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Prevention of Mercuric Chloride induced Cerebellar Damage in Mice with a Multiherbal Hepatic Drug Results and Possibilities

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ABSTRACT

Male Swiss albino mice, 5-6 months old were divided into four groups. Group I acted as controls, no treatment at all; Group II received 0.5 ml Liv.52 syrup/day/mouse upto 400 days. Three sub-groups of Group III were exposed to mercuric chloride as drinking water dissolved in distilled and deionized water at three concentrations and for three duration i.e. 0.5mM for 30 days. 0.1 mM for 100 days and 1µg/ml for 400 days. Group IV was like that of previous group where mice exposed to three concentrations of mercuric chloride were also orally administered 0.5 ml Liv.52 syrup/ day/mouse for corresponding durations. Whole brains were fixed on day 31st, 101 and 401. Histology of cerebellar region revealed that drug alone does not cause untoward effect but mercuric chloride induced severe damage to granular layer of cerebellar cortex at 0.5 mM while lower doses i.e.0.1mM and 1 µg/ml caused mild effect. Drug could not prevent mercury induced cerebellar pathology at highest dose but could do so at two lower doses tested. Infrared spectroscopy revealed in vitro interaction between mercuric chloride and Liv.52 as Cl_2 was broken off. Possible action of drug is discussed.

Keywords: Herbal drug, Mercury chloride, Mice, brain, protection

INTRODUCTION

Human brain is susceptible to inorganic mercury poisoning hence occupationally exposed people like dentists and chloralkali workers remain a high risk of mercury exposure (EHC-118)[1]. It was felt worth testing protective role of a multiherbal drug Liv.52 towards mice brain against mercuric chloride poisoning as this drug was found to do so for mice blood, liver, gut and kidney [Rathore and Varghee[2], Varghese and Rathore)[3].

Infrared spectroscopy (IR) was also done to find out if any *in-vitro* interaction takes place between drug and mercuric chloride so that action of drug can be understood and explained.

EXPERIMENTAL PROCEDURES

Present investigation consisted of two sets of experiments. First one was designed to test ability of the drug to prevent $HgCl_2$ induced cerebellar damage. Second experiment was designed to learn about the direct (*in vitro*) interaction between drug (herbal mixture powder *Capparis Spinosa, Cichorium intybus, Solanum nigrum, Cassia occidentalis, Terminalia arjuna, Achillea millefolium, Tamarix gallica* and Mandur bhasma) and mercuric chloride by IR spectroscopy. Experiments were done as follows:

Histopathology of cerebellar region

Six months old male Swiss albino mice were divided into the following groups:

Group I Controls (C): No treatment. Mice on standard food and distilled & deionized water *ad-libitum.*

Group II only Drug (D): In this group mice received only Liv.52 syrup 0.5 ml/day/mouse upto 400 days.

Group III Mercuric Chloride treatment (P): Mercuric chloride salt [Ranbaxy 99.9% pure) was dissolved in distilled and deionized water to prepare three different concentrations which were individually offered as drinking water to mice for three different duration i.e. 0.5 mM for 30 days. 0.1 mM for 100 days and 1 µg/ml for 400 days.

Group IV Liv.52 Administration During $HgCl_2$ Exposure (P+D): Mice drinking solutions of three concentrations of mercuric chloride (as in group III) also received 0.5 ml Liv.52 syrup/day/mouse for corresponding days i.e. for 30, 100 and 400 days. Bouins fixed brains sectioned at 4 microns were stained with Delafields, Haematoxylin and Eosin. Observations of the cerebellar region have formed the basis of present results and discussion.

Infrared Spectroscopy (IR-study)

Three samples were prepared and analyzed on IR instrument (Perkin Elmer – 377). First sample consisted of dry mercuric chloride salt. Second one consisted of Liv.52 powder (drug is available as tablets having powder, syrup and drops). Third one was prepared by mixing drug powder with saturating amounts of 0.5 mM solution of mercuric chloride and its subsequent air-drying. For all these three samples peaks were recorded and analyzed.

RESULTS

Histological: In control mice cerebellar cortex showed normal appearance of Purkinje cells and granular layer. Drug did not affect at all. Mice exposed to 0.5 mM mercuric chloride for 30 days revealed damage to granular layer, which could not be prevented with the use of drug. Mice exposed to 0.1 mM and 1µg/ml of mercuric chloride for 100 and 400 days respectively showed mild effect i.e. thinning and depletion of granular layer. This effect was not seen when herbal hepatonic drug was administered during mercuric chloride exposures. Figures 1 to 8 support histological findings.

Mice cerebellar Cortex – Hematoxylin Eosine – C.S. 400 X

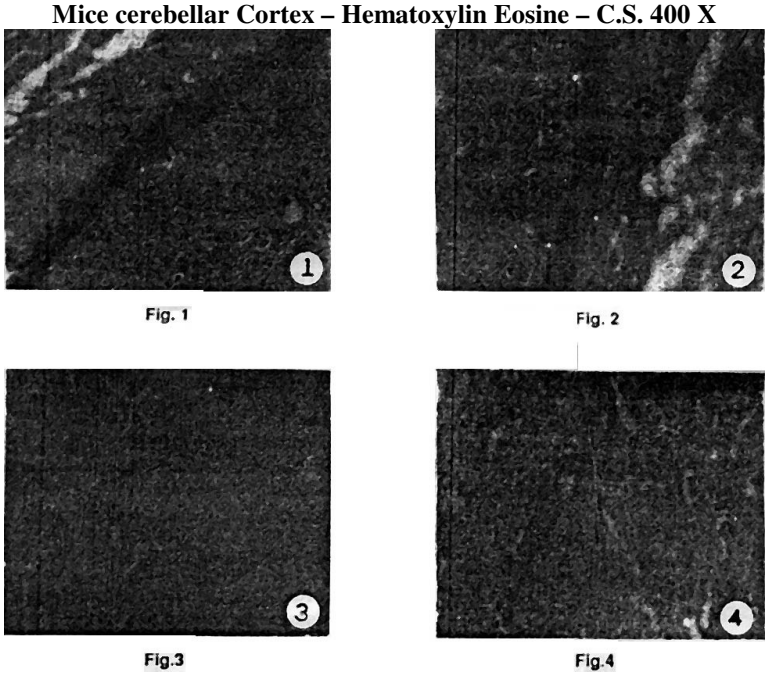

Fig. 1 Fig. 2

Fig.3 Fig.4

Fig. 1: Controls showing normal appearance of Purkinje cells and granular layer – both layers well defined. **Fig. 2:** Only Liv.52 administration for 400 days, control like appearance. **Fig. 3:** 0.5 MM $HgCl_2$ exposure for 30 days caused damage to the granular layer. **Fig. 4:** Liv.52 during $HgCl_2$ exposure for 30 days could not prevent damage.

Infrared Spectroscopy [IR

Spectra]: The IR spectra of Liv.52 and mixture (Liv.52 plus $HgCl_2$) showed OH peak at 3450 cm^{-1}. CH stretching at 2920, 2860, CH bending peak at 1440-1420 cm^{-1}. C-O stretching peak and C=C peak at 1630^{-1} cm

The mercuric chloride showed only two peaks at 1570-1520 and at 880 cm.

In the mixture 880 cm^{-1} peak was absent suggesting that from $HgCl_2$ the "Cl_2" must have been broken off. Interpretation is based on standard procedure of absorption spectroscopy (Dyer).

Mice cerebellar Cortex – Hematoxylin Eosine – C.S. 400 X

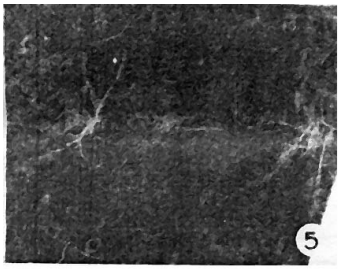

Fig. 5

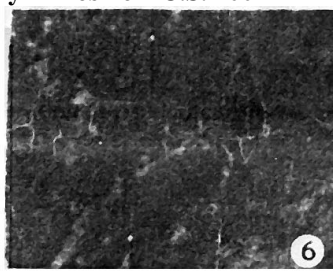

Fig. 6

Fig. 7

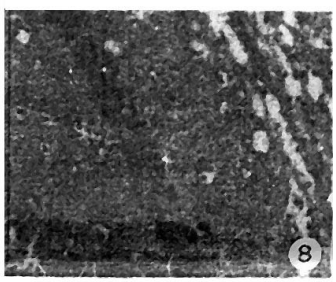

Fig. 8

Fig. 5: 0.1 mM $HgCl_2$ exposure for 100 days caused less distinct and depleted granular layer. **Fig. 6:** Liv.52 administration during $HgCl_2$ exposure for 100 days shows almost normal histology. **Fig. 7:** Lug/ml $HgCl_2$ exposure for 400 days caused slight disorganization of granular layer. **Fig. 8:** Liv.52 administration during $HgCl_2$ exposure for 400 days shows quite normal histology.

DISCUSSION

Mercuric chloride severely damaged the cerebellar cortex region of mice brain at 0.5 mM dose while mild effect was seen at two lower doses i.e. 0.1 mM and 1µg/ml. Similar effect of elemental inorganic and organic mercury exposures (Minimata episode in Japan) are on record (Toxicological Profiles, USA)[3]. For the sake of relevancy, present discussion is restricted to the inorganic mercury only. Metallic mercury caused necrosis in the brain of rabbits and mice (Ashe *et al.*[6], Ganser and Kischner[7]). Oral and subcutaneous administration of mercuric chloride for 11 weeks caused degenerative changes in the cerebellum of rat (Chang and Hartman a & b)[8,9]. Troen *et al.*[10], studied mercuric chloride poisoning among 65 human subjects and reported pathological lesions in their brain. Davis *et al.*[11], reported Hg-poisoning among two women patients following chronic (6 to 25 years) ingestion of tablet laxative that contained 120 mg US grade mercurous chloride (Hg_2Cl_2). At autopsy, low brain weight, low volume and a reduced number of nerve cells in the cerebellum was recorded Light microscopic analysis of histochemical stains revealed granules of mercury within neuronal cytoplasm.

Administration of herbal drug Liv.52 during mercuric chloride exposures could reduce toxic effect at two lower does but failed to act at highest dose tested. Now role of drug can be looked upon.

First dose (0.5mM) was quite high as LD_{50} for mercuric chloride is 10 mg/kg for mouse (EHC-118) and animals were dying such observation does not deserve any discussion.

No behavioural parameter was taken into consideration except an observation "Food-intake" (already reported by Rathore and $Varghese_2$), which is governed by brain centres. Mercuric chloride

caused reduction in food intake at all exposure doses. This observation was in good conformity with the earlier reports that mercury affects brain centres and results in hypophobia (Berthoud *et. al.*)[12]. This effect was reduced appreciably in the presence of drug.

This is not easy to predict the role of drug it must have been playing at the level of brain during mercuric chloride exposures at 0.1 mM and 1µg/ml concentrations. We have noticed that this drug could reduce and nullify $HgCl_2$ induced histopathological changes in the mice gut (Varghese and Rathore[3]). This in turn might have improved food-intake. This seems reasonable as blockage of the transport of nutrients in the brain (Abe *et al.*)[13] has been suggested as possible mechanism of action Hg^{2+} at brain level.

Hg ions bind with-SH group in biomembrances and damages them via lipid peroxidation (Gstraunthaler) and lowers phosphorus incorporation the brain phoshpolid (Mehra and Kunwar[15]). Hg^{2+} ion labialise lysosomal membranes (Verity and Reith[16]) inhibits protein synthesis (Nakada *et al.*[17]) affects structure and synthesis of RNA and DNA (Eichhorn and Clark[18], Gruenwedel and Davidson[19]) and disturbs structure and function of mitochondrial membranes (Humus and Weinburg[20]). In this way inorganic mercury induces cellular damage (EHC-118)[3].

On the other hand this herbal drug Liv.52 was found to revert all above cited effect following carbontetrachloride, alcohol and radiation by lowering lipid peroxidation and by enhancing tissue GSH content (Saxena and Garg[21,22], Saxena *et al.*[23], Goel and Dhawan[24], Bardhan *et. al.*[25], Subbarao and Gupta[26], Jagetia and Ganapathi[27], Sarkar *et. al.*[28]).

Results of IR study revealed that drug and mercuric chloride did react atleast *in vitro* system and CL^2 was broken off. This observation is as typical as another recent one (Rathore and Verghese)[29] that drug influences the uptake, retention and excretion of Hg from mice blood, liver, testis and kidney but not from brain. Mild chelating action of drug can be held for preventing other organs but not the brain.

Authors are of opinion that drug must have acted in some complicated way and further deep research is needed to confirm that protective role of this drug so that it can be used in future as one of the supportive occupational medicine. This concluding statement is based on facts that once damaged, brain tissue does not regenerate in mammals and common food items in India have been reported to contaminated with mercury (Ghoshdastidar and Chakrabarti[30], Lenka *et. al.*[31], Panda *et al.*[32]).

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