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RESEARCH ARTICLE

LIVER DYSFUNCTION OF WISTAR RATS INFLUENCED BY CITRIC ACID AS FOOD ADDITIVE.

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Abstract

The aim of this study was focused on assessing liver function of Wistar rats influenced by oral ingestion of different doses of the food additive citric acid, using rats' serum AST as an indicator. Administered doses were (100, 600 and 1250 mg/kg b.w). 36 female Wistar rats were divided into 4 groups (9 rats /each). One group served as control, and the remainder of the 3 groups received different doses of citric acid. Housing was in Meck Nimir Research Center, Khartoum. Animals were accessed to free tap water and standard diets liberally. Serum was taken from each rats and analyzed by spectrophotometer. Transverse sections of liver organs were used to prepare Histopathological slides. A significant ($p \leq 0.05$) gradual increase according to increased treatment doses in serum AST was observed in treated animals, compared with control. Also there were different signs of liver histopathological abnormalities including atrophy (black material inside kupfler cell), Necrosis, hyperaemia and deattached cells, changes mainly around central vein, infiltration of black material, small particles, hyperaemia, cellular damage, cytoplasmic vacuolation, and polymorphic nuclei, compared to control. All tested animals showed significant ($p \leq 0.05$) increased body weight, compared to control. All animals survived till the end of the experiment. As a conclusion we can state that oral ingestion of citric acid results in liver dysfunction, so it is preferred to ingest foods and beverages containing citric acid with caution. This study was aimed to assess rats' liver dysfunction influenced by oral ingestion of citric acid..

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Introduction:-

Citric acid is a weak organic acid. It is a natural preservative and is also used to add an acidic (sour) taste to foods and soft drinks. In biochemistry, it is important as an intermediate in the citric acid cycle and therefore occurs in the metabolism of almost all living systems. It also serves as an environmentally benign cleaning agent and acts as an antioxidant. Chemically, citric acid shares the properties of other carboxylic acids. When heated above 175 °C, it decomposes through the loss of carbon dioxide and water. It is found naturally in Lemons, grapefruits and other citrus fruits. In production technique, cultures of *Aspergillus niger* are fed on sucrose to yield citric acid. After the mold is filtered out of the resulting solution, citric acid is isolated by precipitating it with lime (calcium hydroxide) to yield calcium citrate salt, from which citric acid is regenerated by treatment with sulfuric acid. Metabolically, the citric acid cycle is a series of enzyme catalyzed chemical reactions of central importance in all living cells that use oxygen as part of cellular respiration, deriving energy from carbohydrates, lipids, and proteins (Garden et al., 2003).

The average daily intake of citric acid from natural sources in the diet and food additives was estimated at about 40 mg/kg b.w for women, 130 mg/kg b.w for infants and 400 mg/kg b.w for individuals on slimming diets; maximum daily intake is reported to reach levels of 500 mg/kg b.w. No formal ADI (acceptable daily intake) level has been specified for citric acid and its common salts by the Joint FAO/WHO Expert Committee on Food Additives nor by the EC Scientific Committee for Food. Ingestion of a single dose of 25000 mg of citric acid by a woman (corresponding to approx. 417 mg/kg b.w) caused vomiting and nearly dying in one reported case. Volunteers given oral doses of potassium or magnesium citrate corresponding to approx. 4700 mg (corresponding to approx. 78.4 mg/kg b.w) of citric acid did not suffer any overt gastrointestinal effects. Based on wide spectrum of data relating to experimental animals and on human experience citric acid has a low acute toxicity; only one case of near fatal human intoxication was found. In a repeated dose study with rats a NOAEL of 1200 mg/kg b.w/day and a LOAEL of 2000 mg/kg b.w/day have been determined. The major subchronic and chronic toxic effects seem to be limited to changes in blood chemistry (Coleman, 1997). Citric acid can be added to ice cream as an emulsifying agent to keep fats from separating, to caramel to prevent sucrose crystallization, or in recipes in place of fresh lemon juice. Citric acid is used with sodium bicarbonate in a wide range of effervescent formulae, both for ingestion (e.g., powders and tablets) and for personal care (e.g., bath salts, bath bombs, and cleaning of grease). Citric acid is also often used in cleaning products and sodas or fizzy drinks. Citric acid sold in a dry powdered form is commonly sold in markets and groceries as "sour salt", due to its physical resemblance to table salt. It has use in culinary applications where an acid is needed for either its chemical properties or for its sour flavor, but a dry ingredient is needed and additional flavors are unwanted (Frank, 2005). One reasonable explanation is that citric acid entering the organism can be absorbed by the detoxifying organs such as liver and act as an adjuvant to complex with metal ions contained in the detoxification enzymes inactivating them. Furthermore, detoxifying this kind of xenobiotics leads to the generation of free radicals such as hydrogen peroxide (H_2O_2) by means of oxidation/reduction reaction, exerting a damaging impact on body's tissues. Although many parameters have been used to indicate the toxic effects of citric acid or citrate on living organisms, there still remains limited information on its detailed effects in the livers. It was reported from clinical biochemistry examination of drink poisoning in children that enlarged baby's liver was related to the addition of citric acid into drinks. In addition, by monitoring the short-term effect of single dose citric acid on mouse tissue (Aktaç et al., 2003) discovered that citric acid treatment caused injury of hepatocyte membranes, cytoplasmic vacuolization in hepatocyte, karyopyknosis, suggesting the toxic effects of citric acid on mice. Toxicity of citric acid was performed through biochemical analysis on citric acid-treated mice and the result showed a significant decrease in the activities of many antioxidative enzymes and a series of pathological changes such as disorganized hepatocyte cords, blood clot in central veins, lymphocyte and neutrophil infiltrating (Zhang et al., 2011). However, there are still very few studies for exploring whether citric acid or citrate could induce apoptotic cell death in mouse liver. Recently, it found that necrotic changes caused by this xenobiotic substance, such as vacuolated cytoplasm in hepatocytes, and chromatin decrease in mouse liver (Aktaç et al., 2003). All doses were orally administered in mg/kg body weight. A previous study revealed some pathological changes in liver of mice exposed to citric acid, such as vacuolisation and glassy cytoplasm in the hepatocyte, nuclear membrane invaginations, picnotic nuclei. Similarly, with the effect of sodium benzoate in the rats and mice, high vacuolisation and glassy appearance in hepatocyte cytoplasm was explained (Fujitani, 1993). Humans are exposed daily to complex mixtures of chemical compounds in their food. One of these substances are antioxidants which are used as food preservatives (Würtzen, 1990). Heo et al., (2013) reported that addition of citric acid or its salts can enhance the growth and the feed to gain ratio of weaned and growing-finishing pigs.

Materials and methods:-

Biologic experiment:-

36 male Wistar rats (weight ranged from 200 – 250 g), were divided into 4 groups (9 rats / each). All animals were provided the basal diet composed of: (beef meat 10.6%, sesame oil 46.6%, corn flour 42.4%, and table salt 0.29%). All rats were put in quarantine for seven days. All animals were freely accessed to prepared diet and tap water. 3 groups orally received 100, 600 and 1250 of citric acids. According to (Xiaoguang et al., 2014), doses of citric acid of (120 mg/kg b.w), middle dose (240 mg/kg b.w) and high dose groups (480 mg/kg b.w). Oral doses of citric acid were calculated to be administered by Wistar rats to assess liver function. In this study the low dose approximately equal to (Xiaoguang et al., 2014), but the middle and high doses were double as in to (Xiaoguang et al., 2014). Doses were administered once daily for twenty eight days. Blood samples were taken from rats' eyes using capillary tubes, labeled then centrifuged at 30000 rpm and serum was kept at 5° C. Blood sampling was done: initially, on the 14th and on the 28th day. By the 28th day all animals were autopsied and liver organs were collected, labeled then kept in 10% formal/saline solution. Slides were prepared according to method described by (Bancroft and Gamble, 2002).

Measurement of serum AST concentrations:-

Working solution was prepared by adding 2 ml from reagent 1(buffer, lactate dehydrogenase LDH, malate dehydrogenase MDH, L aspartate, pH 7.8) and 500 µl from reagent 2(substrate α-ketoglutarate). Working solution was mixed and put in 37⁰ C, 1 ml was taken from working solution then 100 µl from serum was added, mixed and incubated at 37⁰ C for 1 minute. Initial absorbance was read, at 1 minute intervals, the difference between absorbance were calculated. The average absorbance difference per minute: $\Delta A / \text{minute} \times 1750 \text{ (factor)} = \text{U/L}$ (Murray, 1984). A calibrated spectrophotometer (Awareness Technology, model No. 1904 plus, serial No. 1904-5252) was set for measurement of AST concentrations.

Statistical analysis:-

All values were express as Means \pm Sd. The SPSS one – way Anova test was used for the evaluation of differences between Wistar rats groups according to dos. The differences were considered significant if a P. value was less than 0.05.

Results and discussions:-

Toxicological study

In this study, oral administration of citric acid doses of 100, 600 and 1250 (mg/kg b. w) resulted in significant ($p \leq 0.05$) gradual increase in serum AST (Table 1) according to increased treatment doses. Induced liver injury, and serum transaminase elevations, were attenuated by 1-2 g/kg of citric acid ingestion, accompanied by induced systemic inflammation (Abdel-Salam et al., 2014). According to Berk and Korenblat, (2007), serum AST normal range is 10 to 34 IU/L. Increased AST levels, are associated with diseases that affect liver cells (Berk and Korenblat, 2007). Citric acid is of low acute toxicity; only one case of near fatal human intoxication was reported. In a repeated dose study with rats a NOAEL of 1200 mg/kg b.w/day and a LOAEL of 2000 mg/kg b.w/day have been determined. The major subchronic and chronic toxic effects seem to be limited to changes in blood chemistry (Coleman, 1997). Ingested citric acid can be absorbed by the detoxifying organs such as liver and act as an adjuvant to complex with metal ions contained in the detoxification enzymes inactivating them. Furthermore, detoxifying this kind of xenobiotics leads to the generation of free radicals such as hydrogen peroxide (H_2O_2) by means of oxidation/reduction reaction, exerting a damaging impact on body's tissues. Limited information is available concerning detailed effects of citric acid in the livers. It was reported that enlarged baby's liver was related to the addition of citric acid into drinks (Aktaç et al., 2003). Increased rats' weight is confirmed by (Heo et al., 2013) reporting that addition of citric acid or its salts can enhance the growth and the feed to gain ratio of weaned and growing-finishing pigs.

Liver dissections:-

All plates show transverse sections of rats' liver. Plate A represents control, whether plates B, C and D represented animals treated by 100, 600 and 1250 mg/kg body weight of citric acid respectively. Plate B: Indicated more atrophy (black material inside kupfler cell), Necrosis changes, hyperaemia and deattached cells. Plate C: showed changes mainly around central vein, infiltration of black material, small particles, atrophy ,necrosis (all of three) decreases toward the edges of the lobules (beside portal areas) and hyperaemia. Plate D: showed that there is more extensive cellular damage, more cytoplasmic vaculation, more polymorphic nuclei and no black material inside or outside kupfler cell. Gradual histopathological changes were observed in plates B, C and D, compared to control. These study liver changes are in agreement with (Aktaç et al., 2003), (Zhang et al., 2011), and (Fujitani, 1993). Microscopical examination of the liver showed histopathological changes depending on the citric acid. These changes were tissue degeneration, cytoplasmic vacuolisations, nuclear membrane invaginations, picnotic nucleus and necrosis of the hepatocytes (Aktaç et al., 2003).

Table 1:- Effects of oral ingestion of different doses (treatments in mg/ kg b. w) of citric acid on Female rats' serum AST.

Groups Doses	Control	Initial	14 th day	28 th day
100	24.44 \pm 1.51 ^a	25.00 \pm 2.40 ^{N.S}	145.11 \pm 5.93 ^c	264.44 \pm 5.81 ^d
600	25.11 \pm 2.62 ^a	24.67 \pm 2.45 ^{N.S}	289.44 \pm 13.04 ^d	429.00 \pm 18.53 ^e
1250	25.22 \pm 3.19 ^a	27.44 \pm 4.48 ^{N.S}	406.78 \pm 41.02 ^e	563.56 \pm 20.76 ^{de}

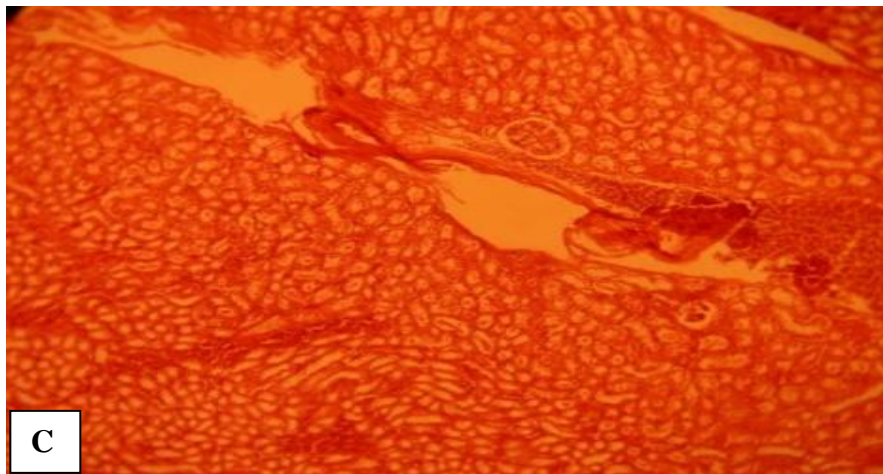
Values are means \pm SD. Means with rows not sharing common letter (s) are significantly different ($P < 0.05$). N.S = non- significant.

Histopathological slides:-

(Eosine & hematoxylin \times 1000)



(Eosine & hematoxylin \times 1000)



(Eosine & hematoxylin \times 1000)



(Eosine & hematoxylin $\times 1000$)

Conclusions and Recommendations:-

According to the significant increase in rats' serum AST, accompanied by the gradual observed histopathological changes in plates B, C and D, compared to control, it is almost concluded that citric acid results in rats' liver dysfunction. Since human daily - ingested doses of citric acid are almost below the NOAEL, I recommend that foods and beverages containing this chemical should be consumed with caution. As AST is a marker of cell damage, confirmed by the histopathological findings of extensive cellular damage, especially in group (1250 mg / kg b.w), I also recommend for further studies concerning more than a cellular damage analysis, thus increasing results strength.

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