.

Use of plants and plant products in clinical practices is as old as human civilizations. These products in one way or the other have been found to cure various health related complications of not only human beings but also other living forms. *Tagetes erecta* has been an established neuro-protective plant (Saxena *et al.,* 2008; Sheety *et al.,* 2015; Saxena *et al.,* 2017; Mudumbi *et al.,* 2019) [24, 26, 22].

Therefore flower extract of *Tagetes erecta* has been selected as a test compound to check its modulating effect against mercuric chloride induced nephrotoxicity if any, on the basis of same set of parameters (*vide supra*).

2. Materials and Methods

2.1 Experimental animal-rearing and maintenance

The present study was conducted on one hundred female albino rats, *Rattus norvegicus* (Berkenhout), weighing 110 ± 20 gm, eight weeks old, selected from an inbred colony. These experimental albino rats were provided standard rat pellet feed and water *ad libitum*, acclimatised to laboratory conditions for two weeks prior to administration of dose. During the entire experimentation these animals were kept under standard conditions of light, temperature and humidity.

2.2 Preparation of *Tagetes erecta* flower extract

Tagetes erecta flowers were collected from the local Agra (India) region, shade dried for seven more days. This plant material was further subjected to grinded as to make a coarse powder. Then this powder was extracted in methanol solvent for twenty two continuous cycles using Soxhlet apparatus. The plant extract so obtained was concentrated by rotator evaporator under optimum temperature and pressure conditions. The flower extract of *Tagetes erecta* was given on the basis of safety trials (Saxena *et al.,* 2017) [22].

2.3 Experimental compound

Technical grade of Mercuric chloride (purity 95%) was obtained from Sigma chemicals Ltd., Mumbai. LD_{50} of mercuric chloride following oral intoxication in albino rats came out to be 9.26 mg/kg b.wt. (Finey, 1971; Saxena *et al.*, 2008; Saxena *et al.,* 2017) [12, 24, 22]. It was orally administrated, in various doses, dissolved with water as per experimental protocol.

2.4 Experimental protocol

All the experimental albino rats, were randomly divided into four groups containing twenty five rats, each. First group was considered for conducting acute study (1day) and rest three represented sub-acute (7, 14, 21 days) groups. Further, all of these four groups were divided into five sets containing 5 rats each (Table 1-12). First set of each the four groups represented control animals which were never intoxicated with any chemical nor given plant extract. Different doses of *Tagetes erecta* flower extract and mercuric chloride, either singly or in combination as per experimental protocol was orally administrated to rest of the experimental animals. Animals corresponding to the second set of each group were given only plant extract; 10mg/kg b.wt/day as acute dose for one day and 1.43, 0.71 and 0.48 mg/kg b.wt/day for 7, 14 and 21 days respectively. Animals corresponding to the third set of each group were orally administrated mercuric chloride only 1/10 of LD_{50} i.e. 0.926mg/kg b.wt/day as acute dose for one day and 0.132, 0.066 and 0.044 mg/kg b.wt/day for 7, 14 and 21 days respectively. Rats corresponding to set 4 of each group were given same dose of plant extract prior to administration of same amount of mercuric chloride as set 2 and 3, whereas reversal of set 4 in set 5. The rearing of animals as well as experimentation was approved by the Ethics Committee of Department of Zoology, Dr. B.R. Ambedkar University, Agra (U.P.) India.

2.5 Collection of blood and separation of serum

The albino rats were sacrificed at predetermined time intervals and blood samples were collected from the ventricle of the heart with the help of hypodermic needle. The blood so collected was immediately stored in sterilised properly labelled centrifuge tubes, allowed to stand for about three minutes to clot. Then after this blood sample was centrifuged at 3000 rpm for 15 minutes and serum was separated by fine rubber bulb pipette.

2.6 Estimation of serum electrolytes

Biochemical levels of electrolytes in the serum *viz*. Sodium, potassium, calcium and chloride were estimated after Trinder (1957), Terri (1958) [30], Tietz (1986) [29] and Schoenfed (1964) respectively.

2.7 Serum total proteins, albumin, globulin and A/G ration

Biochemical estimation of serum total protein, albumin was done after Henry *et al.*,(1974) [13] and Dumas *et al.,* (1971) [11] respectively, whereas Serum total globulin was measured by subtracting the so obtained numerical value(*vide supra*) of serum total albumin from serum total protein. Similarly A/G ratio was obtained by dividing the numerical value of serum albumin by serum globulin.

2.8 Estimation of serum Uric acid, Urea, Creatinine and Blood urea nitrogen

Biochemical estimation of serum Uric acid was performed as per Berthelot method after Crouch (1977) [21], Urea by phospho-tungstate method described by Martinck (1970), Creatinine by LK line picrate method by Toro and Ackermann (1975) [31] and Blood urea nitrogen was done after Talke and Schubert (1965) [28].

2.9 Statistical analysis

Data obtained from biochemical estimations was statistically calculated and analysed for difference in means by ANOVA followed by Dunnet‘t’ test for ‘p’ value of experimental sets by software SPSS 11.5 for windows.

3. Results

3.1 Serum electrolytes

Biochemical levels in serum, of the three out of four electrolytes considered in the present study were found to decrease significantly. These electrolytes were Sodium, Chloride and Calcium. The significance level though was significant at nearly all the dose levels but still more severe following acute mercuric chloride intoxication. In all these cases, both pre and post *Tagetes erecta* flower extract treatment has been found to modulate the mercuric chloride induced nephrotoxicity by bringing the levels of these electrolytes nearly to normal limits or comparatively up to difference of less significance (Table 1-3).

However, the value of Potassium in serum of the mercuric chloride intoxicated rats, has been found to be significantly more than control rats, still intensity maximum in group of rats receiving acute dose of mercuric chloride. Here also, *Tagetes erecta* flower extract pre and post mercuric chloride treatment has been found to either normalise the level of potassium in serum or reduce the difference of significance (Table-4)

Table 1: Serum chloride (mlmol/l) in *Rattus norvegicus* after acute(1d) and sub-acute (7, 14 and 21 ds) treatment with *Tagetes erecta* flower extract, mercuric chloride, mercuric chloride followed by *Tagetes erecta* flower extract and *Tagetes erecta* flower extract followed by mercuric chloride

Type of dose	Days of treatment	Type of treatment	Dose/mg/kg/day	Serum Na^+	Dunnett's multiple comparison test	F-value
Acute	1d	Set-1	-	130.68±0.52	-	13.65***
		Set-2	10	131.07±0.31	0.64	
		Set-3	0.926	126.75±0.71**	4.48	
		Set-4	10+0.926	128.62±0.35*	2.37	
		Set-5	0.926+10	128.88±0.34*	2.71	
Sub-acute	7ds	Set-1	-	130.68±0.52	-	6.94**
		Set-2	1.43	131.14±0.18	0.83	
		Set-3	0.132	127.88±0.68*	3.26	
		Set-4	1.43+0.132	129.92±0.41*	2.56	
		Set-5	0.132+1.43	130.06±0.29	2.94	
	14ds	Set-1	-	130.68±0.52	-	5.66**
		Set-2	0.71	130.84±0.13	0.29	
		Set-3	0.066	128.48±0.46*	3.16	
		Set-4	0.71+0.066	129.92±0.33	2.53	
		Set-5	0.066+0.71	130.00±0.42	2.42	
	21ds	Set-1	-	130.68±0.52		3.87*
		Set-2	0.48	130.86±0.34	0.28	
		Set-3	0.044	128.80±0.50*	2.60	
		Set-4	0.48+0.044	130.31±0.37	2.42	
		Set-5	0.044+0.48	130.45±0.40	2.57	

p>0.005: Non-significant
p< 0.05: Significant
p< 0.01: Highly significant
p< 0.001: Very highly significant

Set-1: Control
Set-2: *T. erecta* flower extract
Set-3: Mercuric chloride
Set-4: *T. erecta* flower extract+ Mercuric chloride
Set-5: Mercuric chloride+ *T. erecta* flower extract

Table 2: Serum chloride (mlmol/l) in *Rattus norvegicus* after acute(1d) and sub-acute (7, 14 and 21 ds) treatment with *Tagetes erecta* flower extract, mercuric chloride, mercuric chloride followed by *Tagetes erecta* flower extract and *Tagetes erecta* flower extract followed by mercuric chloride

Type of dose	Days of treatment	Type of treatment	Dose/mg/kg/day	Serum Cl^-	Dunnett's multiple comparison test	F-value
Acute	1d	Set-1	-	99.52±0.96		7.24***
		Set-2	10	99.89±0.82	0.09	
		Set-3	0.926	94.51±0.32***	4.05	
		Set-4	10+0.926	97.13±0.69**	2.33	
		Set-5	0.926+10	97.08±0.78**	2.53	
Sub-acute	7ds	Set-1	-	99.61±0.93		4.03**
		Set-2	1.43	99.79±0.86	0.05	
		Set-3	0.132	95.62±0.83**	3.08	
		Set-4	1.43+0.132	98.17±0.72*	2.35	
		Set-5	0.132+1.43	98.21±0.73*	2.36	
	14ds	Set-1	-	99.65±0.99		3.48*
		Set-2	0.71	99.83±1.09	0.05	
		Set-3	0.066	95.82±0.99**	2.68	
		Set-4	0.71+0.066	98.62±0.49*	2.54	
		Set-5	0.066+0.71	98.95±0.57	2.74	
	21ds	Set-1	-	99.89±1.01		3.59*
		Set-2	0.48	100.19±0.88	0.28	
		Set-3	0.044	96.33±0.84**	2.59	
		Set-4	0.48+0.044	98.43±0.44*	2.22	
		Set-5	0.044+0.48	98.84±0.62	2.49	

p>0.005: Non-significant
p< 0.05: Significant
p< 0.01: Highly significant
p< 0.001: Very highly significant

Set-1: Control
Set-2: *T. erecta* flower extract
Set-3: Mercuric chloride
Set-4: *T. erecta* flower extract+ Mercuric chloride
Set-5: Mercuric chloride+ *T. erecta* flower extract

Table 3: Serum potassium (mg/dl) in *Rattus norvegicus* after acute(1d) and sub-acute (7, 14 and 21 ds) treatment with *Tagetes erecta* flower extract, mercuric chloride, mercuric chloride followed by *Tagetes erecta* flower extract and *Tagetes erecta* flower extract followed by mercuric chloride

Type of dose	Days of treatment	Type of treatment	Dose/mg/kg/day	Serum K^+	Dunnett's multiple comparison test	F-value
Acute	1d	Set-1	-	5.54±0.36		2.19
		Set-2	10	5.63±0.39	0.18	
		Set-3	0.926	6.95±0.36**	2.78	
		Set-4	10+0.926	5.97±0.49*	2.57	
		Set-5	0.926+10	5.67±0.36*	2.32	
Sub-acute	7ds	Set-1	-	5.54±0.41		1.36
		Set-2	1.43	5.62±0.31	0.17	
		Set-3	0.132	6.75±0.34**	2.47	
		Set-4	1.43+0.132	6.06±0.51**	2.35	
		Set-5	0.132+1.43	5.89±0.52	1.66	
	14ds	Set-1	-	5.64±0.36		1.38
		Set-2	0.71	5.58±0.49	0.07	
		Set-3	0.066	6.70±0.35**	2.32	
		Set-4	0.71+0.066	6.11±0.46**	2.46	
		Set-5	0.066+0.71	5.83±0.35	2.31	
	21ds	Set-1	-	5.58±0.34		1.89
		Set-2	0.48	5.98±0.31	0.75	
		Set-3	0.044	6.88±0.39**	2.54	
		Set-4	0.48+0.044	5.97±0.40*	2.65	
		Set-5	0.044+0.48	5.75±0.41	1.99	

p>0.005: Non-significant
p< 0.05: Significant
p< 0.01: Highly significant
p< 0.001: Very highly significant

Set-1: Control
Set-2: *T. erecta* flower extract
Set-3: Mercuric chloride
Set-4: *T. erecta* flower extract+ Mercuric chloride
Set-5: Mercuric chloride+ *T. erecta* flower extract

Table 4: Serum calcium (mg/dl) in *Rattus norvegicus* after acute(1d) and sub-acute (7, 14 and 21 ds) treatment with *Tagetes erecta* flower extract, mercuric chloride, mercuric chloride followed by *Tagetes erecta* flower extract and *Tagetes erecta* flower extract followed by mercuric chloride

Type of dose	Days of treatment	Type of treatment	Dose/mg/kg/day	Serum Ca^{2+}	Dunnett's multiple comparison test	F-value
Acute	1d	Set-1	-	8.80±0.11		16.53***
		Set-2	10	8.88±0.11	0.49	
		Set-3	0.926	6.58±0.44***	4.86	
		Set-4	10+0.926	8.58±0.17*	4.21	
		Set-5	0.926+10	8.48±0.16*	4.02	
Sub-acute	7ds	Set-1	-	8.25±0.13		13.42***
		Set-2	1.43	8.93±0.09	0.86	
		Set-3	0.132	6.98±0.41**	4.30	
		Set-4	1.43+0.132	8.63±0.14*	3.82	
		Set-5	0.132+1.43	8.43±0.18*	3.31	
	14ds	Set-1	-	8.98±0.16		7.43***
		Set-2	0.71	8.84±0.11	0.23	
		Set-3	0.066	7.29±0.44**	3.31	
		Set-4	0.71+0.066	8.55±0.13	2.73	
		Set-5	0.066+0.71	8.34±0.18*	2.20	
	21ds	Set-1	-	8.98±0.14		5.72**
		Set-2	0.48	9.04±0.13	1.33	
		Set-3	0.044	7.32±0.56*	2.59	
		Set-4	0.48+0.044	8.39±0.14	2.43	
		Set-5	0.044+0.48	8.25±0.13*	1.62	

p>0.005: Non-significant
p< 0.05: Significant
p< 0.01: Highly significant
p< 0.001: Very highly significant

Set-1: Control
Set-2: *T. erecta* flower extract
Set-3: Mercuric chloride
Set-4: *T. erecta* flower extract+ Mercuric chloride
Set-5: Mercuric chloride+ *T. erecta* flower extract

3.2 Serum protein profile

Serum total, albumin and globulin has been found to decrease significantly in rats intoxicated with mercuric chloride, however A/G showed increase only after acute intoxication of mercuric chloride. *Tagetes erecta* flower extract pre and post mercuric chloride treatment has been found to have comparatively less significant level of differences (Table 5-8).

Table 5: Serum total proteins (g/dl) in *Rattus norvegicus* after acute (1d) and sub-acute (7, 14 and 21 ds) treatment with *Tagetes erecta* flower extract, mercuric chloride, mercuric chloride followed by *Tagetes erecta* flower extract and *Tagetes erecta* flower extract followed by mercuric chloride

Type of dose	Days of treatment	Type of treatment	Dose/mg/kg/day	Serum total proteins (g/dl)	Dunnett's multiple comparison test	F-value
Acute	1d	Set-1	-	4.60±0.24		10.45**
		Set-2	10	4.76±0.26	0.44	
		Set-3	0.926	2.74±0.36***	4.27	
		Set-4	10+0.926	4.38±0.13*	4.23	
		Set-5	0.926+10	4.33±0.19*	3.85	
Sub-acute	7ds	Set-1	-	4.69±0.26		5.66**
		Set-2	1.43	4.73±0.10	0.13	
		Set-3	0.132	3.26±0.34**	3.32	
		Set-4	1.43+0.132	4.39±0.30	2.46	
		Set-5	0.132+1.43	4.24±0.20*	2.46	
	14ds	Set-1	-	4.71±0.37		3.03*
		Set-2	0.71	4.71±0.42	1.60	
		Set-3	0.066	3.42±0.27**	2.84	
		Set-4	0.71+0.066	4.41±0.21	2.92	
		Set-5	0.066+0.71	4.26±0.19*	2.52	
	21ds	Set-1	-	4.76±0.32		5.41**
		Set-2	0.48	4.87±0.27	0.27	
		Set-3	0.044	3.42±0.23**	3.42	
		Set-4	0.48+0.044	4.46±0.18	3.52	
		Set-5	0.044+0.48	4.28±0.21*	2.72	

p>0.005: Non-significant
p< 0.05: Significant
p< 0.01: Highly significant
p< 0.001: Very highly significant

Set-1: Control
Set-2: *T. erecta* flower extract
Set-3: Mercuric chloride
Set-4: *T. erecta* flower extract+ Mercuric chloride
Set-5: Mercuric chloride+ *T. erecta* flower extract

Table 6: Serum albumin (g/dl) in *Rattus norvegicus* after acute(1d) and sub-acute (7, 14 and 21 ds) treatment with *Tagetes erecta* flower extract, mercuric chloride, mercuric chloride followed by *Tagetes erecta* flower extract and *Tagetes erecta* flower extract followed by mercuric chloride

Type of dose	Days of treatment	Type of treatment	Dose/mg/kg/day	Serum Albumin	Dunnett's multiple comparison test	F-value
Acute	1d	Set-1	-	2.87±0.14		8.15***
		Set-2	10	2.93±3.12	4.36	
		Set-3	0.926	1.94±0.16**	0.33	
		Set-4	10+0.926	2.61±0.16	2.94	
		Set-5	0.926+10	2.50±0.098*	2.97	
Sub-acute	7ds	Set-1	-	2.95±0.12		4.48***
		Set-2	1.43	3.09±0.21	3.30	
		Set-3	0.132	2.13±0.17**	0.43	
		Set-4	1.43+0.132	2.69±0.13	2.52	
		Set-5	0.132+1.43	2.60±0.29	0.88	
	14ds	Set-1	-	3.07±0.13		3.98*
		Set-2	0.71	3.11±0.09	3.03	
		Set-3	0.066	2.48±0.15**	0.22	
		Set-4	0.71+0.066	2.73±0.14	1.52	
		Set-5	0.066+0.71	2.63±0.17	0.69	
	21ds	Set-1	-	3.16±0.12		3.58*
		Set-2	0.48	3.20±0.24	3.63	
		Set-3	0.044	2.25±0.14**	0.14	
		Set-4	0.48+0.044	2.79±0.11*	4.91	
		Set-5	0.044+0.48	2.69±0.28*	2.75	

p>0.005: Non-significant
p< 0.05: Significant
p< 0.01: Highly significant
p< 0.001: Very highly significant

Set-1: Control
Set-2: *T. erecta* flower extract
Set-3: Mercuric chloride
Set-4: *T. erecta* flower extract+ Mercuric chloride
Set-5: Mercuric chloride+ *T. erecta* flower extract

Table 7: Serum globulin (g/dl) in *Rattus norvegicus* after acute(1d) and sub-acute (7, 14 and 21 ds) treatment with *Tagetes erecta* flower extract, mercuric chloride, mercuric chloride followed by *Tagetes erecta* flower extract and *Tagetes erecta* flower extract followed by mercuric chloride

Type of dose	Days of treatment	Type of treatment	Dose/mg/kg/day	Serum Globulin	Dunnett's multiple comparison test	F-value
Acute	1d	Set-1	-	1.74±0.22		4.96**
		Set-2	10	1.84±0.18	0.34	
		Set-3	0.926	0.79±0.26**	2.64	
		Set-4	10+0.926	1.76±0.12	3.18	
		Set-5	0.926+10	1.83±0.17	3.15	
Sub-acute	7ds	Set-1	-	1.70±0.26		0.59

		Set-2	1.43	1.63±0.19	0.20	
		Set-3	0.132	1.12±0.35*	1.32	
		Set-4	1.43+0.132	1.46±0.31	0.71	
		Set-5	0.132+1.43	1.64±0.39	0.99	
	14ds	Set-1	-	1.64±0.34		
		Set-2	0.71	1.96±0.44	0.56	
		Set-3	0.066	0.94±0.39*	1.37	1.51
		Set-4	0.71+0.066	1.68±0.29	1.66	
		Set-5	0.066+0.71	1.62±0.18	1.77	
	21ds	Set-1	-	1.60±0.37		
		Set-2	0.48	1.67±0.35	0.13	
		Set-3	0.044	1.17±0.16	1.06	0.59
		Set-4	0.48+0.044	1.46±0.33	2.34	
		Set-5	0.044+0.48	1.44±0.23	0.96	

p>0.005: Non-significant
p< 0.05: Significant
p< 0.01: Highly significant
p< 0.001: Very highly significant

Set-1: Control
Set-2: *T. erecta* flower extract
Set-3: Mercuric chloride
Set-4: *T. erecta* flower extract+ Mercuric chloride
Set-5: Mercuric chloride+ *T. erecta* flower extract

Table 8: Serum A/G ratio in *Rattus norvegicus* after acute(1d) and sub-acute (7, 14 and 21 ds) treatment with *Tagetes erecta* flower extract, mercuric chloride, mercuric chloride followed by *Tagetes erecta* flower extract and *Tagetes erecta* flower extract followed by mercuric chloride

Type of dose	Days of treatment	Type of treatment	Dose/mg/kg/day	Serum A/G ratio	Dunnett's multiple comparison test	F-value
Acute	1d	Set-1	-	1.78±0.27		
		Set-2	10	1.66±0.16	0.37	
		Set-3	0.926	3.34±0.74**	2.00	4.65**
		Set-4	10+0.926	2.51±0.17*	2.59	
		Set-5	0.926+10	2.41±0.13*	2.43	
Sub-acute	7ds	Set-1	-	1.98±0.37		
		Set-2	1.43	1.79±0.13	0.39	
		Set-3	0.132	4.53±2.07**	1.21	1.03
		Set-4	1.43+0.132	2.21±0.45	0.40	
		Set-5	0.132+1.43	2.87±1.09*	0.71	
	14ds	Set-1	-	2.46±0.67		
		Set-2	0.71	2.25±1.03	0.167	
		Set-3	0.066	3.24±2.04	1.29	1.68
		Set-4	0.71+0.066	2.01±0.58	1.69	
		Set-5	0.066+0.71	1.73±0.28	1.52	
	21ds	Set-1		2.66±0.54		
		Set-2	0.48	2.49±1.08	0.12	
		Set-3	0.044	2.09±0.33	0.63	0.26
		Set-4	0.48+0.044	2.44±0.79	0.41	
		Set-5	0.044+0.48	2.19±0.84	0.99	

p>0.005: Non-significant
p< 0.05: Significant (*)
p< 0.01: Highly significant (**)
p< 0.001: Very highly significant (***)

Set-1: Control
Set-2: *T. erecta* flower extract
Set-3: Mercuric chloride
Set-4: *T. erecta* flower extract+ Mercuric chloride
Set-5: Mercuric chloride+ *T. erecta* flower extract

3.3 Kidney function tests

The values of entire selected kidney marker tests *viz*. Serum Urea, Uric acid, Creatinine, Blood Urea nitrogen (BUN) has shown an increase following mercuric chloride intoxication. However, the values of these parameters in the serum of *Tagetes erecta* flower extract pre and post mercuric chloride treatment groups have been found to be comparatively less altered (Table 9-12).

Table 9: Serum Urea (mg/dl) in *Rattus norvegicus* after acute (1d) and sub-acute (7, 14 and 21 ds) treatment with *Tagetes erecta* flower extract, mercuric chloride, mercuric chloride followed by *Tagetes erecta* flower extract and *Tagetes erecta* flower extract followed by mercuric chloride

Type of dose	Days of treatment	Type of treatment	Dose/mg/kg/day	Serum Urea	Dunnett's multiple comparison test	F-value
Acute	1d	Set-1	-	15.99±0.05		
		Set-2	10	15.80±0.15	1.18	
		Set-3	0.926	18.37±0.27**	3.41	12.81**
		Set-4	10+0.926	16.61±0.31	6.14	
		Set-5	0.926+10	16.34±0.19*	4.28	
Sub-acute	7ds	Set-1	-	15.46±0.29		
		Set-2	1.43	15.95±0.19	1.44	
		Set-3	0.132	17.94±0.29**	5.51	8.38**
		Set-4	1.43+0.132	15.93±0.21	4.54	
		Set-5	0.132+1.43	16.05±0.12*	4.34	
	14ds	Set-1	-	15.86±0.20		14.88**

		Set-2	0.71	15.77±0.32	0.24	
		Set-3	0.066	18.82±0.31**	7.96	
		Set-4	0.71+0.066	16.08±0.16	7.84	
		Set-5	0.066+0.71	16.28±0.18*	7.01	
	21ds	Set-1	-	16.47±0.17		
		Set-2	0.48	16.33±0.18	0.56	
		Set-3	0.044	19.14±0.22**	9.5	16.41**
		Set-4	0.48+0.044	16.66±0.25	7.39	
		Set-5	0.044+0.48	17.21±0.14*	7.25	

p>0.005: Non-significant
p< 0.05: Significant (*)
p< 0.01: Highly significant (**)
p< 0.001: Very highly significant (***)

Set-1: Control
Set-2: *T. erecta* flower extract
Set-3: Mercuric chloride
Set-4: *T. erecta* flower extract+ Mercuric chloride
Set-5: Mercuric chloride+ *T. erecta* flower extract

Table 10: Serum Uric acid (mg/100ml) in *Rattus norvegicus* after acute(1d) and sub-acute (7, 14 and 21 ds) treatment with *Tagetes erecta* flower extract, mercuric chloride, mercuric chloride followed by *Tagetes erecta* flower extract and *Tagetes erecta* flower extract followed by mercuric chloride

Type of dose	Days of treatment	Type of treatment	Dose/mg/kg/day	Serum Uric acid	Dunnett's multiple comparison test	F-value
Acute	1d	Set-1	-	1.63±0.005		
		Set-2	10	1.61±0.02	0.95	
		Set-3	0.926	3.30±0.17***	9.92	95.24***
		Set-4	10+0.926	1.71±0.04	10.38	
		Set-5	0.926+10	1.73±0.07*	9.62	
Sub-acute	7ds	Set-1	-	1.72±0.018		
		Set-2	1.43	1.73±0.01	0.75	
		Set-3	0.132	4.15±0.19***	12.54	146.98***
		Set-4	1.43+0.132	1.75±0.02	12.39	
		Set-5	0.132+1.43	1.88±0.04*	12.07	
	14ds	Set-1	-	1.75±0.019		
		Set-2	0.71	1.70±0.008	2.28	
		Set-3	0.066	4.38±0.21***	12.21	134.96***
		Set-4	0.71+0.066	1.79±0.01	12.04	
		Set-5	0.066+0.71	1.88±0.06*	11.21	
	21ds	Set-1	-	1.79±1.86		
		Set-2	0.48	1.75±0.03	1.37	
		Set-3	0.044	4.78±0.18***	16.51	260.61***
		Set-4	0.48+0.044	1.82±0.01	16.37	
		Set-5	0.044+0.48	1.50±0.02	16.17	

p>0.005: Non-significant
p< 0.05: Significant (*)
p< 0.01: Highly significant (**)
p< 0.001: Very highly significant (***)

Set-1: Control
Set-2: *T. erecta* flower extract
Set-3: Mercuric chloride
Set-4: *T. erecta* flower extract+ Mercuric chloride
Set-5: Mercuric chloride+ *T. erecta* flower extract

Table 11: Serum creatinine (mg/dl) in *Rattus norvegicus* after acute (1d) and sub-acute (7, 14 and 21 ds) treatment with *Tagetes erecta* flower extract, mercuric chloride, mercuric chloride followed by *Tagetes erecta* flower extract and *Tagetes erecta* flower extract followed by mercuric chloride

Type of dose	Days of treatment	Type of treatment	Dose/mg/kg/day	Serum Creatinine	Dunnett's multiple comparison test	F-value
Acute	1d	Set-1	-	0.33±0.44		
		Set-2	10	0.31±0.03	0.34	
		Set-3	0.926	2.37±0.09***	19.74	89.48***
		Set-4	10+0.926	0.44±0.12*	12.77	
		Set-5	0.926+10	0.46±0.14*	11.33	
Sub-acute	7ds	Set-1	-	0.38±0.09		
		Set-2	1.43	0.30±0.09	0.54	
		Set-3	0.132	2.33±0.11***	13.74	65.28***
		Set-4	1.43+0.132	0.42±0.12	12.55	
		Set-5	0.132+1.43	0.65±0.15*	8.79	
	14ds	Set-1	-	0.47±0.07		
		Set-2	0.71	0.42±0.94	0.41	
		Set-3	0.066	2.23±0.65***	18.08	101.71***
		Set-4	0.71+0.066	0.56±0.10	13.98	
		Set-5	0.066+0.71	0.59±0.11*	12.76	
	21ds	Set-1	-	0.34±0.13		
		Set-2	0.48	0.39±0.59	0.08	128.37***
		Set-3	0.044	2.34±0.13***	14.73	

		Set-4	0.48+0.044	0.48±0.60	13.32	
		Set-5	0.044+0.48	0.51±0.06*	13.14	

p>0.005: Non-significant
p< 0.05: Significant
p< 0.01: Highly significant
p< 0.001: Very highly significant

Set-1: Control
Set-2: *T. erecta* flower extract
Set-3: Mercuric chloride
Set-4: *T. erecta* flower extract+ Mercuric chloride
Set-5: Mercuric chloride+ *T. erecta* flower extract

Table 12: Blood urea nitrogen (mg/dl) in *Rattus norvegicus* after acute (1d) and sub-acute (7, 14 and 21 ds) treatment with *Tagetes erecta* flower extract, mercuric chloride, mercuric chloride followed by *Tagetes erecta* flower extract and *Tagetes erecta* flower extract followed by mercuric chloride

Type of dose	Days of treatment	Type of treatment	Dose/mg/kg/day	Blood urea nitrogen	Dunnett's multiple comparison test	F-value
Acute	1d	Set-1	-	9.61±0.40		5.19**
		Set-2	10	9.59±0.39	0.03	
		Set-3	0.926	12.35±0.37**	4.98	
		Set-4	10+0.926	9.73±0.54	3.64	
		Set-5	0.926+10	9.97±0.38*	3.33	
Sub-acute	7ds	Set-1	-	9.61±0.41		6.37**
		Set-2	1.43	9.49±0.44	0.21	
		Set-3	0.132	11.89±0.46**	3.67	
		Set-4	1.43+0.132	9.73±0.37	3.72	
		Set-5	0.132+1.43	10.39±0.25*	4.44	
	14ds	Set-1	-	9.80±0.47		6.27**
		Set-2	0.71	9.77±0.39	0.05	
		Set-3	0.066	12.22±0.36**	4.10	
		Set-4	0.71+0.066	10.03±0.57*	4.05	
		Set-5	0.066+0.71	10.34±0.29*	3.26	
	21ds	Set-1	-	10.18±0.40		6.94**
		Set-2	0.48	9.52±0.32	1.23	
		Set-3	0.044	12.35±0.37**	3.94	
		Set-4	0.48+0.044	10.34±0.49*	3.49	
		Set-5	0.044+0.48	10.46±0.40*	3.24	

p>0.005: Non-significant
p< 0.05: Significant (*)
p< 0.01: Highly significant (**)
p< 0.001: Very highly significant (***)

Set-1: Control
Set-2: *T. erecta* flower extract
Set-3: Mercuric chloride
Set-4: *T. erecta* flower extract+ Mercuric chloride
Set-5: Mercuric chloride+ *T. erecta* flower extract

4. Discussion

Kidneys are the primary sites of accumulation of mercuric chloride following intoxication through any route, thus very prone to damage. Kidneys play an important role in the maintenance of homeostasis in body fluids of various organisms. Homeostasis is mainly achieved through keeping equilibrium between various electrolytes, macromolecules, etc. in the body fluids. Electrolytes and salts are active components of body cells and fluids including blood. Within the body cells, electrolytes regulate the electrical potential of cell membranes and up to the conduction of nerve impulses. Any chemical capable of causing nephrotoxicity can thus, easily disturb the equilibrium of body fluids (Alam *et al.*, 2007; Augusti *et al.*, 2007; Sxaena *et al*., 2006; Saxena *et al*., 2017) [3, 8, 23, 22].

Potassium, sodium, chloride and calcium are among the vital electrolytes present within the cell or extra-cellular fluid. All these four have to be in proper balance to assure normal cellular metabolic and neuromuscular functioning. The imbalance of three of these ions namely sodium, chloride and calcium with respect to potassium is believed to be a major factor in several serious ailments (Mahour and Saxena, 2008, Singhal, 2011; Anand and Saxena, 2015) [17, 24, 27, 6].

Sodium and its associated anion (mainly chloride) account for more than 90% of the solute in the extracellular fluid. Hyponatremia and hypochloremia as found in the present study may be a consequence of interference of mercuric chloride and its biotransformation products as well as reactive oxygen species (ROS) formed with the cellular proteins. The membrane proteins so disturbed may mainly be associated to the juxtaglomerular apparatus of the nephron, leading to the loss of sodium and chloride ions from the extracellular fluid through depletion or haemodilution. Further, mercuric chloride may have impairs the ability of kidneys to reabsorb and disturbs the rennin-angiotensin aldosterone system which results in various fluid volume and electrolytes disturbances (Mahour and Saxena, 2008; Anand and Saxena, 2015) [17, 6].

Potassium is also a very important electrolyte present in the mammalian body as it helps in maintenance of homeostasis, muscle contractions and propagation of nerve signals. Additionally, potassium is helpful in reducing blood pressure, water retention, and protection against stroke, prevent osteoporosis and kidney stones. Hyperkalemia in the mercuric chloride intoxicated rats can be attributed to the damaged glomeruli. Due to the interference of mercuric chloride, its bio transformed products and ROS must have damaged the cellular components of glomerulus, resulting in decreased glomerular filtrate rate (GFR), thus rise in level of potassium ions in serum of such animals. The decrease in serum calcium level following mercuric chloride intoxication could have been due to impairment of kidney function as well as that of inhibition in secretion of parathyroid hormone. Intoxication of mercuric chloride must have inhibited bone resorption of and activation of vitamin D, resulting in decreased intestinal reabsorption of calcium. This might have finally leaded to reduction in renal tubular calcium reabsorption thus

responsible for this decrease in serum calcium level (Saxena and Mahour, 2006; Mahour and Saxena, 2008; Anand and Saxena, 2015; Saxena *et al.*, 2017) [23, 17, 22].

Proteins are large, complex variously shaped macromolecules, composed of amino acids as the basic monomer unit. Within the body of an organism, these macromolecules play various vital functions related to the functionality and integrity of cell, as are structural and functional components of membranes, transport systems, chomosomes, enzymes and catalytic activities, etc. Any disturbance to the level of this macromolecule or its component can be an indicator of serious complication at cellular, organ or complete body level (Bhushan *et al.*, 2010; Bhushan *et al.*, 2013) [9, 10]. The reduction in serum total proteins may be due to binding of mercury with sulfhydryl group of proteins, thereby inhibiting the enzyme activities resulting in the decreased level of serum total proteins. Serum albumin and globulin decreases significantly after all the doses probably due to low protein intake, inadequate digestion, or absorption of protein, increased protein catabolism, diminished albumin synthesis, protein loss in urine and haemodilution. Serum A/G ratio does not reveal anything much special about mercuric chloride toxicity (Al-Zubaidi and Rabee, 2015; Al-Madani *et al.*, 2009) [5, 4].

Increase in serum urea, uric acid may be due to tubular back-leak damage respectively whereas BUN due to glomerular damage and decreased GFR leading to tubular damage. Increase in serum creatinine has been observed due to high production and break down rates of phosphocreatinine. This is probably due to the fact that proximal tubule is the most common site of toxicant induced cell injury. This might be due to selective accumulation of xenobiotics into this segment of nephron. Under such conditions, therefore, the backleak of filtrate result in decreased excretion and increase in retention of nitrogenous waste, so responsible for such increase in the level of serum urea, uric acid and creatinine (Saxena *et al.*, 2008; Apaydin *et al.*, 2016; Yadav *et al.*, 2019) [24, 7, 33].

The alterations induced by mercuric chloride have been modulated by pre and post treatments of *Tagetes erecta* in the present study. There is clear cut indication of renal protection evident at biochemical level. *Tagetes erecta* reveal promising results in modulation of mercuric chloride toxicity on the basis of antioxidants present in the form of lutein, β-carotene as special and specific agents. *Tagetes* flower extract has a vast potential to be exploited as remedy against a good number of biochemical alterations in mammalian kidney induced by heavy metals other than mercury (Saxena *et al.*, 2008; Nishanthi and Anuradha, 2012; Khasif *et al.*, 2014; Munhoz *et al.*, 2014; Saxena *et al.*, 2017) [24, 20, 16, 18, 22].

Further studies at higher level, if undertaken, would help to understand the mechanism of nephron-protective potential of *Tagetes erecta* flower extract.

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