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Effect of treatment of cow's urine "Gomutra" and antioxidants in alleviating the lindane-induced oxidative stress in kidney of Swiss mice (*Mus musculus*)

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Abstract The study aimed to evaluate the effect of cow urine and combination of antioxidants against lindane induced oxidative stress in Swiss mice. Male healthy mice, 8–10 weeks old, weighing 30 ± 5 g were randomly selected and divided into eight groups, namely, control (C); lindane (L); antioxidant (A), antioxidant+lindane (A+L), cow urine (U), cow urine+lindane (U+L), cow urine+antioxidants (U+A) and cow urine+antioxidants+lindane (U+A+L). Group C animals were administered only the vehicle (olive oil); doses selected for other treatments were: lindane: 40 mg/kg b.w.; antioxidants: 125 mg/kg b.w. (vitamin C: 50 mg/kg b.w., vitamin E: 50 mg/kg b.w., α-lipoic acid: 25 mg/kg b.w.) and cow urine: 0.25 ml/kg b.w. In group A+L and U+L antioxidants and cow urine were administered 1 h prior to lindane administration and in group U+A and U+A+L cow urine was administered 10 min before antioxidants. All treatments were administered orally continuously for 60 days. Lindane treated group showed increased lipid peroxidation, whereas glutathione, glutathione peroxidase, superoxide dismutase, catalase, protein and endogenous levels of vitamin C and E were significantly decreased compared to control. Administration of cow urine and antioxidants alleviated the levels of these biochemical parameters.

Keywords Antioxidants · Cow urine · Kidney · Lindane · Oxidative stress

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Introduction

Kidney is vital organ of the body responsible for segregating the metabolic waste products from the blood. Accumulation of metabolites of xenobiotics contributes significantly to its susceptibility to damage kidney [24]. Any nephrotoxic insult would result in accumulation of waste materials in the blood which in turn may lead to other toxic manifestations in the body. Toxic injury to the kidney is known to occur as a result of exposures to halogenated hydrocarbons, such as lindane, carbon tetrachloride and trichloroethylene, and the heavy metals cadmium and lead [3, 35, 36, 48, 59]. Some of these toxicants cause acute injury to the kidney, while others produce chronic changes that can lead to end-stage renal failure or cancer.

Lindane, the gamma isomer of HCH possesses the property of persistence, bioaccumulation and long term toxicity [32] and fulfills the criteria of POPs i.e., persistent organochlorine pesticides. India has total installed capacity of lindane (technical) production of 1,300 tonnes per annum (tpa), with two companies producing: Kanoria Chemicals and Industries Ltd with a capacity of 1,000 tpa, and India Pesticides Limited with 300 tpa capacity. According to data available from Department of Chemicals and Petrochemicals, Ministry of Chemicals and Fertilizers, between 1995 and 2005, India has produced 5,387 tonnes of technical grade lindane. In India, lindane formulations are registered for use in pharmaceutical products for control of head lice and scabies on people, to control fly, flea, cockroach, mosquito, bed bug, and beetle populations and for the control of pests in cotton, sugarcane, pumpkin, cabbage, onion, apple, walnut, maize, okhra, potato, tomato, cauliflower, radish, cucumber and beans [15]. Lindane is highly lipophilic and absorbed by the respiratory, digestive or cutaneous pathways. Its accumulation depends on the duration of the exposure and affect

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tissues in the following order: fat > brain > kidney > muscle > lung > heart > spleen > liver > blood [56]. Toxicity induced by lindane is attributed to oxidative stress as it induces the release of free radicals and generation of reactive oxygen species (ROS) [67].

Oxidative stress is defined as a disruption of the pro-oxidant–antioxidant balance in favor of the former, leading to potential damage [37]. It is a result of one of three factors: (i) an increase in ROS, (ii) an impairment of antioxidant defence systems, or (iii) an incapacity to repair oxidative damage.

A number of studies indicate the toxicity of lindane on kidney [22, 48]. It has been considered that ROS play an important role in the pathogenesis of renal injury. Since the entire range of toxic metabolites in the body is excreted mainly from the kidney, this organ is endowed with significant antioxidant defense system next only to liver. This is understandable because ROS play a key intermediary role in the pathophysiologic processes of a wide variety of clinical and experimental renal diseases [50, 65].

The human body has a strong inherent synergistic and multilevel defense mechanism, to combat and counteract the damage caused by free radicals [41]. This defense is mediated by endogenous antioxidant system which either prevent these reactive species from being formed, or cause their removal before they can damage vital components of the cell [17]. The excessive free radicals or the suppression of antioxidant defense of body results into toxicity. In such conditions, though the body tissue is endowed with enzymatic and non-enzymatic protective systems, but it seems that the homeostasis of the body fails. In such situations, the use of exogenous substances with antioxidative potential becomes important [64].

Antioxidants have gained immense importance in recent years. The potency of various antioxidants on different organ systems has been investigated against lindane toxicity [8, 9, 11, 42, 63].

Cow urine or Gomutra is considered sacred in Hindu mythology and from ancient times it has been used as a medicine in India. The medicinal properties of cow's urine have been mentioned in Sushrut (45/221) and Charak (sloka-100) where it is considered useful in treating renal colic, jaundice, anemia, diarrhea, gastric infection, piles and skin diseases including vitiligo. It is also considered as an appetizer and is known to reverse inflammation, a diuretic as well as a nephroprotective agent. It also acts at cellular level and generates bioenergy [31]. The analysis of cow urine has shown that it contains nitrogen, sulphur, phosphate, sodium, manganese, carbolic acid, iron, silicon, chlorine, magnesium, melci, citric, titric, succinic, calcium salts, vitamin A, B, C, D, E, minerals, lactose, enzymes, creatinine, hormones and gold acids. The cow urine contains those substances, which are present in the human body and thus its consumption maintains the balance of these substances and cures incurable diseases [49]. Cow urine is also used along with herbs to treat various diseases like fever, epilepsy, anemia, abdominal pain, constipation etc. by the traditional healers [34].

There is a paucity of information regarding the role of fresh cow urine and combination of vitamin E, vitamin C and alpha lipoic acid against lindane toxicity in kidney. Therefore, in the present study their role in alleviating the oxidative stress induced by lindane intoxication in kidney of mice has been investigated. Moreover, the use of cow urine from ancient times has shown to be quite effective but its efficiency against lindane induced toxicity and its combined effect along with the various antioxidants is hitherto unreported. Hence, present study was undertaken to fill the lacuna in this regard.

Materials and methods

Chemicals

Lindane (γ-HCH) was obtained from Sigma chemicals St. Louis, Mo, USA (CAS No. 58-89-9 and purity 97 %). vitamin E, vitamin C, α-lipoic acid, sodium azide, thiobarbituric acid, dinitrophenylhydrazine (DNPH), 2, 2 dipyridyl, and phenazine methosulphate were obtained from Himedia, India. Dithiobisnitro benzene (DTNB), reduced glutathione and bovine serum albumin were purchased from Sisco Research Laboratories, Mumbai, India. All other chemicals and solvents used were of analytical grade. Cow urine: Urine of young cow was collected from local cowshed and stored in an air tight bottle for further use.

Animals and treatment

Male Swiss mice, weighing 30 ± 5 g and 8–10 weeks old were procured from Cadila Health Care Institute, Ahmedabad. Animals were maintained on sterilized rice husk bedding in polypropylene cages and kept at a temperature of about 23 ± 3 °C with 12 ± 1 h L:D cycle. Animals were fed on standard pelletal diet (Pranav Agro, Baroda). Food and water were ad libitum. Experimental protocol was approved by the Institutional Animal Ethics Committee. Handling of animals was according to the guidelines of

Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Govt. of India.

Dose selection

Dose for lindane was selected after conducting pilot experiments in our laboratory. LD50 for lindane was found to be at 60 mg/kg body wt. considering this aspect, a dose level which may show adverse effect on kidney was selected as the dose for the present study. Duration of treatment was based on occupational exposure of workers i.e., for 2 months during active malaria vector control programme. Dose selected for lindane was 40 mg/kg body wt. and duration of treatment was for 2 months i.e., 60 days. There was no mortality in exposure group during the study.

Doses for antioxidants were calculated keeping the doses prescribed for humans and also in accordance with the previous reports [8, 9, 42]. The combined dose of antioxidants selected was 125 mg/kg body wt. which included vitamin C 50 mg/kg body wt., vitamin E 50 mg/kg body wt. and α-lipoic acid 25 mg/kg body wt. Cow urine was administered at a dose equivalent to the corresponding dose for human in ml/kg b.w. i.e., 0.25 ml/kg b.w.

Doses of lindane, vitamin E and lipoic acid were prepared by dissolving in olive oil. Dose of vitamin C was prepared in distilled water. Cow urine was administered without any modification.

Experimental protocol

A sub chronic study was done for 60 days and oral route of dose administration was chosen for all treatments. Mice were divided into eight groups with minimum of 8–10 animals in each group.

In the group IV and VIII the antioxidants and cow urine were administered 1 h prior to lindane administration. In group VI and VII cow urine was administered 10 min before the antioxidants administration. All the treatments were given continuously for a period of 60 days.

Mice were sacrificed by cervical dislocation at the end of the scheduled period of 60 days and 24 h after the last dose treatment. Both the kidneys were blotted free of blood, weighed and to maintain uniformity in all groups the right kidney was used for biochemical analysis. The right kidney (just to maintain uniformity amongst animals of all groups) was washed with ice cold physiological saline and a 10 % w/v homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at $6{,}000 \times g$ for 10 min to obtain the supernatant. Supernatant was diluted five times and used for estimating the biochemical parameters.

Biochemical analysis

The kidney tissue homogenate was used for the estimation of lipid peroxidation (LPO) [68], superoxide dismutase (SOD) [30], catalase (CAT) [16], glutathione peroxidase (GPx) [54], glutathione (GSH) [40], protein [38], vitamin E (α-tocopherol) [18] and vitamin C (ascorbic acid) [45].

Statistical evaluation

Values are mean ± SD and the results obtained were analyzed using one way ANOVA. Inter group comparisons were performed by using the least significance difference (LSD) test. A probability value of $P < 0.05$, 0.01 was considered as statistically significant.

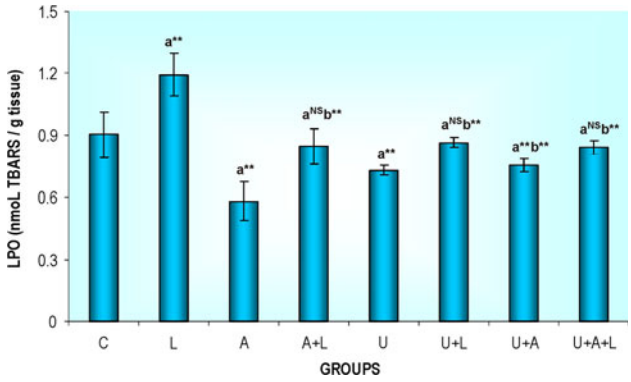
S. no.	Group no.	Group code	Treatment	Dose	Duration
1	I	C	Control: vehicle only	Olive oil only	60 days
2	II	L	Lindane	40 mg/kg b.w.	60 days
3	III	A	Antioxidants alone	Combined dose of 125 mg/kg b.w.	60 days
4	IV	A+L	Antioxidants+lindane	Antioxidant dose of 125 mg/kg b.w. followed by lindane at 40 mg/kg b.w.	60 days
5	V	U	Cow urine alone	0.25 ml/kg b.w. cow urine	60 days
6	VI	U+L	Cow urine+lindane	0.25 ml/kg b.w. cow urine +40 mg/kg b.w. lindane	60 days
7	VII	U+A	Cow urine+antioxidants	0.25 ml/kg b.w. cow urine +125 mg/kg b.w. antioxidants	60 days
8	VIII	U+A+L	Cow urine+antioxidants+lindane	0.25 ml/kg b.w. cow urine +125 mg/kg b.w. antioxidants +40 mg/kg b.w. lindane	60 days

Results

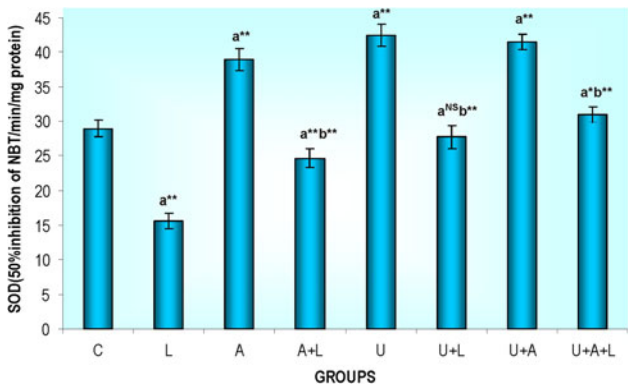
The changes in various biochemical parameters in different groups have been presented in Graphs 1, 2, 3, 4, 5, 6, 7, 8.

Effect on LPO (Graph 1)

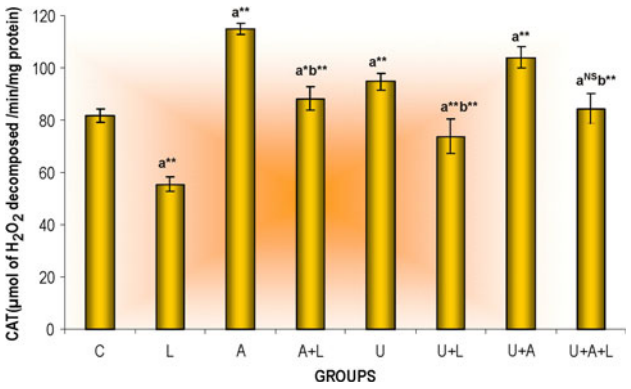
A significant increase ($P < 0.01$) of 32.21 % was observed in the LPO levels after lindane intoxication as compared to control. All the pretreatment groups showed a significant decline ($P < 0.01$) in the LPO levels. 29.21, 27.66, and 29.47 % decline was observed in the A+L, U+L, and U+A+L groups respectively as compared to lindane group. The increasing order of the LPO levels in various groups was as follow: L > C > U+L > A+L > U+A+L > U+A > U > A.



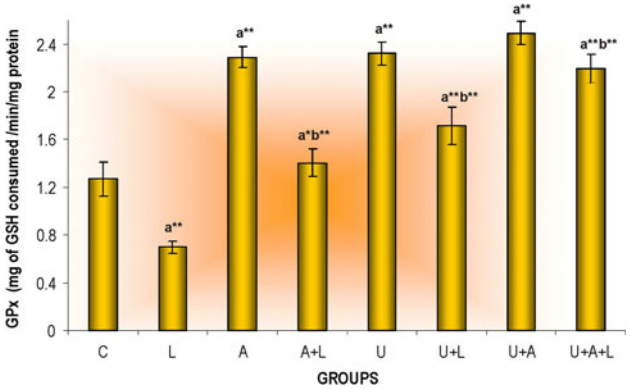
Graph. 1 Mean ± SD values of LPO (nmoL TBARS/g tissue) in various groups (a 60 day assessment). *a* when compared to control, *b* when compared to lindane, *NS* non significant, * significant ($P < 0.05$), ** highly significant ($P < 0.01$)



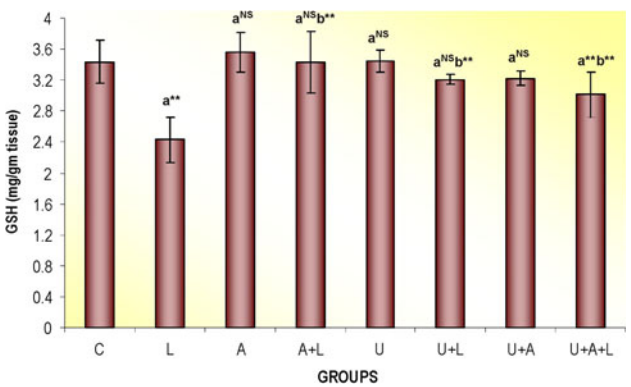
Graph. 2 Mean ± SD values of SOD (50 % inhibition of NBT/min/mg protein) in various groups (a 60 day assessment). *a* compared to control, *b* when compared to lindane, *NS* non significant, * significant ($P < 0.05$), ** highly significant ($P < 0.01$)



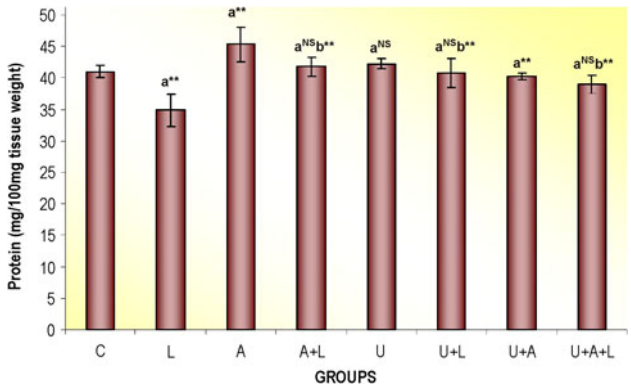
Graph. 3 Mean ± SD values of CAT (µmol of H_2O_2 decomposed/min/mg protein) in various groups (a 60 day assessment). *a* compared to control, *b* when compared to lindane, *NS* non significant, * significant ($P < 0.05$), ** highly significant ($P < 0.01$)



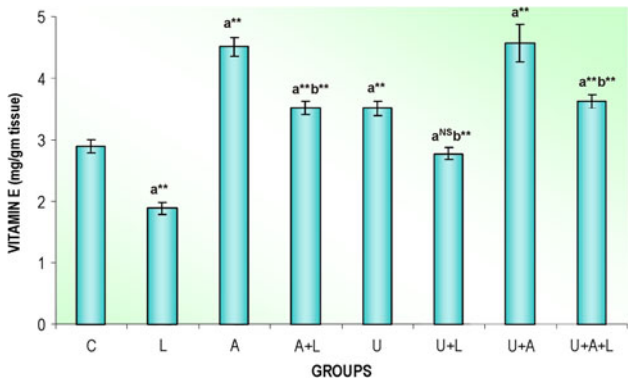
Graph. 4 Mean ± SD values of GPx (mg of GSH consumed/min/mg protein) in various groups (A 60 day assessment). *a* compared to control, *b* when compared to lindane, *NS* non significant, * significant ($P < 0.05$), ** highly significant ($P < 0.01$)



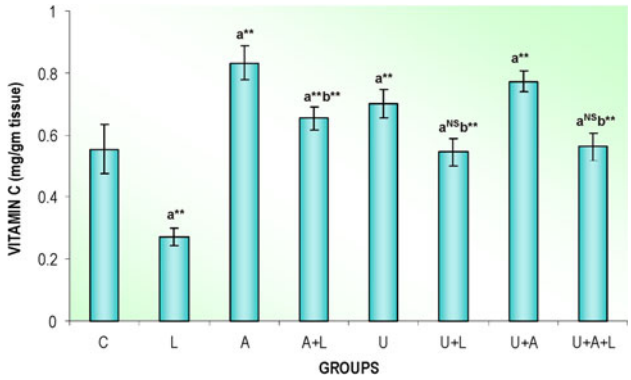
Graph. 5 Mean ± SD values of glutathione (GSH) (mg/gm tissue) in various groups (a 60 day assessment). *a* compared to control, *b* when compared to lindane, *NS* non significant, * significant ($P < 0.05$), ** highly significant ($P < 0.01$)



Graph. 6 Mean ± SD values of total protein (mg/100 mg tissue weight) in various groups (a 60 day assessment). *a* compared to control, *b* when compared to lindane, *NS* non significant, * significant ($P < 0.05$), ** highly significant ($P < 0.01$)



Graph. 7 Mean ± SD values of endogenous vitamin E (mg/g tissue) in various groups (a 60 day assessment). *a* compared to control, *b* when compared to lindane, *NS* non significant, * significant ($P < 0.05$), ** highly significant ($P < 0.01$)



Graph. 8 Mean ± SD values of endogenous vitamin C (mg/g tissue) in various groups (a 60 day assessment). *a* compared to control, *b* when compared to lindane, *NS* non significant, * significant ($P < 0.05$), ** highly significant ($P < 0.01$)

Effect on SOD (Graph 2)

The SOD levels declined significantly ($P < 0.01$) up to 45.96 % in lindane intoxicated mice as compared to control group. The pretreatment groups A+L, U+L and U+A+L showed a significant increase ($P < 0.01$) of about 57.74, 77.64, and 98.26 % respectively, as compared to lindane group. The increasing levels of SOD in various groups were as follow: L < A+L < U+L < C < U+A+L < A < U+A < U.

Effect on CAT (Graph 3)

Lindane induced kidney toxicity showed a significant decline ($P < 0.01$) of 32.12 % in the CAT activity as compared to control. A significant increase ($P < 0.01$) of 58.91 % in A+L, 32.85 % in U+L and 51.97 % in U+A+L was observed when compared to lindane. The cow urine alone group also showed a decrease of 9.82 % as compared to lindane but the decrease was not significant. The maximum % of increase was observed in the A+L group. The increasing order of the enzyme level in all the eight groups was as follow: L < U+L < C < U+A+L < A+L < U < U+A < A.

Effect on GPx (Graph 4)

As compared to control animals, the lindane intoxicated animals showed a significant decrease ($P < 0.01$) of 45.19 % in the GPx levels. All the pretreatment groups showed promising results and brought about a significant rise of 101.89, 146.37, and 215.30 % in the GPx levels in the groups A+L, U+L and U+A+L, respectively. The increasing order of GPx levels in various groups was as followed: L < C < A+L < U+L < U+A+L < A < U < U+A.

Effect on GSH (Graph 5)

A significant decline ($P < 0.01$) of 29.24 % was observed in the GSH levels after lindane intoxication as compared to control animals. The pretreatment groups showed a non-significant decrease in the levels of GSH as compared to control which accounted to 0.10 % in the A+L group, 6.69 % in U+L group and 12.37 % in U+A+L group. But when compared to lindane a significant increase ($P < 0.01$) of 41.18, 31.87, and 23.84 % was seen in the respective groups A+L, U+L and U+A+L. The increasing order of GSH levels in different groups was as follow: L < U+A+L < U+L < U+A < A+L < C < U < A.

Effect on protein (Graph 6)

A significant decrease ($P < 0.01$) of 15.01 % was observed in the total protein levels of lindane intoxicated animals as compared to control. The pretreatment groups A+L, U+L and U+A+L showed a significant rise ($P < 0.01$) of 20.00,

17.08, and 11.95 % respectively, in the levels of total protein as compared to lindane group. The best results were shown by the pretreatment of combination of antioxidants which resulted in about 20.00 % rise in the total protein levels which had decreased to 15.01 % after lindane intoxication. The increasing order of protein levels in various groups is as follows: L < U+A+L < U+A < U+L < C < A+L < U < A.

Effect on endogenous vitamin E (Graph 7)

A significant decrease ($P < 0.01$) of 34.94 % was registered in the endogenous levels of vitamin E in lindane group as compared to control. A+L, U+L and U+A+L groups significantly ($P < 0.01$) alleviated the levels of vitamin E by 86.65, 47.30, and 92.32 %, respectively in comparison to lindane group. The increasing order of vitamin E levels in all the groups was as follow: L < U+L < C < U < A+L < U+A+L < A < U+A.

Effect on endogenous vitamin C (Graph 8)

As compared to control, a significant decrease ($P < 0.01$) of 50.95 % was observed in the endogenous levels of vitamin C in animals toxicated with lindane. This decrease was alleviated significantly ($P < 0.01$) when the different pretreatments were given. In comparison to lindane treated animals, the animals of A+L, U+L and U+A+L group showed an increase of 140.95, 100.51, and 107.18 %, respectively. The increasing order of the vitamin C levels in all the eight groups was as follow: L < U+L < C < U+A+L< A+L < U < U+A < A.

Discussion

The results of the present study clearly demonstrate that LPO significantly increased in kidney after in vivo treatment of lindane. Thus, results of the present study are in agreement with previous reports where increase in LPO was also observed due to lindane in different tissues [11, 22, 42, 48, 67]. Moreover, renal LPO levels also increase due to aflatoxin [61], CCl_4 [7], chlorpryfos-ethyl [46], lead [55], cisplatin [4] and gentamicin [1] toxicity. The increase in LPO results owing to increase in ROS or alternatively lindane might inhibit antioxidant molecules and antioxidant enzymes. The support for such an assumption comes from the findings that lindane reduces antioxidant molecules and antioxidant enzymes [8, 42, 48] which is also observed in the present study.

The pretreatment with vitamin C, E, lipoic acid and cow urine in the groups A+L, U+L and U+A+L significantly lowered the LPO levels as compared to mice treated with lindane alone. Earlier reports have also shown that supplementation with vitamin C and E attenuated the LPO levels decreased due to cisplatin [4] and chlorpyrifos [46]. Lipoic acid also ameliorates renal oxidative stress [6]. Moreover, combination of lipoic acid and vitamin E [11], vitamin C, E, lipoic acid and resveratrol [8] and vitamin C, E and lipoic acid [42] ameliorated the lindane induced increased LPO levels.

The results reveal that the pretreatment of combination of antioxidants and cow urine (U+A+L) was the most effective in lowering the LPO levels followed by combination of antioxidant (A+L) and cow urine pretreatment (U+L).

Due to increase in LPO the amount of free radicals crosses the threshold level. Nevertheless, decrease in the activities of CAT, SOD, GPx and GSH further deteriorates the situation and enhance the formation of lipid peroxides. It could be assumed that lindane might have caused LPO by inhibiting the antioxidant enzymes, molecule, vitamin E and C. The mechanism by which lindane induces oxidative stress involves the activity of cyt P450 system resulting in the generation of superoxide radicals [21].

Similar to earlier reports, results of the present study also show a decrease in SOD and CAT levels in the lindane treated group and in all such cases the main culprit being superoxide radicals [9, 42, 48]. Renal SOD and CAT levels were also lowered due to chlorpryfos-ethyl [46], aflatoxin [61], CCl_4 [7], lead [55], cisplatin [4], alcohol [60] and gentamicin [1] toxicity.

The decreased activity of SOD in kidney in lindane treated mice may be due to the enhanced lipid peroxidation or inactivation of the antioxidative enzymes or could result from inactivation by hydrogen peroxide or glycation of the enzyme [62]. This would cause an increased accumulation of superoxide radicals, which could further stimulate lipid peroxidation. A decrease in SOD activity favors the accumulation of superoxide radicals, which in turn inhibit CAT [33] as also seen in the present study.

All the pretreatment groups showed alleviation in the levels of SOD and CAT. Earlier reports have shown that vitamin C and E are capable of increasing the renal SOD and CAT levels [4, 7, 46]. Combination of lipoic acid, vitamin C, E and resveratrol [9] and lipoic acid and vitamin C and E [42] attenuated the SOD and CAT levels in brain and testis, respectively of lindane treated mice. Amongst the three pretreatments the maximum amelioration in the levels of SOD was observed in case of U+A+L group followed by U+L group and was least in A+L group. Moreover, in the present study the protection offered by combination of antioxidants (A+L group) in ameliorating the CAT levels was the maximum followed by

combination of antioxidants and cow urine (U+A+L group) and minimum in the only cow urine (U+L) group.

The reduction in the activity of SOD, CAT enzymes may result in a number of deleterious effects due to the accumulation of superoxide anion and hydrogen peroxide [58]. The elimination of H_2O_2 is either effected by CAT or GPx [69].

The level of GPx in the exposed group were lowered which is in accordance with the earlier studies where lindane caused a similar decrease in the levels of GPx in various tissues [42, 48]. Renal GPx levels were found to be decreased due to chlorpryfos-ethyl [46], aflatoxin [61], acetaminophen [27], cisplatin [4] and CCl_4 [7] toxicity. The decreased levels can be attributed to either increased H_2O_2 generation or decreased GSH concentration because GSH is one of the substrates for GPx [57]. A decreased GSH concentration was observed in the present study. Decreased activity of GPx may also result from radical induced inactivation and glycation of the enzymes [26].

Because of the decreased GPx activity the accumulation of H_2O_2 may cause inhibition of SOD activity [10]. During oxidative stress, inactivation of GPx may occur, and on the other hand superoxide anion ($O_2^{\bullet -}$) itself can inhibit peroxidase function [12]. So GPx must be considered to be complementary to SOD [43].

The pretreatment with vitamin C, E, lipoic acid and cow urine in groups A+L, U+L and U+A+L significantly improved the levels of GPx. It has been shown in earlier reports that vitamin C and E are capable of increasing the renal GPx levels [4, 7, 46] and combination of vitamin C, E and lipoic acid ameliorated the testis GPx levels lowered due to lindane [42]. It is also concluded that the significant increase in the GPx levels was the maximum in case of U+A+L group followed by U+L group and was least in A+L group.

In the present study, the levels of GSH were decreased significantly in lindane treated group indicating the oxidative stress caused by lindane as also reported earlier [8, 22, 42, 48, 67]. Decrease in renal GSH levels has also been documented in a case of alcohol [60], aflatoxin [61], gentamicin [1, 29], acetaminophen [27] and cisplatin [4] toxicity.

The decrease in the GSH level as observed in the present study can be due to increased utilization of GSH for metabolism of lipid hydroperoxides by GPx or interaction of GSH with free radicals. Similar analogy is being also drawn in the earlier reports [5, 23].

The levels of GSH were significantly ameliorated after the pretreatment with combination of antioxidants namely, vitamin C, E and α- lipoic acid (A+L group), cow urine (U+L group) and combination of antioxidants and cow urine (U+A+L group). Earlier studies have shown that vitamin C, E and lipoic acid attenuates the decreased renal GSH levels [4, 29]. Lindane induced decreased GSH levels were ameliorated by combination of lipoic acid, vitamin C, E and resveratrol [8] and lipoic acid, vitamin C and E [42] in brain and testis, respectively. The significant increase in GSH levels in the pretreatment groups was least in U+A+L group; intermediate in U+L group and maximum in A+L group.

The present study reveals that lindane inhibits GPx and CAT due to its capacity to generate ROS, which will result in H_2O_2 accumulation. The increased H_2O_2 in turn could cause SOD inhibition resulting in increased production of superoxide radicals. Thus, increased production of superoxide radicals would inhibit both CAT and GPx. Treatment with the adopted formulation reduced the level of lipid peroxides indicating the effective antioxidant property of the combination as well as cow urine alone in the moderation of tissue damage.

Antioxidant defense system protects the aerobic organism from the deleterious effects of reactive oxygen metabolites. Vitamin E, a major lipophilic antioxidant and vitamin C, play a vital role in the defense against oxidative stress [53]. In our study, the levels of vitamin E and C were decreased significantly during lindane intoxication. This is in agreement with the previous reports that oxidative stress results in deficiency in vitamin C and E [60, 61]. The increased oxidative stress due to lindane intoxication might have resulted in excess utilization of vitamin C and E, consequently, depleting their levels. The observed decrease in the level of kidney ascorbic acid and alpha tocopherol in lindane treated group could be as a result of increased utilization of these antioxidants in scavenging the free radicals generated due to lindane.

Moreover, it is well established that GSH in blood keeps up the cellular levels of the active forms of vitamin C and vitamin E by neutralizing the free radicals. When there is a reduction in the GSH the cellular levels of vitamin C is also lowered, indicating that GSH, vitamin C, and vitamin E are closely interlinked to each other [61]. In agreement with these reports, the decreased levels of GSH, vitamin C and vitamin E on lindane administration were observed in our study.

The pretreatment with combination of antioxidants in group A+L, with cow urine in group U+L and combination of antioxidants and cow urine both in group U+A+L, resulted in significant increase in the endogenous levels of vitamin C and E. The amelioration in the endogenous levels of vitamin C was the maximum in A+L group, followed by U+A+L group and minimum in U+L group. In contrast, the significant increase in the levels of vitamin E was the maximum in U+A+L group; intermediary in A+L group and least in U+L group. The increase in levels of vitamin C and E is obvious in the pretreatment groups A+L and U+A+L as these were exogenously supplied with both vitamin C and E, however, the cow urine alone

(U+L) group also showed significant elevation in the levels of the two vitamins. This could be attributed to the composition of cow urine which is said to contain vitamins.

The analysis of total proteins is important for estimating the degree of damage in the body. The protein profile of the cells is indicative of the physiological status of animal and there exists dynamic equilibrium between the synthetic and degenerative pathways with these biomolecules. In the present study a decline in the total proteins was observed after lindane intoxication which can be due to decreased protein synthesis or increased protein loss. Similar reduction in the levels of total proteins due to lindane have been reported [9, 42, 48]. It is also reported that renal toxicity due to CCl_4 [7] and gentamicin [1] causes a similar decrease in protein levels. Decreased protein levels could be attributed to decreased feed consumption, maldigestion or malabsorption, hepatic dysfunction [13, 47]. Reduced protein levels can also be ascribed to increased urinary excretion owing to kidney damage [66] and glomerular apparatus or reduced protein synthesis [39]. Prabhakaran and Devi [51] have proposed that a toxicant can affect the protein content of the tissue either by inhibiting RNA synthesis or inhibiting of amino acids into the polypeptide chain.

The pretreatment groups showed an elevation in the protein levels which is in accordance with earlier reports where administration of either vitamin C, E or in combination and combination of vitamin C, E and lipoic acid and resveratrol elevated the decreased protein levels [7, 9, 42]. The most effective pretreatment was that of combination of antioxidants (A+L group); cow urine pretreatment group (U+L) was moderately effective and least effective was pretreatment with combination of antioxidants and cow urine both (U+A+L group).

Hypoglycemic [44], cardio-respiratory effect [20], immunomodulatory [14], antigenotoxic and antioxidant properties in vitro [34], anticlastogenic [19] and chemoprotective [52] effects of distillate and redistillate of cow urine have been reported. Attenuation of CCl_4 induced hepatotoxicity by Panchagavya ghrita (prepared by cow milk, cow urine, cow dung, ghee and curd) [2] and cow urine distillate [25] have also been reported. Efficacy of cow urine therapy has been evaluated on cancer patients [28]. The amelioration of oxidative stress by fresh cow urine has not been reported so far and this work seems to be the first report.

Thus it is inferred that the combination of antioxidants taken in the present study are quiet helpful in mitigating and modulating the oxidative stress in the kidney caused due to lindane. Moreover, cow urine treatment also modulated the oxidative stress parameters caused by lindane. From the results it is apparent that the given combination of antioxidants and cow urine act synergistically in reducing lindane induced dysfunction. The analysis of the results of the present study reveals the efficacy of cow urine against oxidative stress. It can be safely concluded that the suggested combination of vitamin C, vitamin E, alpha lipoic acid and cow urine can prove to be beneficial in a number of ailments. The highlight of the investigation is the efficiency of cow urine against the pesticide toxicity which can open new insights in the field of medicine. Cow urine can prove to be an effective co-remedy for oxidative stress. This study emphasizes the importance of antioxidants and cow urine which could be beneficial in the therapeutic world for the treatment of various disorders implicating oxidative stress.

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