Prevention of toxic effects of mercuric chloride on Some male accessory organs in mice with a Multiherbal drug “SPEMAN”

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ABSTRACT: Adult Swiss albino male mice exposed to mercuric chloride via drinking water at 5 µg/ml for 100 days revealed significant reduction in the wet weight and severe histopathological changes in male accessory organs, poor level of serum testosterone and infertility. These effects were reduced remarkable and fertility was restored when drug (12.50 mg/mouse/day orally) was administered during mercury exposure for 100 days or after Hg-exposure for next 60 days (Post therapy). Natural recovery after mercury exposure for 60 days remind ineffective. Probable action of herbal drug based on the presence of the active principles of constituents (i.e Orchis mascula, Mucuna pruriens, parmelia perlata, Argyreia speciosa, Tribulus terristris, Leptadenia reticulata, Lactuca scariola and Hygrophila spinosa) is discussed in detail.

Key words: Arachidic acid, Linoleic acid, Stigmasterol, Dopamin., Glutathione, S, Vit A, B1, C and E, Zn, Ca Cu and Co.

INTRODUCTION

Inorganic mercury induced testicular1,2 and epididymal3 damage in experimental animals, loss of libido, infertility and impotency among occupationally exposed4,5 man are established facts. Recently a non-sterpoidal, multiherbal drug “speman” Could restore mercuric chloride induced loss n food and water consumption, body weight6 and testicular and epididymal dysfunction7. Male accessory organs play critical role in the process of reproduction8 hance further studies included seminal vesicles, prostrate and vas deference.

MATERIALS AND METHODS

Six months old isogenic Swiss albino mice were divided into the following five groups

Group I Controls (c): 30 mice on standard food and tap water ad-libitum, 0.5 ml distilled water per mouse/day orally.

Group II Mercuric chloride exposure (P): 90 mice on standard food but drinking mercuric chloride (HgCl2, Rabaxy, 99.9% pure salt) solution in tap water at 5 µg/ml ad-libitum, 0.5ml distilled water/mouse/day orally.

Group III Speman administration during mercury exposure (P+D): 30 mice drinking mercuric chloride solution as in earlier group also received orally smooth suspension of 12.50 mg Speman in 0.5mpl distilled water /mouse/day. All mice (leaving five form each group for fertility testing) of groups I and III and 25 of group
11 were sacrificed on day 101. Remaining 60 mice of group II were shifted on mercury free water and were divided into two groups.

**Group IV Post therapy with drug (PT):** 25 mice received drug (12.50 mg/mouse/day as in P+D) for next 60 days.

**Group V Natural Recovery (NR):** 25 mice were allowed to recover naturally. Mice of groups IV and V were killed on day 161 (leaving 5 mice for fertility testing).

**Fertility Testing:** Five mice from each group were kept with untreated virgin females (1:1) for next 60 days to observe live births.

**Histology:** Freshly dissected seminal vesicles, vas deferens and whole prostates were weighed and fixed in Bouin’s fluid for histology.

**Sperm counting:** Epididymis were kept and opened in clot diluent.

**RIA for serum testosterone:** Freshly collected serum was used to measure serum testosterone level using kit made by diagnostic system laboratories, Mumbai.

**Use, composition and dose of drug:** Himalaya Drug company, Bangalore, manufactures this multiherbal drug under trade name “Speman” which is used to cure infertility and for symptomatic relief of prostatic enlargement. Drug is composed of orchis mascula, lactuca scariola, Hygrophila spinosa, Mucuna pruriens, Parmelia perlata, Argyreia speciosa, Tribulus terrestris, Leptadenia reticulata, lactuca scariola and Hygrophila spinosa is given in the subsequent paragraphs.

**RESULTS**

Results are presented in the form of table I, which clearly show mercuric chloride-induced loss in the wet weight of accessories, in the level of serum testosterone and infertility. Administration of drug during Hg-exposure i.e. group P+D could reduce toxicity of mercuric chloride while post-therapy (PT) could revert toxic effects fully and could restore fertility too. Histological observations confirmed Hg-induced damage which remained as such following natural recovery while drug could prevent such damage. Only for the sake of brevity figures are omitted from the text.

**DISCUSSION**

Mercuric chloride damage testis and lowered serum testosterone level in mice which can be held responsible for Hg-induced reduction in the wet weight of accessory organs in the present case as castration is known to cause similar changes. HgCl₂ induced histological changes in present study are not unexpected as mercuric chloride is known to do so towards testes and epididymis hence this observation does not possible protective action of drug against mercury toxicity based on the presence of known active components in the drug which is a mixture of Orchis mascula, Mucuna pruriens, Parmelia perlata, Argyreia speciosa, Tribulus terrestris, Leptadenia reticulata, Lactuca scariola and Hygrophila spinosa is given in the subsequent paragraphs.

Drug could prevent toxic effects (P+D) and cure when used after mercury exposure (PT). This reflects its probable androgen like activity because optimal level of androgens is essential for the maintenance of normal structure and function of gonads and accessory reproductive organs.
Interestingly, drug not only possesses precursors of male hormone but is also rich in substances which can reduce/ nullify toxicity of mercuric chloride.

-Linoleic acid is present in Hygrophila and Mucuna\textsuperscript{9,10} which is parent compound for the synthesis of prostaglandins and has varied function in reproduction. Arachidic acid present in lactuca\textsuperscript{9} controls androgen production\textsuperscript{11}. Stigmasterol is present in Hygrophila\textsuperscript{12,13}, Leptadenia\textsuperscript{14,15} and in Tribulus\textsuperscript{16} whose structure is similar to cholesterol required for testosterone and biomembrane synthesis. Mercuric chloride is known to inhibit testosterone synthesis\textsuperscript{5}.

-Gultathione is present in Mucuna\textsuperscript{9}, ascorbic acid in Lactuca\textsuperscript{17} and Mucuna\textsuperscript{18} and Vitamin –E in lactuca\textsuperscript{9}. All these being antioxidant can prevent mercury induced damage to biomembranes via lipid peroxidation\textsuperscript{19,20}. Ascorbic acid also helps in fertility\textsuperscript{21} and in the synthesis of testosterone\textsuperscript{22}. also it could protect mice testis against radionuclides\textsuperscript{23}.

-Sulphur is present in Lactuca and Mucuna\textsuperscript{9} and mercury ions have strong affinity for –SH groups. Exogenous sulphur can alter cellular binding of mercury ions thereby reducing its toxicit.\textsuperscript{24}

-Thymine (Vitamin-B1) is present in Lactuca\textsuperscript{9} helps the growth of leydig cells and accessory reproductive organs\textsuperscript{25}. Vitamin-A present in lactuca\textsuperscript{9,17} is essential for synchronized germ cell cycle in mammalian testes\textsuperscript{26} and for the maintenance of proper levels of iron. Zinc and copper\textsuperscript{27}.

-Mercuric chloride induced gut pathology in mice is known\textsuperscript{28} but poor absorption of amino acids, sugars and minerals ma be compensated as the drug possesses man of them.

-Twenty seven amino acids including essential ones are present in Argyreia\textsuperscript{29}, Mucuna\textsuperscript{18,30} and tribulus\textsuperscript{31}. Appreciable amount of proteins are present in Hygrophila\textsuperscript{32}, Lactuca\textsuperscript{9}, Leptadenia\textsuperscript{9}, mucuna\textsuperscript{9,33,34} and Tribulus\textsuperscript{16,35}. Optimal levels of amino acid protein are essential for mammalian spermatogenesis\textsuperscript{36}. These can restore the process of DNA and protein synthesis which are inhibited mercuric chloride\textsuperscript{37}.

-Many sugars are present Mucuna\textsuperscript{31}, Tribulus\textsuperscript{16} and Leptadenia\textsuperscript{33} which may be utilized for energy requirements as mercury ions damage mitochondria and lowers tissue resporation\textsuperscript{38}. Zinc, calcium magnesium, copper and cobalt are present in Hygrophila and Mucuna\textsuperscript{9,39} and these are also needed for normal spermatogenesis\textsuperscript{36,40}. Lastly, presence of L.dopamin in Mucuna\textsuperscript{9} can restore male sex behaviour as mercury causes loss of libido\textsuperscript{4,5}.

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Table 1: Effects of mercuric chloride and speman on the wet weight of epididymis, sperm population, serum testosterone level and fertility in mice

(Mean ± S.E.M)

| Parameters                                      | Control (‘C’) | HbCl2 exposed (‘P’) | Speman while exposed to HgCl2 (‘P+0’) | Speman after HgCl2 exposure (‘PT’) | Natural Recovery (‘NR’) |
|-------------------------------------------------|---------------|---------------------|--------------------------------------|----------------------------------|------------------------|
| Wet Weight of seminal vesicles (mg)             | 78.90±1.16    | 56.30±0.88          | 69.82±1.32                          | 80.00±1.71                       | 61.58±1.91             |
| Wet weight of prostrate (mg)                    | 32.48±1.32    | 21.25±1.50          | 31.87±1.23                          | 31.02±1.11                       | 25.18±1.01             |
| Wet weights of vas deference (mg)               | 1894±0.25     | 10.64±0.64          | 16.25±1.2                           | 19.35±1.52                       | 14.26±1.71             |
| Serum testosterone level (mg/ml)                | 2.75±0.27     | 0.12±0.03           | 2.70±0.32                           | 2.71±0.39                        | 0.16±0.005             |
| Sperms million/ml                               | 45-50         | Nil                 | 30-35                               | 40-45                            | Nil                    |
| Motility                                        | 70%           | Nil                 | 50%±b                              | 65%±b                            | Nil                    |
| Fertility (5M/5F)                               | 80%(4/5)      | 0%(0/5)             | 40%a,b(2/5)                         | 0%±b (3/5)                       | 0%(0/5)                |

Statistically significant based on ‘t’ test at 5% level of significance.
A=C versus all groups and b=P versus all groups.