Single Dose of Chloroform Induces Hepatic Dysfunction with Pro-Inflammatory Cytokines Response in Vivo

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ABSTRACT
Chloroform (CH) is considered an urgent environmental pollutant that leads to critical health effects. This investigation was carried out to verify the involvement of serum cytokines in the hepatic dysfunction induced by single exposure to CH in laboratory rats. Eighteen adult male laboratory rats were separated into three groups of eight rats each. Control (CON) group included animals without any treatment, while the rest of the two groups were administrated trichloromethane at a dose of 477 mg/kg , orally then sacrificed after 1 day (CH-1) and 7day (CH-7) post-exposure. The results indicated that single-dose exposure induced liver dysfunction through significant elevation of serum activities of hepatic enzymes compared with unexposed control rats. Furthermore, chloroform intoxication caused a significant time-dependent rise in concentrations of some pro-inflammatory cytokines in serum of exposed rats. In conclusion, a single high dose of chloroform causes liver dysfunction and promotes the response of pro-inflammatory cytokines in rats.

1. Introduction

Chloroform (CH), also called trichloromethane ($CHCl_3$), is a highly volatile organic compound that is an uncolored liquid with a distinctive odour [1]. It was introduced in 1847 as a powerful anesthetic, until its clinical usage declined in 1976 due to its adverse side effects [2]. Inhalation of CH induces central nervous system depression, leading to anesthesia, and can be fatal at high concentrations [3]. It is a common pollutant in atmosphere and water, and released into air as a by-product of its formation in chlorination of drink water, sewage, and swimming pools [4,5]. Its volatility allows it to go from water to the atmosphere, where it remains for several months before being removed through chemical conversion [6,7]. It is also a by-product of paper bleaching and emissions from vehicle exhaust [8]. It is worth noting that CH is classified as a hazardous substance on the list of the most important priority substances by agency for toxic substances and disease registry. In addition, it has been considered a potential carcinogen [9]. In sequence, liver, kidneys, and central nervous system are the target organs for CH poisoning [10]. Both humans and animals get identical poisoning symptoms from it; the average oral fatal dose for humans is roughly 45 g. [11]. Mammals are generally good absorbers, metabolizers, and excretors of chloroform following exposure, and the bloodstream carries the chemical throughout the body extensively [12].

The main byproduct of the metabolism of chloroform is carbon dioxide (CO_2), which is mostly expelled by the lungs. However, some of the CO_2 is integrated into endogenous metabolites and may be expelled as various amino acids, urea, bicarbonate, and methionine [13]. It is extremely harmful to the liver, albeit the first 12 to 48 hours following exposure may not show full signs of damage [14]. Acute heart failure, lung failure, and centrilobular liver necrosis can all be fatal outcomes of acute exposure to excessive doses of CH. [15]. It is well established that liver is the key site of metabolism of xenobiotics and hepatotoxicity resulting from exposure to organic solvents is rarely diagnosed or even suspected [16,17]. There is strong suspicion that chloroform metabolites may have mutagenic and immune toxic effects [18,19]. It is requisite to investigate the role of inflammatory mediators including cytokine response, in the previously reported hepatic injury induced by high dose chloroform in vivo [20,21]. Thus, this experimental study aimed to assess contribution of serum cytokines to liver injury in a rat model caused by a single chloroform treatment.

Animals and treatments

For this experiment, a total of eighteen fully grown male Wistar rats with weigh ranged around 180 - 220 g. were selected from the animal resources center. They were housed in appropriate clean cages and allowed to acclimatize to standard laboratory environmental conditions in terms of temperature, lighting and humidity for one week prior starting the experiment. They were maintained on a standard diet with easy access to water. All animals were treated ethically according to the National Institutes of Health guidelines (1978). Randomly, they were set into three groups, each consisting of six rats. Following receiving a single oral dosage of 477 mg/kg of trichloromethane [22], the first group (CON) was kept as a control without receiving any treatment. The other two groups were euthanized one day (CH-1) and seven days (CH-7) following the exposure. Blood samples from cardiac punctures were taken, put in designated tubes, processed to extract the serum, and then kept cold at– 40 ° C until analysis.

Assessment of liver enzymes and proinflammatory cytokines

A standardized ELISA methodology was used to measure liver enzymes levels including ALT & AST, ALP, and GGT in serum samples. As directed by the kit, standards were made by serial dilution. Serum concentrations of the pro-inflammatory cytokines including IL-1β, IL-6 and TNF-α were evaluated using commercially standardized ELISA protocol. According to industry recommendations, serum samples were processed.

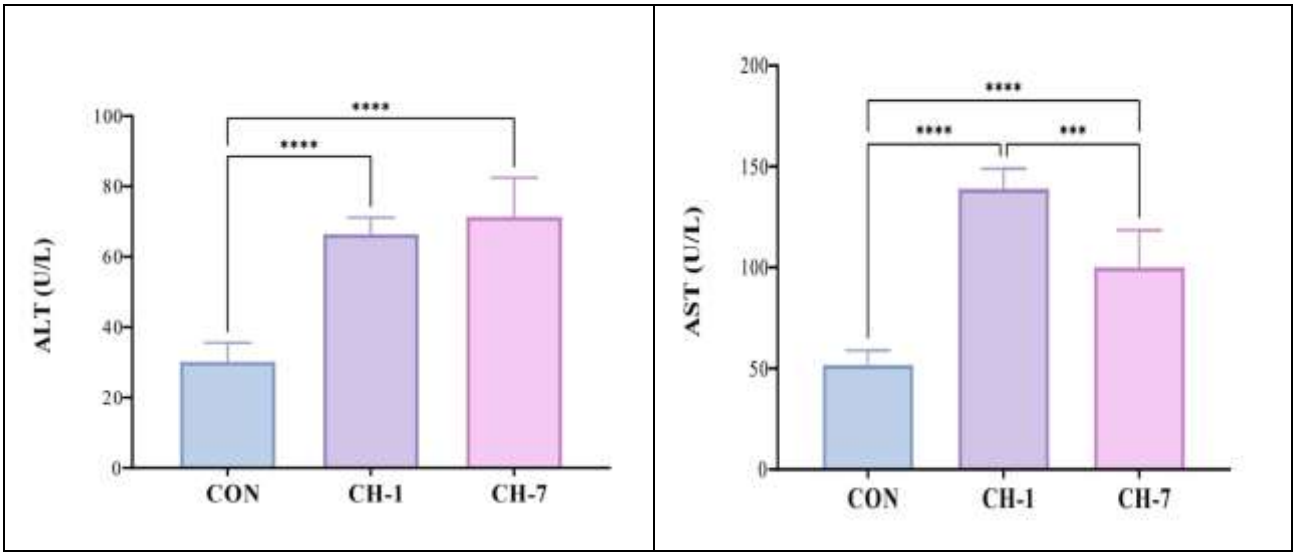
Data analysis

The SPSS (version 25) software was applied to process all results, and M± SD was the output. Differences between research groups were determined using one-way analysis of variance (ANOVA), followed by Tukey's post hoc analysis interpretation. A significance level set at $p < 0.05$ was used. GraphPad Prism was utilized to design the graphs.

Results

Effect on liver enzymes levels

The detrimental effect of chloroform on the serological levels of studied rats after 1 day and 7 days of CH exposure is represented in Fig. (1). The findings confirmed that CH-treatment led to a remarkable increasing in levels of analyzed liver enzymes in serum of CH-1 and CH-7 rats compared to controls. It is worth noting that this increase was gradual and time-dependent in enzymes (ALT, ALP, and GGT) in CH-treated rats.



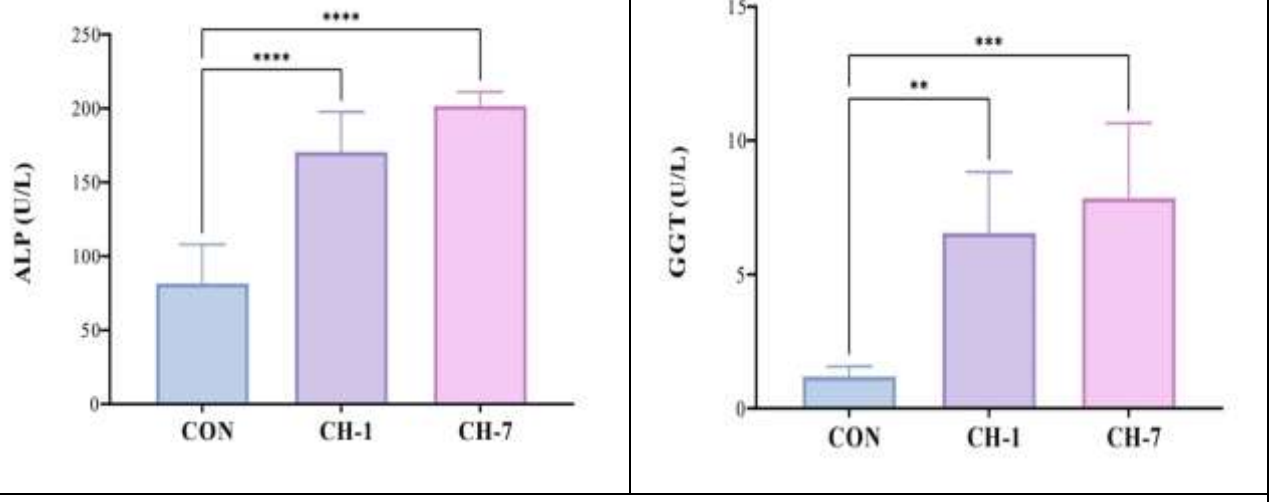
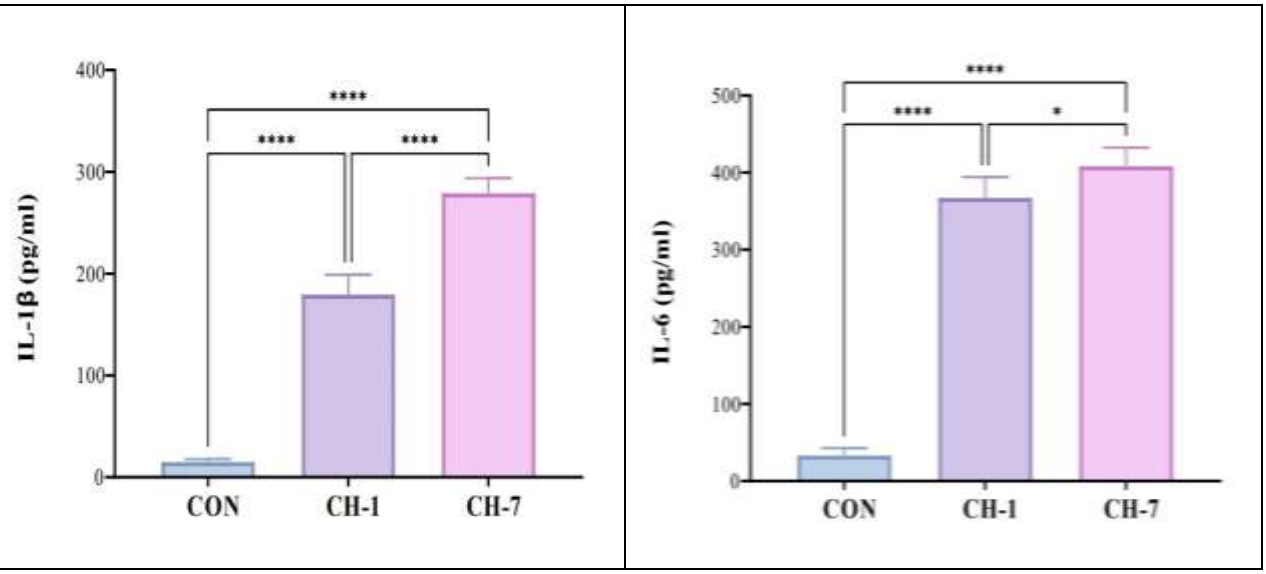


Figure 2: Impact of chloroform on serum concentrations of liver enzymes in three experiment groups. **: significant at ($p < 0.01$), *** ($p < 0.001$); **** ($p<0.0001$).

Effect on proinflammatory cytokines levels

Results confirmed that exposure to chloroform induced a significant time-dependent increase ($p < 0.0001$) in the serum levels of IL-1β, IL-6, and TNF-α in CH-1 and CH-7 rats compared to control (CON) group as shown in Fig. (2).



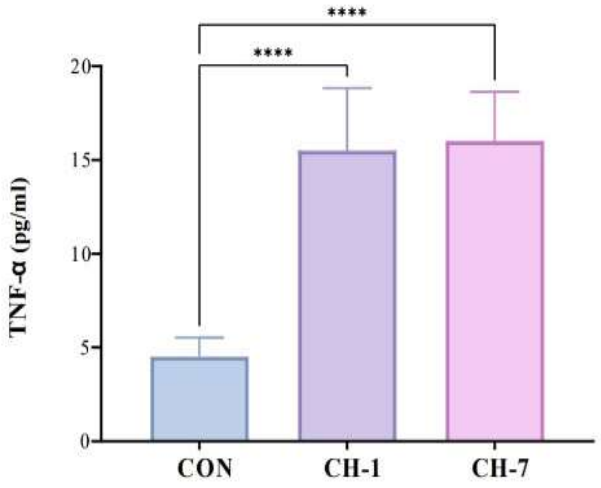


Figure 2: Impact of chloroform on serum concentrations of pro-inflammatory cytokines in three experiment groups. *: significant at ($p < 0.05$); **** ($p<0.0001$).

Discussion

The liver is a vital organ involved in extramedullary hematopoiesis, has a high regenerative capacity, and is essential for detoxification and metabolism [23,24]. In the present era, the liver damage caused by environmental chemical pollutants has become overwhelming due to continued exposure, thus increasing the risk of poisoning [25-27]. Serum levels of these liver enzymes are measured during liver function testing, which is proven to be a useful method for assessing the liver's health [28]. Thus, a rise in these serum enzyme levels could be a sign of liver damage or inflammation [29]. According to the results, a single high dose of chloroform induced liver damage through disruption levels of hepatic functional indices in exposed rats. The levels of liver enzymes were significantly raised compared to control group, this indicated that chloroform increased cell membrane permeability and induced hepatocytes damage [30]. Our findings were consistent with a previous study by Chima *et al.* as they found that chloroform-intoxicated albino rats (100 mg/kg/b.wt) had induced activity of serum hepatic markers ALT, ALP, AST and GGT [31]. Furthermore, Okechukwu *et al* confirmed that experimental rats intoxicated with chloroform demonstrated a substantial increase ($p < 0.05$) in serum levels of ALT, ALP, and AST, suggesting that the treated animals were under oxidative stress. They explained this by the disruption of the plasma membrane and damage to hepatocellular cells, which resulted in rapid release of these enzymes into the bloodstream [32]. In a different study, Somade *et al.* found that oral trichloromethane exposure to rats at a level of 200 mg/kg induced hepatotoxicity, as evidenced by a discernible rise in the liver's expression of nitric oxide, H2O2, apoptotic cells, nuclear factor kappa B, and oxidative stress indicator. Additionally, p53 expression and antioxidant activities were clearly reduction than in the unexposed group [33]. The hepatotoxicity of chloroform has been reported to be due to phosgene-mediated depletion of cellular glutathione or increased amounts of covalent binding to hepatic macromolecules, such as proteins, DNA, and lipids, resulting in cellular malfunction and death [34]. Primarily, CH is metabolized in the liver through oxidation-reduction pathways, with the major oxidation products being carbon dioxide and reactive metabolites containing phosgene and trichloromethyl radicals. [35,36]. Oxygen tension, species, tissue, and dose all affect the balance of the pathways, which are based on cytochrome P450-dependent enzyme activation [37]. The results also proved the time-dependent increase serum concentration of proinflammatory cytokines in chloroform intoxicated rats compared to controls. Several studies that have published on this topic support this finding by reporting a similar elevation in pro-inflammatory cytokine response during exposure to chemical toxicants [38-41]. From the above, it is clear that damage caused by impaired liver function can lead to disruption of the body's health system as a result of immune suppression [42].

Conclusions

This experimental investigation concluded the destructive effect of chloroform on liver function enzymes in the serum of a rat model. It also confirmed the immunotoxicity through the induction of pro-inflammatory cytokine response in a time-dependent increase.

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