Biological Forum – An International Journal **15(1): 554-558(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Alteration in the Serum Cholesterol Level of Dams due to Intoxication of Mercuric Chloride and its Recovery by Ashwagandha and EPL

Vishwas Tiwari*
Department of Zoology, Nirmala College,
Vikram University, Ujjain (Madhya Pradesh), India.

(Corresponding author: Vishwas Tiwari)*
(Received: 07 December 2022; Revised: 13 January 2023; Accepted: 18 January 2023; Published: 23 January 2023)
(Published by Research Trend)

ABSTRACT: The recovery function of 'Essential phospholipids' (EPL) and Ashwagandha on Mercuric Chloride induced alteration in serum cholesterol levels of dams were investigated. Ashwagandha and EPL is considered as "Indian Ginseng" and remarkable antioxidant reduces toxic free radicals in the blood . Both Allopathic and Ayurvedic medicines may be beneficial in the recovery of intoxicated blood and significant clue for Hematological investigations. Animals were treated with 0.5 ppm aqueous mercuric chloride for a period of 7, 14 and 21 days. For the recovery 175mg of EPL and 35mg of Ashwagandha were administered to mice (Already treated with mercuric chloride) for a period of 7,14 and 21 days. Daily treatment of mercuric chloride decreased the cholesterol level in the blood of dams. Simultaneous administration of EPL and Ashwagandha significantly recovered the serum cholesterol level of dams. It appears that the protective effect of EPL and Ashwagandha against mercuric chloride increased serum level of cholesterol is mediated through antioxidative action of EPL and Ashwagandha. Thus, the serum level of cholesterol in all groups was increased.

Keywords: Dams (pregnant mice), EPL, Ashwagandha, Cholesterol, Mercuric chloride.

INTRODUCTION

It is now evident that toxic substances like heavy metal and their compounds released into the environment, affect the reproductive processes and fertility including embryofoetotoxicity and teratogenicity in pregnant animals. Gloria *et al.* (2003) investigated that Mercuric chloride is cumulative poison and considered as direct acting toxicant. Goodman (1983) observed mercuric chloride toxicity on placenta of female rats, while Marszalek (1984) and Lewandowski *et al.* (2002) investigated mercuric chloride toxicity on foetus of female rats. Meanwhile Belles *et al.* (1996) thoroughly studied the intoxication of methyl mercury induced embryo foetotoxicity, The chelate binding character of metals with various chelating agents like EPL, Ashwagandha, EDTA, Tiron, DTPA etc., were the key aspect of the recovery of metal intoxication in mice. On the other hand, Domingo *et al.* (1990; 1992) investigated effectiveness of chelation therapy with time after acute uranium intoxication in mice while James and Soni (1991) observed changes in tissue proteins due to administration of $HgCl_2$ and two chelators in mice. Viswanathan *et al.,* (2000) investigated that Cholesterol act as precursor of various steroid hormone metabolism. It is essential for the synthesis of steroid based hormones. Steven (1975) thoroughly studied comparative placentation in mammals, Mclean *et al.* (1975) observed role of hypothalamic peptide GRH in human placenta while Atkinson *et al.* (1975) noted circulating level of steroids and chorionic gonadotrophins during pregnancy in rhesus monkey. EPL (*Essentiale phospholipids*) is renowned chelating agent contains phosphatidylcholine, phosphatidyl ethanolamine, phosphatidyl serine. This composition sold in market by Nattermann International GMBH West Germany, Rhone-Poulenc India Limited Mumbai. Phospholipids are specific vital components of cellular and sub-cellular membrane systems, where together with glycolipids, cholesterol and proteins they form part of 'essential" structural elements high concentration of unsaturated fatty acids in the EPL namely dilinoleyl phosphatidylcholine make the membrane less rigid and increase their mobility. This facilitates and improves the transport across membrane. Phospholipids are esters of glycerophosphoric acid. David *et al.* (2003) investigated that, The EPL is obtained from a drug "Essentiale". The phospholipids in "essentiale" is called 'essential'. The kinetics of EPL reveals that a significant proportion of the substance is integrated into the membrane of damaged cells and organelles. Solomon *et al.* (1967) observed that, liver in animal body performs metabolic activities for carbohydrate, protein and lipids. As Cholesterol acts as precursor for hormonal chain metabolism or

intermediary metabolism through liver, then it transfer to various organs of the body via blood circulation including foetal and maternal tissues, consequently EPL protects the foeto-placental tissues through hormonal chain action (Ramlingam *et al.,* 2003). Thus, the present work has been taken in to consideration.

MATERIALS AND METHODS

Experimental animals. Eighty four young isogenic healthy sexually mature Swiss albino mice (weighing 24 ± gm) were used for present study. The mice were procured from Veterinary College, Mhow (M.P.). They were fed with balanced standard food ad. libitum daily.

Exposure of mice to mercury. Animals were treated with 0.5 ml/day of 0.5 ppm aqueous $HgCl_2$ for a period of 7, 14 and 21 days.

Chemicals and herbal compounds. Mercury (Hg) was used as $HgCl_2$ marketed by Qualigens fine chemicals (A division of Glaxo India Ltd.). In the present study the drug 'Essentiale' manufactured by Nattermann International GMBH, West Germany and ashwagandha manufactured by the Dabur India Ltd., were used as detoxifying agent.

Determination of dose: The dose of 0.5 ml $HgCl_2$ solution determined by LC 50% mortality method, while the dose of 'Essentiale' was directly used as a capsule of 175 mg standard quantity manufactured by Nattermann International GMBH Company, West Germany and 35 mg dose of Ashwagandha was used, which was earlier found most effective Panda *et al.* (1997).

Experimental design: A total number of 84 pregnant mice were divided in three groups.

Sr. No.	Group	Treatment
1.	Control	standard food + water
2.	Treated	exposed to standard food + water + 0.5ml. $HgCl_2$ solution
3.	Recovery-I	exposed to 0.5ml $HgCl_2$ + EPL with Standard food
4.	Recovery-II	exposed to 0.5 ml. $HgCl_2$ + Ashwagandha with standard food

The mice of experimental group I, II and III were sacrificed in 7, 14 and 21 days and along with them the animals of control group were sacrificed on the same day.

Embryo foetotoxicity Study. The pregnant mice were anaesthetized by diethyl ether. The abdomens were incised longitudinally and both uterine horns were carefully exposed. The position and number of live and dead foetuses were recorded for each dam. Individual foetal weights were recorded. Foetuses were carefully examined for external abnormalities. Half of the foetuses were fixed in aqueous Bouin's solution and half in 10% formalin solution.

Serum Sample and Assay. For biochemical studies the blood of dams was directly collected by cardiac puncture using sterilized needle. The blood samples of control, treated and recovery groups of dams were collected to estimate the level of cholesterol, estrogen and progesterone hormones and immediately processed for biochemical estimation. The serum level of cholesterol was investigated by cholesterol kit, while serum level of both estrogen and progesterone hormones were measured by using RIA method (Young *et al.,* (1975) and by chemiluminescence method, USA).

RESULTS AND DISCUSSION

The realization of the effects of the metal in many organisms provided impetus to the emerging fields of developmental and reproductive toxicology. Mercury, cadmium and arsenic were among the best studied experimental teratogens of the 1960's and 1970's. The developmental pathology, maternal metabolism, transport across and toxicity to the placenta, embryo/foetal accumulation and metabolism of metals was described during this period (Chang, 1996), (Lee and Han, 1995). Although, Bio- kinetics, Metabolism and chemical toxicity of mercury are well known. Until recently little attention was paid to the potential toxic effects of mercury on reproduction and development in mammals (Clarkson, 1991). The developmental toxicity was included among the harmful effects derived from the exposure of certain elements and embryotoxicity and teratogenicity have resulted in pregnant animals exposed to certain metals (Freeman *et al.,* 1987). Thus arsenic, mercury, cadmium and lead have been known to be developmental toxicants (Domingo, 1994). The reports regarding transport of metals across the placenta and metabolism of metals by the placenta are reviewed by Rogers (1996), whereas Colomina *et al.* (1995) studied the interference between the mother and the conceptus changes as development proceeds and the ability of some metal to reach the conceptus changes concordantly. Mclean *et al.* (1975) observed that, Toxic effect of metals on the placenta impedes maternal-foetal exchange processes and the placental insufficiency ultimately causes the developmental toxicity. Cholesterol is intensively involved as a precursor for many biochemical metabolic reactions performed by liver. Thus, looking to the need of the steroid hormones in the present experiment, the cholesterol, estrogen and progesterone level has been taken into consideration to observe the effect of mercuric chloride intoxication (Vishwanathan *et al.,* 2000; Bentley, 1998).

In the biochemical estimations, the cholesterol, estrogen and progesterone level were estimated in 7, 14 and 21 days of duration in all groups of the dams.

Table 1: Effect of $HgCl_2$ on Cholesterol level in different groups.

Sr. No.	Days	Control	Treated	Recovery by Ashwagandha	Recovery by EPL
1.	7	136.00 ± 0.4	131.6 ± 0.6	115.30 ± 0.2	108.30 ± 0.9
2.	14	138.79 ± 0.8	129.26 ± 0.5	183.40 ± 0.5	154.70 ± 0.7
3.	21	146.89 ± 0.5	126.38 ± 0.3	159.68 ± 0.7	164.00 ± 0.6

Levels are expressed in mgm%.
All values are expressed in mean ± SEM.

7 days: In 7 days, experiment of control group, the serum cholesterol level was estimated up to 136.0 ± 0.4 mgm% while in treated group decline upto 131.6 ± 0.6 mgm% but in ashwagandha and EPL recovery groups, no significant improvement was observed i.e., 108.3 ± 0.9 and 115.3 ± 0.2 mgm% respectively. Decline in treated group were due to intoxication of mercuric chloride (Table 1). Results of present experiment revealed that the administration of $HgCl_2$ (0.5 ppm) for 7, 14- and 21-days duration brings alterations in the concentration of serum cholesterol, estrogen and progesterone. In the experimental duration the serum cholesterol level were declined in 7, 14, and 21 days. The % level of cholesterol was higher in control group. In 21 days, sudden rise in levels were reported with comparison to simultaneous control group. Since, the cholesterol being the precursor in the biosynthetic steroidogenic pathway of steroid hormones. In the present study level of all hormones get reduced due to mercuric chloride intoxication. Similarly, Arunadevy *et al.* (1999) noted the decreased level of testicular cholesterol by mercuric chloride. Whereas, Nagar and Bhattacharya (2001) found that the cholesterol and testosterone were reduced due to the dose of 0.5 ppm of mercuric chloride in mice for a period of 7, 14, and 21 days of duration. In another study, Tiwari and Bhattacharya (2004) observed that serum cholesterol and thyroxine levels were declined in mercuric chloride treatment (0.5 ppm) in mice.

In the present investigation, the cholesterol level did not exhibit much improvement in its level during ashwagandha and EPL administration in 7-day duration after withdrawal of $HgCl_2$ the level were less than treated group but it became elevated in 14- and 21-day duration.

14 days: Tremendous alterations were observed in the serum levels of cholesterol, estrogen and progesterone of the dams. The serum level of cholesterol with comparison to 7 days control group were rise up to 138.79 ± 0.8 mgm% while in treated group it declined up to 129.26 ± 0.5 mgm%. Recovery groups showed appreciable rise in the levels *i.e*., 154.7 ± 0.7 mgm% in EPL group and 183.4 ± 0.5 mgm% in ashwagandha group of the dams.

21 days: Appreciable alterations were reported in serum levels of cholesterol, estrogen, progesterone in 21 days duration of experiment of the dams. The serum level of cholesterol in 21 days control group were reported upto 146.89 ± 0.5 mgm% while in treated group it declined upto 126.38 ± 0.3 mgm%. Significant rise of 164.0 ± 0.6 and 159.68 ± 0.7 mgm% were reported in EPL and ashwagandha recovery groups in 21 days experiment of the dams (Table 1). In the present investigation the cholesterol level did not exhibit much improvement in its level during ashwagandha and EPL administration in 7 day duration after withdrawal of $HgCl_2$ the level were less than treated group but it rises in 14 and 21 day duration Similarly, studies of Rao *et al.* (2003) had revealed increased level of cholesterol treated with $NiCl_2$ and protection by administration of ascorbic acid at the dose of (10 mg / kg / body weight). The administration of 175 mg of EPL / kg / body wt. to $HgCl_2$ exposed mice, consequently, serum level of cholesterol was improved than treated group. Thus, antioxidative action of EPL produced improvement in mercury intoxicated serum level of cholesterol (David *et al.,* 2003; Tiwari and Bhattacharya 2004).

In another study, Chinoy (1973) stated that ascorbic acid increases cAMP level might resulted in the recovery in the activities of several enzymes in different tissues. Moreover, ascorbic acid is a powerful reducing agent, participate in redox reaction, thereby activating serum metabolic processes. Further, Patel and Chinoy (1997) suggested that ascorbic acid and calcium act synergistically to reduce Hg toxicity. Here, in present study the increased level of cholesterol might be due to antistress action of ashwagandha and EPL exhibited repairment of tissue that synthesized the cholesterol. The overall recovery in the cholesterol level was quite beneficial to protect against mercury intoxication. However, for therapeutic purpose further investigations are necessary.

CONCLUSION

The Synergistic recovery effect of EPL and Ashwagandha is remarkably observed in this investigation. The 'Essential Drug" not only recovers the fluctuated level of Cholesterol but also reset the biochemical pathway of Cholesterol during biosynthesis. Secondly Ashwagandha, 'The Indian Ginseng" through Anti-oxidative action not only keep the normal recommended level of Cholesterol in the blood but also reduces free radicals in the blood. (David *et al.,* 2003; Tiwari and Bhattacharya 2004).

Ashwagandha and EPL is considered as "Indian Ginseng "and remarkable antioxidant reduces toxic free radicals in the blood. Both Allopathic and Ayurvedic

medicines may be beneficial in the recovery of intoxicated blood and significant clue for Hematological investigations in the Medical field. The recovery action of 'Essential phospholipids' (EPL) and Ashwagandha on Mercuric Chloride induced alteration in serum Cholesterol levels of dams were investigated. The overall recovery in the Cholesterol level was quite beneficial to protect against mercury intoxication. However, for therapeutic purpose further investigations are necessary.

Acknowledgement. Authors are thankful to Dr. Kirti Diddi, Principal of Nirmala College, Vikram University, Ujjain, for encouragement and providing necessary laboratory facilities during this investigation.

Conflict of Interest. None.

REFERENCES

Arunadevy, R. A., Jaya, O. M. A., Suganthy and Ramlingam, V. (1999). Effect of mercuric chloride on testicular lipid classes in adult albino rats. *Indian. J. Toxicol,* (6), 41 –47.

Atkinson, L. E., Hotchkiss, J, Fritz, G. R., Surve, A. H, Neill, J. D. and Khobil, E. (1975). Circulating levels of steroids and chorionic gonadotropin during pregnancy in the rhesus monkey, with special attention to the rescue of the corpus luteum in early pregnancy. *Biol. Reprod*, (12)**,** 335.

Belles, M., Sanchez, D. J., Gomez, M., Domingo, J. L., Jones, M. M. and Singh, P. K. (1996). Assessment of the protective activity of monoisoamyl meso-2,3 – dimercap to succinate against methyl mercury-induced maternal and embryo / foetal toxicity in mice. *Toxicology, 106*(1-3), 93.

Bentley, P. J. (1998). Hormones and reproduction (In: comparative vertebrate endocrinology) (Eds. IIIrd) Cambridge University Press, Cambridge, U.K. 379-455.

Chang, L. W. (1996). Toxicology of metals (eds.) CRC Press Inc. USA.

Chinoy, N. J. (1973). Metabolic significance of ascorbic acid free radical peroxidase in animal tissue. *J. Ind. Nat. Sci. Acad*., *39*(1), 119 - 121.

Clarkson, W. T. (1991). Mercury: Major issues in Environmental health. *Environment Health Perspectives*, (100), 31-38.

Colomina, M. T., Albina, M. L., Domingo, J. L. and Corbella, J. (1995). Effects of maternal stress on methyl mercury-induced developmental toxicity in mice. *Physiol. Behav., 58*(5), 979-983.

David, K. K., Tiwari, V. and Bhattacharya, L. (2003). Effect of mercuric chloride on testis of Swiss albino mice *Musculus albinus* with special reference to recovery of the damaged activity. *Adv. Pharmacol. Toxicol., 4*(2), 25-30.

Domingo, J. L. (1994). Developmental and reproductive effects of metal chelators. (In: Toxicology of metals (1996) by Chang, L.W. pp 1153 – 1165), CRC Press Inc. New York.

Domingo, J. L., Colomina, M. T., Llobet, J. M., Jones, M. M., Singh, P. K. and Campbel, R. A. (1992). The action of chelating agents in experimental uranium intoxication in mice: variations with structure and time of administration. *Fund. Appl. Toxicol*., (19), 350-357.

Domingo, J. L., Ortega, A., Llobet, J. M. and Corbella, J. (1990). Effectiveness of chelation therapy with time after acute uranium intoxication. *Fundam. Appl. Toxicol.*, (14)**,** 88-95.

Freeman, S. J., Coakley, M. E. and Brown, N. A. (1987). Post implantation embryo culture for studies of teratogenesis (In: Biochemical toxicology a practical approach, 83-107, IRL, Press. Oxford, Wastingtion D.C.

Gloria, B. R, Olive, P., Hirokatsua, A., Ana Francisco, R., Lillian, V. L., Anna, B., Cristina, V. C. and Dainah, C. (2003). Tagum Study II: follow – up study at two years of age after prenatal exposure to mercury. *Pediatrics, 111*(3), 289–295.

Goodman, D. R. (1983). $HgCl_2$ toxicity on placenta of female rats. *Terato. Carcinog.*

James, A. and Soni, V. C. (1991). Changes in tissue proteins due to administration of $HgCl_2$ and two chelators in mice. *Com. Physiol. Ecol., 16*(1), 32-37.

Lee, J. H. and Han, D. H. (1995). Maternal and foetal toxicity of methyl mercuric chloride administered to pregnant Fischer 344 rats. *J. Toxicol. Environ. Health*, *45*(4), 415-425.

Lewandowski, T. A., Pierce, C. H., Pingree, S. D., Hong, S. and Faustman, E. M. (2002). Methyl mercury distribution in the pregnant rat and embryo during early mid brain organogenesis. *Teratology, 66*(5), 235-241.

Marszalek, K. (1984). Mercuric chloride toxicity in pregnant female rats and foetus. *Acta. Biol. Cracov. Ser. Zool*. (25), 1-14.

Mclean, M., Bisits, A., Davies, J., Woods, R. , Lowry, R. and Smith, R. (1975). Role of hypothalamic peptide GRH in human placenta. *Nature Medicine,* (1), 460-463.

Nagar, R. N. and Bhattacharya, L. (2001). Effect of mercuric chloride on testicular activities in mice *musculus albinus. J. Environ. Biol*., *22*(1), 15-18.

Panda, S., Gupta, P. and Kar, A. (1997). Protective role of ashwagandha in cadmium-induced hepatotoxicity and nephrotoxicity in male mouse. *Curr. Sci*., *72*(8), 546-547.

Patel, S. and Chinoy, N. J. (1997). Protective role of ascorbic acid and calcium on mercury toxicity in rats. *Proc. Nat. Acd. Sci., 60*(b), 112-122.

Rao, M. V., Panchal, B. G., Shaikh, S. A. and Suthar, M. B. (2003). Role of ascorbic acid on $NiCl_2$ induced reproductive toxicity in female mice. *J. Tissue Res., 3*(1), 41-46.

Rogers, J. M. (1996). An introduction to developmental and reproductive toxicology of metals (In: Chang, L.W. Toxicology of metals (Eds.I) 975 – 976. CRC Press Inc.) USA.

Ramlingam, V., Vimaladevi, V., Rajeswary, S. and Suryavathi, V. (2003). Effect of mercuric chloride on circulating hormones in adult albino rats. *J. Environ. Biol., 24*(4), 401-404.

Steven, D. H. (1975). Comparative placentation. Academic Press, London, New York and San Francisco.

Solomon, S., Bird C.E., Ling, W., lwamiya M. and. Young, P. C. M. (1967). Formation and metabolism of steroids in the fetus and placenta. *Rec. Prog. Horm. Res*., (23), 297-347.

Tiwari, V. and Bhattacharya, L. (2004). Adverse effects of mercuric chloride on thyroid of mice, *Musculus albinus* and pattern of recovery of the damaged activity. *J. Environ. Biol*., *25*(1), 109-111.

Viswanathan, V. S., Leela, V, Eswari, S. and Premkumar David, B. (2000). Correlation of thyroid hormones with total serum proteins and cholesterol during pregnancy in Sheep. *Cherion.,* (29), 94-96.

Young, D. S., Pestaner, L. C. and Gilberman, U. (1975). Effects of drugs on clinical laboratory tests. *Clinical Laboratory*, *21,* 3660.

How to cite this article: Vishwas Tiwari (2023). Alteration in the Serum Cholesterol Level of Dams due to Intoxication of Mercuric Chloride and its Recovery by Ashwagandha and EPL. *Biological Forum – An International Journal, 15*(1): 554-558.