EFFECT OF ASCORBIC ACID IN THE DETOXICATION AND PROTECTION AGAINST MERCURIC CHLORIDE POISONING

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Abstract

This study was designed to evaluate the protective and detoxificated effect of ascorbic acid (vit. C) against mercuric chloride toxicity, in normal adult albino rats. The experiment was carried out on 60 adult rats weighing between 200 - 300 g, and divided into 6 groups, each containing 10 rats. The first group acts as a control group. Vitamin C was given to the rats of the second group in a dose of 45 mg/100g body weight. In the third group, mercuric chloride was given in a dose of 1 mg /100 g body weight. In the fourth group, Vit C was administrated one hour after mercuric chloride injection. While in the fifth group, it was given one hour before mercuric chloride. The rats of the sixth group, were treated with vitamin C daily for one week before mercuric chloride administration. Blood for biochemical analysis was collected before killing the rats, then animals were killed by cervical dislocation. Liver and kidney were dissected and prepared for histological and histochemical examination. There was a statistically very highly significant increase in both liver and kidney function tests of mercuric chloride treated rats from the corresponding control group. This also, was confirmed by histological and histochemical changes in the liver and kidney. However, in rats were given ascorbic acid before mercuric chloride, we observed that the biochemical data did not differ significantly from the control values and also, the histological and histochemical changes were more or less similar to the control. Thus, indicating the protective role of ascorbic acid against mercuric chloride poisoning.
**Introduction**

Ascorbic acid (vitamin C), an antiscorbutic factor, is a water-soluble vitamin. It exists as either L-ascorbic acid or dehydroascorbic acid. The active agent is the enolic form of 3-keto-L-gulofuranlactone. Vitamin C has many important functions. It is a powerful reducing agent. It is essential for normal functioning of formative cells of various tissues such as osteoblasts and fibroblasts. It is essential for collagen formation. It takes part in the maturation of R.B.Cs and plays a certain role in carbohydrate metabolism. It may be useful in enhancing resistance against toxins and state of stress. It may block the formation of carcinogenic N-nitroso compounds in the human stomach after ingestion of some foods (Mirvish et al., 1982). As well, it can inhibit mutagenesis induced by such compounds in Salmonella strains (Khudoly et al., 1981). It inhibits mutagenesis induced by the fluorescent light in mice (Dunhan et al., 1982). It had a certain role in the detoxification of the organophosphorus insecticide dimethoate and the protection against its induced cytogenetic damage (Geetanjali et al., 1993). Other possible healthy effects of vit. C include a lowering of hyperlipidemia and protection against heavy-metal toxicity (Martell, 1982).

Mercury is a metal that, in its elemental form, and under normal conditions exists as a liquid. There are three types of mercury:

1. Elemental mercury (Hg) is found in glass thermometers, dental amalgams, scientific instruments and paints.

2. Inorganic mercury occurs in divalent (Hg ++ mercuric) or monovalent (Hg + ; mercurous) forms. Mercuric chloride, the most toxic inorganic form, has been used as a disinfectant. Mercurous chloride was once used as a teething powder and laxative.

3. Organic mercury occurs either as short-chain or long-chain forms. The short-chain alkyl compounds (methyl mercury and ethyl mercury) are well known as environmental contaminants that

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have been studied over the aquatic and terrestrial routes. Long-chain mercurials may be septic and nephrotoxic after chronic exposure in the kidney, while inorganic mercury is often found in the intestinal route and in the skin. After severe exposure, inorganic mercury may cause the nephrotoxicity, proteinuria and nephrotic syndrome (Nielsen et al., 1981).

In a series of experiments, a standard prophylactic dose of tetracycline HCl was used with and without ascorbic acid. Carroll et al. (1982) demonstrated that ascorbic acid was protective against the damage produced by the nephrotoxins and potassium dihydouracil (serine) but, very curiously, it did protect at all against the damage produced by potassium dihydrogen phosphate. Hence...
have been concentrated in the aquatic food chain. Long-chain alkyl and aryl mercurials are used as antiseptic and fungicides.

The inorganic poisoning most often occurs via the gastrointestinal route, although pulmonary and skin absorption can occur. After severe acute exposure, inorganic mercury is concentrated in the kidney, where it causes oliguric renal failure. However, chronic exposure may lead to proteinuria and nephrotic syndrome (Nielsen et al., 1991).

In a series of experiments using a standard preparation of tetracycline (Tetracycline HCl2 and ascorbic acid), Carroll et al. (1995) found that ascorbic acid was unable to exert any protective effect on the rat kidney subjected to ischaemia, and also it did not protect it against the damage by a number of nephrotoxins as (uranium nitrate, potassium dichromate and dl-serine) but, very surprisingly, that it did protect against the renal damage produced by mercuric chloride. Hence the present study was performed to evaluate the effect of ascorbic acid on the mercuric chloride toxicity in rats.

**Material and Methods**

The work was carried out on 60 adult male rats weighing between 200 - 300 g. They were given a standard diet and maintained at room temperature. They were divided into six groups, each contained 10 rats and were treated as follows:

**First group:** regarded as a control group.

**Second group:** the rats were injected with only ascorbic acid (Memphis Co.) intraperitoneally in a dose of 45 mg /100 g. body weight.

**Third group:** the rats were injected subcutaneously with only mercuric chloride (El-Gomhoria Co. of Pharmacy) in a dose of 1 mg/100g. body weight.

**Fourth group:** the animals were injected with the same dose of ascorbic acid (as second group) one hour after mercuric chloride injection (dose as third group).
Fifth group: the animals were given the previous dose of ascorbic acid as in the second group one hour before injection with mercuric chloride.

Sixth group: the animals were given the same dose of ascorbic acid daily for one week then they were injected with mercuric chloride.

The rats were killed 48 hours after the last injection by cervical dislocation. Blood samples were collected before killing for biochemical analysis of GOT, GPT, ALK.Ph.

Blood urea and serum creatinine to detect the liver and kidney functions. The results were statistically analyzed using the Student’s "t" test and the correlation coefficient test (Armitage, 1983).

Kidneys and liver were dissected and prepared for histological and histochemical examinations.

Paraffin sections at 6 micrometers were stained with Hx &E (Lamberg and Rothstein, 1978) and periodic acid Schiff reagent (PAS) according to Bancroft (1975).

Fresh frozen sections at 10 micrometers were stained for succinic dehydrogenase enzyme (S.D.) according to Pearse (1972), alkaline phosphatase enzyme (Alk. Ph.) according to Gomori’s method (Bancroft, 1975) and Adenosine triphosphatase enzyme (ATPase) according to Wachsteinand Meisel (1960).

Results

The laboratory data of liver and kidney function tests were summarized in tables 1-4 and graphs 1-4 and confirmed with histological changes in both organs and the histochemical enzymatic reactions.

Biochemical Data:

The laboratory finding of the second group of rats given only ascorbic acid were nearly similar to the first control group. In the third group mercuric chloride alone, induced highly significant elevations (P > 0.0005) of liver function parameters "GOT, GPT &ALK Ph." As well as the kidney function tests "blood urea and serum creatinine.

Histological Results

Liver:

First and second groups showed normal histological results.

Third and fourth groups liver showed marked dilatation of the central vein with infiltration including polymorph neutrophils and areas showed dilatation of portal banches of portal vein.

The central area showed marked dilatation with few hepatocytes showing pyroninophilic cytoplasm (Fig. 340).

Fifth and sixth groups liver showed mild dilatation of the central vein with infiltration and few histological changes.
In the 4th group, the differences were statistically non significant for both liver and kidney function tests. For the 5th and 6th groups, although the results were more or less similar, they were very highly significantly decrease from the mercuric chloride treated group for all parameters.

**Histological and Histochemical Results**

**Liver:**

**Histological results (Hx & E):**
First and second groups: The results were the same as in the normal animal.

Third and fourth groups: The liver showed marked cellular infiltration including eosinophils and polymorph neutrophils. The portal areas showed dilatation of the branches of portal vein.

The central veins showed marked dilatation and many of hepatocytes showed vacuolated cytoplasm (Fig 1).

Fifth and sixth groups: The liver showed mild dilatation of central vein with few cellular infiltration and few hepatocytes showed vacuolated cytoplasm (Fig 2).

**Histochemical Results.**

**PAS reaction:**
First and second groups: The glycogen content of the liver was observed to be moderate in the walls of the central veins and in the cytoplasm of the hepatocytes. The deposited glycogen granules were seen either as patches or in a homogenous pattern. However, the reaction was strongly observed in the walls of portal vein, hepatic artery and bile ducts.

Third and fourth groups: The reaction of was moderate in the central portion of hepatic lobules and around the central veins which showed dilatation and engorgement. However, it was weaker at the periphery of the hepatic lobules (Fig 3).

Fifth and sixth groups: The hepatic lobules showed patchy PAS reaction, i.e. weak at areas and strong at other areas. Cellular infiltration was observed (Fig 4).

**Enzymes results:**

**Succinic Dehydrogenase (SD):**
First and second groups: It ap-