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Research Article

Biochemical assay of embryo toxicity on Phorate exposed mice and its removal by vitamin C

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Abstract: *Phorate a organophosphate pesticide commonly used to control pests like various leaf feeding insects, mites and soil insects terrestrial crops such as beans, cotton, hops, peanuts, potatoes, sorghum, soyabean, sugar beats, sugarcane etc [1,2]. Extensive use of pesticides in the agricultural field increased the possibility of exposure even to a low level. The commercial use of pesticide also affects the non-target organisms like fish, birds and mammals [3]. Our study is to investigate the toxicity appearing in embryos during early and late pregnancy of female mice. Three group of female mice were taken: control, phorate (phorate was administered intra-peritonially at a concentration of 1mg/kg body weight), Phorate+vit C (also administered intra-peritonially at a concentration of 1mg/kg body weight). All sets of pregnant mice studied as 7 days, and 14 days exposure of phorate. After sacrificed them, following events were found: death of embryo on premature and mature state, unnatural fat deposition on mother's uterus but no malformed embryo was found [4,5,6]. To establish a possible cause of the experimental finding following biochemical assays were performed on liver, kidney and brain tissue both on mother and embryo [7]. The biochemical assays were estimation of total serum protein, alanine aminotransferase (AST), Alanine Amino Transferase (ALT), Lipid peroxidation (LPO), catalase activity and acetylcholinesterase (AchE) activity. The above assays were also performed on Phorate and Phorate + vit C exposed mice to study the ameliorating effect. In conclusion these experimental inference may be beneficial for the other non-target creatures that are crucially important for maintaining the natural balance of flora and fauna as well as for the human beings in future.*

Keyword: *Embryological toxicity; Phorate; Vitamin C*

1. Introduction

Phorate,chemical name Phosphorothioic acid , O- dietmyl S (ethyl thio)methyl ester, an organophosphate , systemic and broad spectrum insecticide inhibit actylcholinesterase (AchE) activity by phosphorylating the serine hydroxyl group in the substrate binding domain of acetylcholine and induces neurotoxicity [8]. Photare has been classified as a class I, high risk toxic OP compound with and LD_{50} of 1.1 to 3.7 mg/kg body wt. for rats [9]. Phorate is used against sucking and chewing insects, leaf hopper, mitessome nematodes, rootworms in agricultural field to protect the crops (phorate 171-205 JMPR 2004; [10]. Through the food chain it reaches to some nontarget organism other than pests and causes harmful hazards [11]. Not only that, phorate also has a harmful effect on embryo. Maternal and embryo toxicity was found at dietary doses of

0.5mg/kg/day fed to rats [12]. Review of study suggests substantial phorate induced generation of intracellular ROS is atributed to mitochondrial dysfunction and leads to oxidative stress [13]. The aim of the study is to evaluate the embryo toxicity due to exposure of phorate and their recovery by vit C [14].

2. Experimental design

A total number of 15 healthy Swiss albino mice (*Mus musculus*) weighing between 20 to 22 gms were randomly selected for experimental purpose and were subdivided into three groups, each group comprising five mice. One group for control which was provided normal diet *ad libitum*. The next group was provided with phorate (1mg/kg. body wt.) and the third group was provided with phorate and vit C (500mg/kg. body wt.) for recovery. All groups were provided the treatment for seven days and after that they were sacrificed and blood, liver and brain tissue were collected.

3. Materials and methods

3.1. Collection of tissue

After sacrifice of the mice, liver and brain tissues were collected and kept separately in Petri dish (-80 ˚) till homogenization. A part of the tissue was diluted for quantitative estimation of protein and biochemical assay.

3.2. Isolation of serum from blood

Blood was drawn by ventricular puncture of etherized (approximately 1ml from each mice) by the routine procedure using sterile disposable synringe and needle. Blood was collected in 15ml centrifuge tube (Axygen scientific, lot no. 061016058) without EDTA. Serum was obtained by centrifugation.

3.3. Sample homogenization and centrifugation

50mg tissues were homogenized in 2ml of Phosphate buffer (PBS) and the homogenized tissues were spun in refrigerated centrifuged (REMI C 24model, India) at 5000rpm for 15 min at 4˚ C. After that the supernatants were stored at -80˚C for biochemical assay.

3.4. Estimation of total protein

For quantitative estimation of total protein, the [15] was used.

3.5. Estimation of Lipid Peroxidation (LPO)

The spectrometric assy of Lipid peroidation (LPO) was performed following the protocol of [16] with some minor modification.

3.6. Estimationof total thiol content

For the estimation of total thiol content, the protocol of [17] was followed with minor modification.

3.7. Estimation of catalase activity

The quantitative measurement of Catalase activity was done by the method of [18]

3.8. Estimation of acetylcholine Esterase (AchE) activity

For the estimation of acetylcholine esterase (AchE) activity was done by the method of [19], A new and rapid colorimetric determination of acetylcholine esterase activity. Bio-chem. Pharmacol.7,88Ð95.

3.9. Estimation of AST and ALT

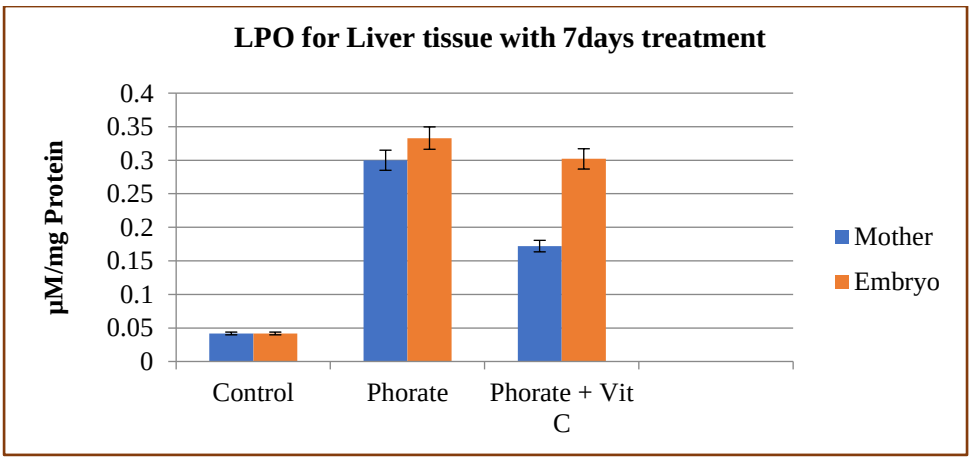
Estimation of AST (Aspertate Amino transaminase) and ALT (Alanine Amino Transferase) the method of [20] was used.

4. Results and discussion

4.1. Treatment of Phorate to Pregnant mother and their embryos

4.1.1. Result analysis of LPO for 7ds.

Sample	Liver Tissues				
	Cont.	Treat.	Sig.	Recovery	Sig.
Mother	0.0418 ± 0.005	0.300 ± 0.041	P<0.001	0.172 ± 0.000	P<0.001
Embryo	0.0418 ± 0.005	0.333 ± 0.006	P<0.001	0.302 ± 0.008	P<0.001

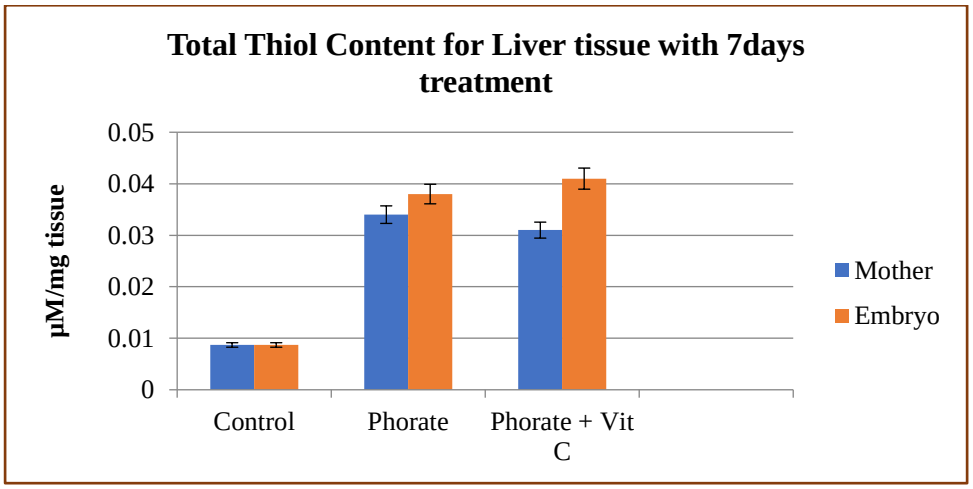


The LPO level was significantly increased in both mother and embryo, treated with Phorate in compare to control but decreased significantly in mother and embryo tissue in the recovery phase which were treated with both phorate and vit C.

The increased level of LPO both in mother and embryo after phorate treatment for 7 days may due to elevation of MDA level which is responsible for the generation of free radical, to protect the tissue from oxidative damage. Bagchi et. al. (1995) have shown that different classes of pesticides induce the production of reactive oxygen species (ROS) and tissue damage. Other reports indicate that the enzyme activities associated with antioxidant defence mechanisms are altered by insecticides both in vivo and in vitro [21]. The decreased level of LPO shows by the treatment of vit C in mother and embryo tissue. From the above finding it can be said that the anti-oxidant property of vit C protect mother and most importantly the embryo hepatic tissue from the oxidative damage.

4.1.2. Result analysis of Total Thiole content for 7ds.

Sample	Liver Tissues				
	Cont.	Treat.	Sig.	Recovery	Sig.
Mother	0.0087 ± 0.000	0.034 ± 0.005	P<0.001	0.031 ± 0.000	P<0.001
Embryo	0.0087 ± 0.001	0.038 ± 0.001	P<0.01	0.041 ± 0.000	P<0.001

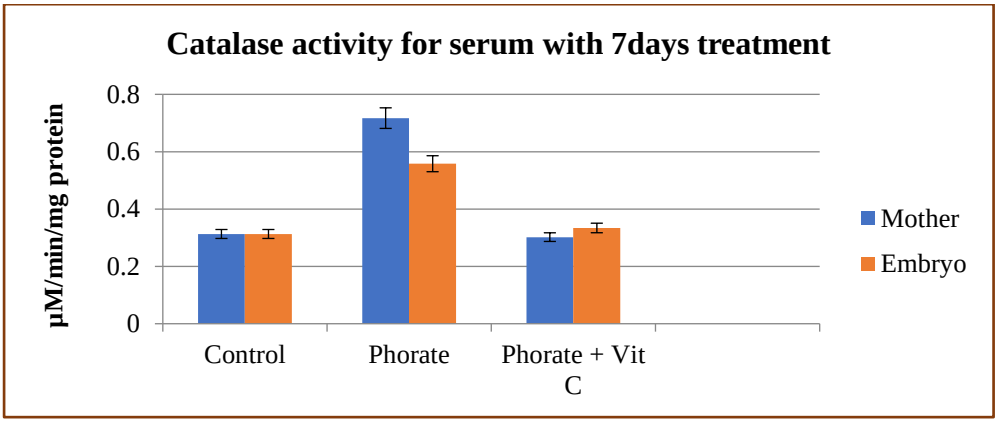


The total thiol content was significantly increased in both mother and embryo, treated with Phorate in compare to control but decreased significantly in mother tissue in the recovery phase which were treated with both phorate and vit C but in embryo the total thiol content was increased in the recovery phase means vit C can't provide protection.

The increased in total thiol content both in mother and embryo may be due to defense activity of thiol against free radicals. Review of study found that thiol share significant role in detoxification, signal transduction, apoptosis and various other functions at molecular level [Journal (on-line/ unpaginated); ID code 6664]. But the vit C protection significantly recover the free radical damage due to its antioxidant property in mother. But in case of embryo Vit C could not protect the embryo from this oxidative damage. It may be due to short term exposer of Vit C on embryo.

4.1.3. Result analysis of Catalase activity of serum for 7ds.

Sample	Serum Tissues				
	Cont.	Treat.	Sig.	Recovery	Sig.
Mother	0.313 ± 0.001	0.717 ± 0.011	P<0.001	0.302 ± 0.002	P<0.01
Embryo	0.313 ± 0.001	0.558 ± 0.038	P<0.001	0.334 ± 0.001	P<0.001

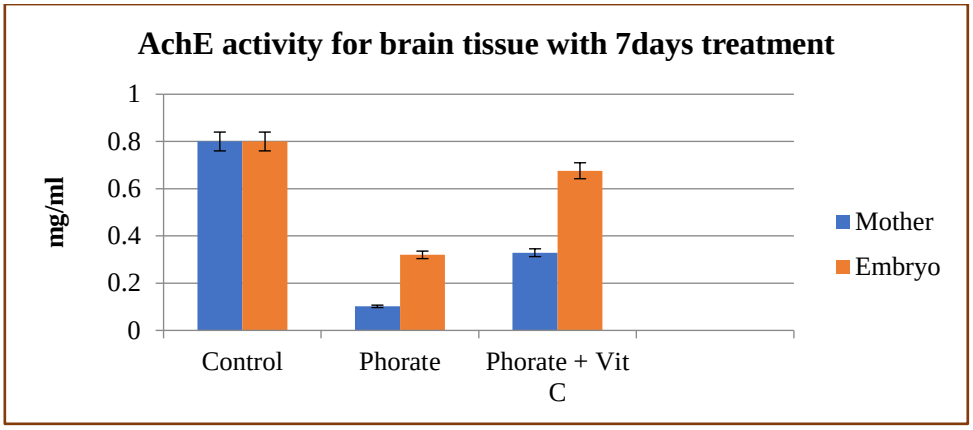


The catalase activity was significantly increased in both mother and embryo, treated with Phorate in compare to control but decreased significantly in mother and embryo tissue in the recovery phase which were treated with both phorate and vit C [22].

The Catalase activity can be considered as a sensitive biomarker for bio monitoring the environment. The catalase enzyme assay result revealed that embryos need more protection from pesticide effects [23]. It may be considered that vit.C was unable to cross the placental barrier.

4.1.4. Result analysis of Brain Acetyl cholinesterase (AchE) activity for 7ds.

Sample	Brain Tissues				
	Cont.	Treat.	Sig.	Recovery	Sig.
Mother	0.800 ± 0.001	0.102 ± 0.010	P<0.001	0.329 ± 0.005	P<0.001
Embryo	0.800 ± 0.001	0.320 ± 0.082	P<0.001	0.676 ± 0.047	P<0.05

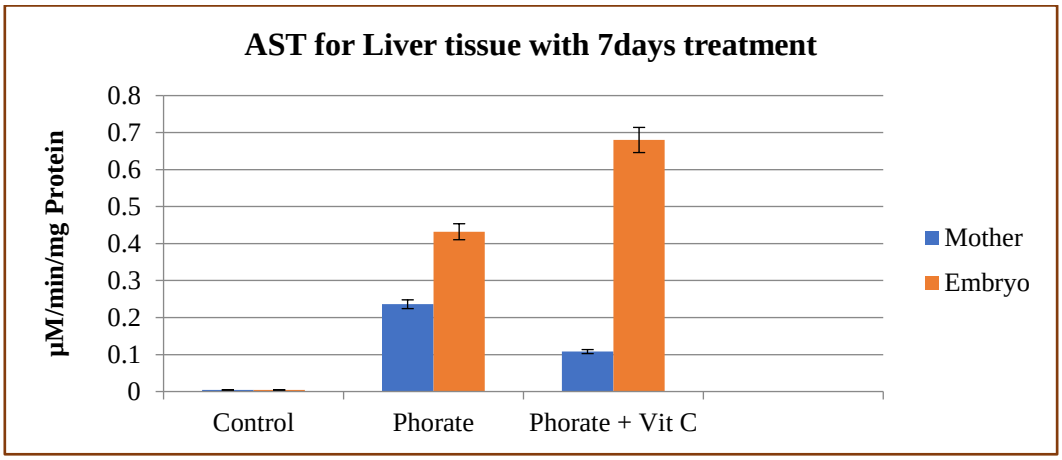


The AchE activity in brain tissue was significantly decreased in both mother and embryo, **treated** with Phorate in compare to control [24] but decreased significantly in mother and embryo tissue in the recovery phase which were treated with both phorate and vit C.

The significantly decreased level of AchE may be due to inhibition of catalytic activity of acetylcholine, the most excitatory neurotransmitter in the central nervous system (brain) [24]. The possible reason may be due to oxidative stress upon neurotransmitter enzyme [25]. The toxic effect of phorate was recovered or ameliorated by conjoint treatment of Vit.C with phorate, So that the cholinesterase enzyme activity increased both in mother and embryos. Specially in embryos the result of phorate + vit.C was near about to control result which may confirm that, Vit.C has an antioxidant property against phorate treatment [22].

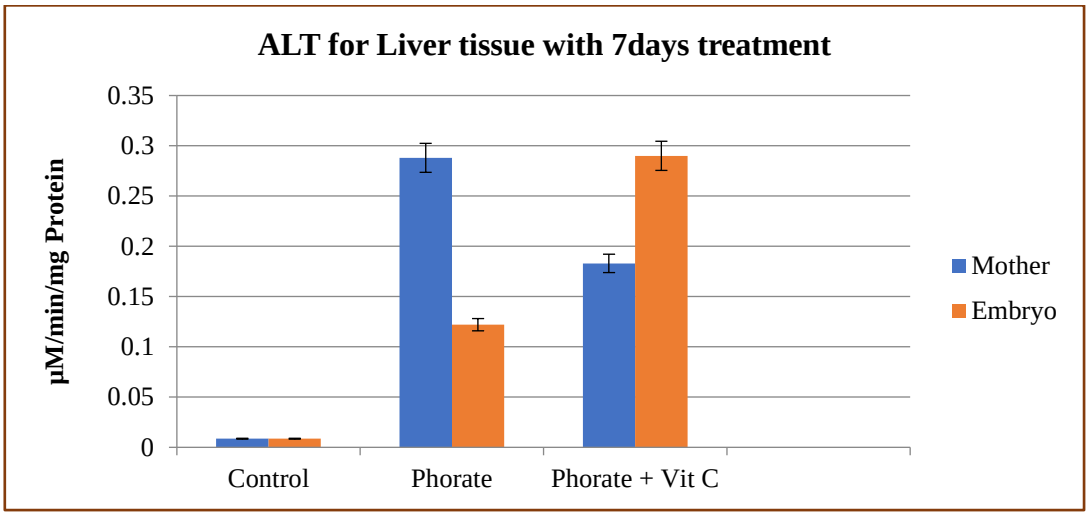
4.1.5. Result analysis of AST for 7ds.

Sample	Liver Tissues				
	Cont.	Treat.	Sig.	Recovery	Sig.
Mother	0.0041 ± 0.001	0.236 ± 0.038	P<0.001	0.108 ± 0.000	P<0.001
Embryo	0.0041 ± 0.001	0.432 ± 0.041	P<0.001	0.680 ± 0.007	P<0.001



4.1.6. Result analysis of ALT for 7ds.

Sample	Liver Tissues				
	Cont.	Treat.	Sig.	Recovery	Sig.
Mother	0.0086 ± 0.001	0.288 ± 0.023	P<0.001	0.183 ± 0.000	P<0.001
Embryo	0.0086 ± 0.001	0.122 ± 0.018	P<0.001	0.290 ± 0.003	P<0.001



Both the AST and ALT level were significantly increased in both mother and embryo, treated with Phorate in compare to control but significantly decreased AST and ALT level were shown in mother tissue in recovery with vit C [26]. But in case of embryo vit C could not recover the ASl and ALT level as its value was increased [25].

For detecting hepatic damage, liver AST and ALT was done and it shows a significant increased in phorate treated mother and embryo tissue. But by the treatment of vit C which is an anti-oxidant, reduces the AST level in mother and embryo which suggests its hepatoprotective activity [27]. In case of hepatic ALT level, it significantly reduced in mother liver tissue but doesn't affect the embryo. It may due to the single dose of vit C cannot protect the hepatic damage and needs more expouser of vit C [22, 28].

5. Conclusion

The organo-phosphate pesticide phorate is an organic compound, unusual for the living body system used to eradicate the pest organisms. It is very much harmful for the living body system revealed by the unusually elevated level of enzyme activities for LPO, Thiol, Catalase, Acetly cholinesterase (AchE), AST & ALT.The application of vit.C may act as an ameliorating agent having anti-oxident property [26,29]. This is true in case of direct treatment for pregnant mice (mother). But this

defensive activity is not truely followed for the embryos as revealed by the higher level of enzyme activities in LPO, Thiol, AchE, AST & ALT and slightly increased level of catalase activity. From the overall results and discussions, it is revealed that vit.C can act as an ameliorating agent [30, 31] against direct treatment of pesticide but in the embryos the administered dose of vit.C had little effect revealed by higher enzyme activities. So, more investigation is required for the embryo-toxicity. We are investigating whether the placenta act as a barrier for vit.C [32].

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