

## Evaluation of the Toxic Effect of Cinnamaldehyde Food Flavor on the Liver and Kidneys of Rats

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### Abstract

*Cinnamaldehyde a food flavor which has a high human Consumption. In this study, the toxic effect of Cinnamaldehyde has been evaluated on rats liver and kidneys. Rats were separated into four groups each group contain five rats. The first group is control which does not received doses of Cinnamaldehyde. Second group received 500 mg/kg/day (ED50), third group received 1200 mg/kg/day (TD50) and last group received 1900 mg/kg/day (LD50) of Cinnamaldehyde in food for period of two weeks. Then sufficient amount of blood samples were collected in a tube (containing lithium heparin anticoagulant). Plasma was separated by centrifugation 300 RBM for 3 times. After that ALP and GOT levels were examined for the abnormalities were determined to showed hepatocellular and cholestatic changes .Hepatocellular in which the membrane of the liver became permeable when damag, allowing for escape of intracellular enzymes into blood stream . Cholestasis in which there was damaged in intra or extra hepatic bile ducts cause induction of synthesis of ALP and increased it is level. Urea and Creatinine tests were performed to determine kidneys functions. All these tests were carried out using Mindary BS-200 instrument. The data was collected and analyzed by variance statistical methods using SAS statistical package version (9.1) which showed a significant increase in ALP enzyme level and urea that indication of abnormalities in liver and kidney function but did not show a significant change in GOT level and Creatinine. Thus the study showed that Cinnamaldehyde can cause liver and kidney abnormalities and this effect is dose related so there must be awareness and rational use of Cinnamaldehyde within the margin of safety and lethality showed in the study.*

**List of Abbreviation**

TD50	Toxic dose
ED50	Effective Dose
LD50	Lethal Dose
RPM	Revolution per Minute
GOT	Aspartate Aminotransferase
ALP	Alkaline Phosphate
SAS	Statistical Analysis System
HPLC	High Performance Liquid Chromatography
STD	Standard Deviation

**1. Introduction****1.1 Background**

Cinnamaldehyde (CNMA) has been in public use since 1900. It is a strong pleasant Fragrance derived from the bark of *Cinnamomum* cultivated trees. The occurrence of cinnamaldehyde is noticed in several brands of cinnamon breads, cereals, cookies, puddings, applesauce, and fruit juices [1].Cinnamaldehyde is chemically related to toxicologically more active compounds like Caroline and crotonaldehyde [2]. Beside cinnamaldehyde acute toxicity, other major components of *Cinnamomum*, cinnamic acid (5000 mg/kg body weight) and cinnamyl alcohol (4000 mg/kg body weight) have been described in rats [3],and the concern about the safety of cinnamaldehyde in general raised [4] studying of both the toxic effect of cinnamaldehyde [5] and the involvement of cinnamaldehyde in apoptosis .

According to important uses of cinnamaldehyde in our daily life products and it is high consumption these study was carried out to determine the relationship between cinnamaldehyde and liver and kidneys abnormalities.

Many species of the genus *Cinnamomum*, the main source of cinnamaldehyde, grow in different regions of India [6].Cinnamaldehyde is widely used in most Indian food, medicinal and cosmetic products [7].

## Structural Formula

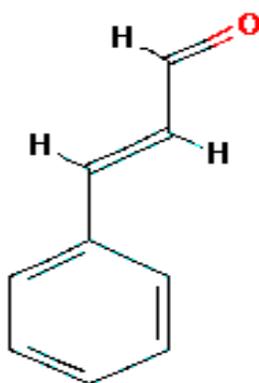


Figure 1.1 Structural Formula of Cinnamaldehyde

Cinnamon is mainly used in the aroma and essence industries due to its fragrance, which can be incorporated into different varieties of foodstuffs, perfumes, and medicinal products [8]. The most important constituents of cinnamon are cinnamaldehyde and trans-cinnamaldehyde, which are present in the essential oil, thus contributing to the fragrance and to the various biological activities observed with cinnamon [9]. A study on *Cinnamomum osmophloeum* (*C. osmophloeum*) indicated that the essential oil from cinnamon leaves contains a high level of Cinnamomum consequently, *C. osmophloeum* is also used as an alternative spice for *C. cassia*. One of the major constituents of essential oil extracted from *C. zeylanicum* named (E)-cinnamaldehyde has an antityrosinase activity, while cinnamaldehyde is the principal compound responsible for this activity [9].

Cinnamon bark contains procyanidins and catechins. The components of procyanidins include both procyanidin A-type and B-type linkages. These procyanidins extracted from cinnamon and berries also possess antioxidant activities [10].

### 1.2 Metabolism

The cinnamyl derivatives used as flavoring substances are simple aromatic compounds with a propyl side-chain containing a primary oxygenated functional group, and they participate in common routes of absorption, distribution, and metabolism. The members of this group may be hydrolyzed to yield the component alcohol, aldehyde, or acid. If the product is an alcohol or aldehyde, it is oxidized to yield the corresponding 3-phenylpropanoic acid or a 3-phenylpropanoic acid derivative which undergoes further side-chain beta-oxidation and cleavage to yield mainly the corresponding benzoic acid derivatives (Figure 1.2). The benzoic acid derivatives are conjugated with glycine and, to a less or extent, glucuronic acid and excreted primarily in the urine. Ortho-Alkyl- and ortho-alkoxy-substituted cinnamaldehyde derivatives undergo beta-oxidation to a minor extent, to yield beta-hydroxy-3-phenylpropanoic acid metabolites that are excreted as the glucuronic acid conjugates [11].

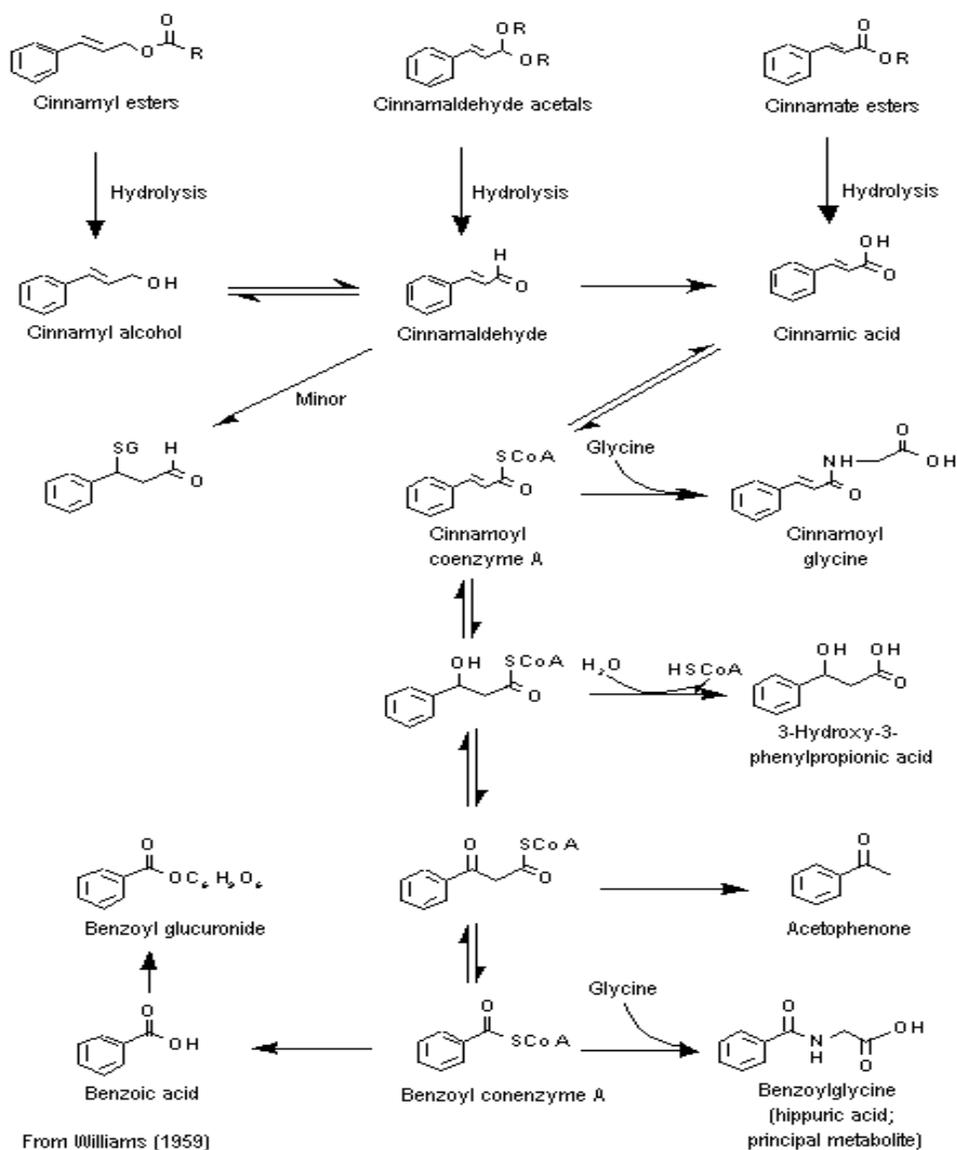


Figure 1.2 Metabolism of Cinnamaldehyde

### 1.3 Cinnamon Trees

Cinnamon trees belong to a large genus of some 250 species, most of which are aromatic. True Cinnamon is native to Sri Lanka, formerly known as Ceylon and the south-eastern coast of India, while the closely related Cassia is native to China. Cinnamon and Cassia are both small tropical evergreen trees that grow up to 20 - 30 feet tall, with aromatic bark and leaves. Young leaves employ a typical trick of tropical trees to make themselves look unappealing to predatory insects by assuming a limp, reddish appearance, as they are wilting. Once they mature they perk up and darken to a deep green. The leaves are elongated ovate with a pointed tip, shiny and dark green on the upper surface, lighter below. The inconspicuous whitish flowers grow in panicles, which later develop into bluish berries. The bark is reddish brown and smooth.



Figure 1.3 Cinnamon tree

The bark of various cinnamon species is one of the most important and popular spices used worldwide not only for cooking but also in traditional and modern medicines. Overall, approximately 250 species have been identified among the cinnamon genus, with trees being scattered all over the world.

#### **1.4 Traditional Uses**

In addition to being used as a spice and flavoring agent, cinnamon is also added to flavor chewing gums due to its mouth refreshing effects and ability to remove bad breath [12]. Cinnamon can also improve the health of the colon; thereby reducing the risk of colon cancer [13]. Cinnamon has also been traditionally used as tooth powder to treat toothaches, dental problems, oral microbiota, and bad breath.

#### **1.5 Medicinal Uses**

Cinnamon is a coagulant that prevents bleeding [14]. Cinnamon also increases the blood circulation in the uterus and advances tissue regeneration [15]. This plant plays a vital role as a spice, but its essential oils and other constituents also have important activities, including antimicrobial [16], antifungal [17], antioxidant [18] and antidiabetic [19]. Cinnamon has been used as anti-inflammatory [20], and anticancer agent [21].

##### **1.5.1 Antioxidant Activity**

Antioxidant compounds present in foodstuffs play a vital role in human life, acting as health-protecting agents. In addition to this role, antioxidants are one of the key additives used in fats and oils. Even in the food processing industry, antioxidants have been used to delay or prevent food spoilage. Spices and medicinal plants have received rapid consideration as sources of beneficial antioxidants against various diseases. Antioxidants have been considered the most

important drivers in the progress and existence of humans, as they respond to free radicals and damage in metabolic diseases and age-related syndromes of humans and other animals [20].

### **1.5.2 Antimicrobial Activity**

Several studies on medicinal plants and their components have indicated the anti-inflammatory activities of cinnamon. Various studies reported the anti-inflammatory activity of cinnamon and its essential oils. To date, there are several flavonoid compounds (e.g., gossypin, gnaphalin, hesperidin, hibifolin, hypolaetin, oroxindin, and quercetin) that have been isolated and have anti-inflammatory activities [18].

### **1.5.3 Neurological Disorder**

Cinnamophilin is a novel thromboxane A<sub>2</sub> receptor antagonist isolated from *C. philippinensis*. The protective effects of trimer 1 in attenuating the diminution in glutamate uptake are possibly arbitrated via their effects on the mitochondria [21].

### **1.5.4 Antidiabetic Activity**

A substance from cinnamon has been isolated and coined as “insulin-potentiating factor” (IPF), while the antidiabetic effects of cinnamon bark have been shown in streptozotocin-induced diabetic rats. Several studies have also revealed that cinnamon extracts lower not only blood glucose but also cholesterol levels [19].

### **1.5.5 Anticancer Activity**

The aqueous extract and the fraction of cinnamon (procyanidins) from HPLC inhibit vascular endothelial growth factor subtype 2 (VEGFR2) kinase activities, thereby inhibiting the angiogenesis involved in cancer [21].

### **1.5.6 Cardiovascular Diseases**

One of the active components isolated from *C. cassia* named 2-methoxycinnamaldehyde (2-MCA) decreases the expression of vascular cell adhesion molecule-1 (VCAM-1) in TNF $\alpha$ -activated endothelial cells, suggesting that ischemia/reperfusion (I/R) injury is ameliorated due to the induction of hemeoxygenase [13,15].

### **1.5.7 Cholesterol and Lipid Lowering Effect**

Cinnamon is positively affecting the lipid profile, whereby the high density lipoprotein (HDL) cholesterol levels decreased, and plasma triglycerides were reduced [15].

## **1.6 Liver Abnormalities**

The liver filters and processes blood as it circulates through the body which metabolizes nutrients, detoxifies harmful substances, makes blood clotting proteins, and performs many other vital functions. The cells in the liver contain enzymes that drive these chemical reactions. When liver cells are damaged or destroyed, the enzymes in the cells leak

out into the blood, where they can be measured by blood tests. Two main liver enzymes are Aspartate aminotransferase (AST), formerly called SGOT; the AST enzyme is also found in muscles and many other tissues besides the liver and alanine aminotransferase (ALT), formerly called SGPT; ALT is almost exclusively found in the liver. If ALT and AST are found together in elevated amounts in the blood, liver damage is most likely present [23].

### **1.7 kidney Abnormalities**

The kidneys maintain the blood creatinine in a normal range. Creatinine has been found to be a fairly reliable indicator of kidney function. Elevated creatinine level signifies impaired kidney function or kidney disease. As the kidneys become impaired for any reason, the creatinine level in the blood will rise due to poor clearance of creatinine by the kidneys. Abnormally high levels of creatinine thus warn of possible malfunction or failure of the kidneys. For this reason standard blood tests routinely check the amount of creatinine in the blood. Creatinine is a chemical waste molecule that is generated from muscle metabolism. Creatinine is produced from creatine, a molecule of major importance for energy production in muscles. Approximately 2 % of the body's creatine is converted to creatinine every day. Creatinine is transported through the bloodstream to the kidneys. The kidneys filter out most of the creatinine and dispose of it in the urine. Because the muscle mass in the body is relatively constant from day to day, the creatinine production normally remains essentially unchanged on a daily basis. Blood urea nitrogen (BUN) level is another indicator of kidney function. Urea is also a metabolic byproduct which can build up if kidney function is impaired. The BUN-to-creatinine ratio generally provides more precise information about kidney function and its possible underlying cause compared with creatinine [24]

### **1.8 Rationale**

Cinnamaldehyde has a high rate of human consumption [8]. Previous studies showed that cinnamaldehyde alters liver and kidney functions. Thus this work has been carried out to assess the toxic dose and effects on humans, as well as determining the suitable amount of cinnamaldehyde that should be used within the margin of safety as a food flavor in cookies and drinks without causing any harmful abnormalities in the kidneys and liver function.

### **1.9 Objective**

#### **1.9.1 General**

- ❖ To Determine the Toxicological Effect of Cinnamaldehyde on rats kidney and liver.

#### **1.9.2 Specific**

- ❖ To observed signs of mortality, toxicity and behavioral changes on rats that receive different concentration of cinnamaldehyde.
- ❖ To determine the toxic effect of cinnamaldehyde on different serum parameters.
- ❖ To compare between the toxic Effect and different concentration of cinammaldehyde doses in hematological parameters.

## Literature Review

### 2.1 Therapeutic Uses

*Cinnamomum zeylanicum* (cinnamon) is widely used in traditional system of medicine to treat diabetes in India. Adams TB et al carried study to isolate and identify the putative antidiabetic compounds. Cinnamaldehyde was administered at different doses (5, 10 and 20 mg/kg BW) for 45 days to streptozotocin (STZ) (60 mg/kg BW)-induced male diabetic rats. It was found that plasma glucose concentration was significantly ( $p < 0.05$ ) decreased in a dose-dependent manner (63.29%) compared to the control. In addition, oral administration of cinnamaldehyde (20 mg/kg BW) significantly decreased glycosylated hemoglobin (HbA<sub>1c</sub>), serum total cholesterol, and triglyceride levels and at the same time markedly increased plasma insulin, hepatic glycogen, high-density lipoprotein-cholesterol levels and alter liver enzyme (AST, ALT and lactate dehydrogenase) [25].

### 2.2 Minimum Potential Fatal Human Dose

WHO/FAO indicate that probable oral lethal dose for humans are 0.5 to 5 g/kg for a 70-kg person. Both the oil and pure aldehyde are irritants, especially if undiluted. They can also cause inflammation and erosion of gastrointestinal mucosa. Prolonged skin contact (more than 48 hr) can produce superficial partial thickness burns [26].

### 2.3 Absorption, Distribution and Excretion

The bioavailability micro encapsulated cinnamaldehyde was investigated in male F344 rats. Adams TB et al study those Rats when gavages with CNMA in corn oil were using either micro encapsulated or the neat chemical at doses of 50, 250, and 500 mg/kg. No differences between the two formulations at any of the doses were found in either CNMA blood concentration profiles or in the rate of urinary hippuric acid excretion. Both formulations showed a low bioavailability (<20 %) at 250 and 500 mg/kg. Regardless of the formulation used, oral gavages of CNMA significantly increased the urinary excretion of hippuric acid. About 75 % of the dose of CNMA was metabolized to hippuric acid and recovered in the urine. The total amount of acid recovered in a 50-hr urinary collection correlated well with the CNMA dose. The data suggest that there was complete release of CNMA from the microcapsules and that microencapsulation of CNMA does not affect its bioavailability or its metabolism [11]. Cinnamaldehyde is presumably oxidized in vivo to cinnamic acid, which is excreted in urine as benzoic and hippuric acids [12]. Cinnamaldehyde administered intraperitoneally to a rabbit was excreted in the urine as benzoic acid, cinnamoyl glycine and hippuric acid [13]. Once formed, cinnamyl alcohol, cinnamaldehyde and cinnamic acid have all been shown to be rapidly absorbed from the gut, metabolized and excreted primarily in the urine and, to a minor extent, in the feces. Results of numerous studies indicate that cinnamyl derivatives are absorbed, metabolized and excreted as polar metabolites within 24 hr [16]. In a study, the effect of dose, species and sex on the disposition of cinnamaldehyde. A 2.0 or 250 mg/kg BW dose of cinnamaldehyde was administered to groups of male and female F344 rats (4/group) or CD1 mice (6/group) by intraperitoneal injection. Regardless of the dose level, species, or sex, greater than 85 % of the radiolabel is recovered in the urine and feces within 24 hr. Greater than 90 % is recovered after 72 hr. When 250 mg/kg BW of [<sup>3</sup>-(14) C] cinnamaldehyde is administered orally to F344 rats, 98 % is recovered from the urine (91 %) and feces (7 %) within 24 hr [27].

## 2.4 Metabolites

Adams TB et al study on metabolism of trans-[3-14C] cinnamaldehyde in male and female Fischer 344 rats and CD1 mice at doses of 200 and 250 mg/kg BW given by IV injection and in males at 250 mg/kg by oral gavages. Some 94 % of the administered dose was recovered in the excreta in 72 hr in both species with most (75-81 %) present in the 0-24-hr urine. Less than 2 % of the administered dose was found in the carcasses at 72 hr after dosing [28].

Peters MM and Caldwell J were identified Urinary metabolites by their chromatographic characteristics. In both species the major urinary metabolite was hippuric acid accompanied by 3-hydroxy 3-phenylpropionic acid benzoic acid and benzoyl glucuronide. The glycine conjugate of cinnamic acid was formed to a considerable extent only in the mouse. The oxidative metabolism of cinnamaldehyde essentially follows that of cinnamic acid, by beta-oxidation analogous to that of fatty acids. Apart from the metabolites common to cinnamic acid and cinnamaldehyde, 7 % of 0-24-hr urinary 14C was accounted for by two new metabolites in the rat and three in the mouse, which have been shown in other work to arise from a second pathway of cinnamaldehyde metabolism involving conjugation with glutathione. The excretion pattern and metabolic profile of cinnamaldehyde in rats and mice are not systematically affected by sex, dose size and route of administration [29].

## 2.5 Toxicity study

Haasch ML, Ford AW and Mar Environ Res were showed that Japanese medaka (*Oryzias latipes*) was used in the medaka embryo-larval assay (MELA) to determine possible adverse developmental effects of ethanol and the spice component, cinnamaldehyde (CAD) The Japanese Medaka were exposed to ethanol at 100mM, CAD at 10, 1.0, 0.67 or 0.50 mM, to ethanol and CAD combined, or were non-treated controls. Ethanol at 100 mM was without effect. CAD alone at 10 mM and 1.0 mM was lethal by 1 dpf. Embryos exposed to 100 mM ethanol and 0.67 mM CAD exhibited cardiovascular and pigmentation defects and delayed hatching. Embryos exposed to 0.50 mM CAD alone had less severe cardiovascular problems as compared to the combined ethanol and CAD treatment. Taken together the results indicate that the combined effects of ethanol and CAD are greater than the individual effects [30].

## 2.6 Human Exposure Study

Frosch PJ was carried out study to determine the frequency of reactivity to a series of commonly used fragrances in dermatological patients. A total of 48 fragrances (FF) were chosen. In pilot study on a total of 1069 patients in 11 centers, the appropriate test concentration and vehicle were examined. For most fragrances, 1 % and 5 % were chosen, and petrolatum proved to be the best vehicle. In the main study, a set of 5 to 10 fragrances at 2 concentrations was patch tested in each center on a minimum of 100 consecutive patients seen in the patch test clinic. These patients were also patch tested to a standard series with the 8 % fragrance mix (FM) and its 8 constituents. In patients with a positive reaction to any of the 48 FF, a careful history with regard to past or present reactions to perfumed products was taken. A total of 1323 patients were tested in 11 centers. The 8 % FM was positive in 89 patients (8.3 % of 1072 patients) [31].

Johansen JD et.al.were study the skin response to serial dilution patch tests and 6-week graded use tests with 0.02, 0.1 and 0.8 % cinnamic aldehyde in ethanol was studied in a group of cinnamic-aldehyde-sensitive eczema patients. The minimum effect level demonstrated was 0.02 % cinnamic aldehyde on patch testing and 0.1 % cinnamic aldehyde on use testing, which are allowed usage concentrations in different kind of cosmetics. 72 % (13/18) developed eczema in the use test performed with an alcoholic solution of cinnamic aldehyde on healthy upper arm skin. 6 of the 13 use-test-positive subjects (46 %) reacted later than day 7, indicating that the standard exposure period of 7 days in use testing may not be sufficient, if low concentrations or volatile substances are used. A significant correlation between patch test sensitivity and the outcome of use testing was found (1, < 0.001), which should be considered in designing future use test studies and advising patients. Detailed exposure information is needed to evaluate more fully the consequences of cinnamic aldehyde sensitivity [32].

The frequency of fragrance Contact sensitization in Hungary in a multicenter study in the years 1998 and 1999 was carried out by Temesvari E et al. A total Of 3,604 patients were tested with fragrance mix (FM), and positive reactions were Observed in 294 (8.2 %). In 160 FM hypersensitive patients, the study was continued with patch testing of the mix constituents of the patients tested, 70.6 % produced positive reactions to the constituents. FM contact sensitization was mainly observed in female patients (74.4 %). The incidence of contact urticaria in FM hypersensitive patients was 6.1 %. Simultaneous patch test trials of other environmental contact allergens, in both early and late evaluations, mainly confirmed hypersensitivity reactions to balsams [33].

## 2.7 Surveillance

Meding B in his surveilanc showed that the workers in a Swedish spice factory (n = 70), and in the office (n = 23) of the same company, were investigated by questionnaire regarding skin symptoms. In a second part of the study, subjects reporting skin symptoms were examined and investigated by patch and prick testing. Skin symptoms were reported by half the factory workers. Purities and skin irritation, particularly from cinnamon powder, were common. Patch test reactions to cinnamic aldehyde were found in 11/25 factory workers, but in several cases, the nature of the reactions was difficult to evaluate. Irritant patch test reactions were seen from powders of cardamom, paprika and white pepper. On prick testing, 6/25 workers reacted to cinnamic aldehyde. The results illustrate the difficulties of patch testing with spices and indicate the need for further research and validation of methods [34].

## 2.8 Alternative and In Vitro Tests

Chao LK et.al.were showed that low concentration of cinnamaldehyde (uM) inhibited the secretion of interleukin-1beta and tumor necrosis factor alpha within lipopolysaccharide (LPS) or lipoteichoic acid (LTA) stimulated murine J774A.1 macrophages. Cinnamaldehyde also suppressed the production of these cytokines from LPS stimulated human blood monocytes derived primary macrophages and human THP-1monocytes [35].

## 2.9 Laboratory Animals

Intraperitoneal injection of either a 250 or 500 mg/kg dose of cinnamaldehyde to mice resulted in ataxia, analgesia, hypothermia, a decrease in spontaneous motor activity, antagonism of methamphetamine-induced hyperactivity, and prolongation of sodium hexobarbital-induced anesthesia. A 500 mg/kg dose was also observed to reduce development of a tonic convulsion in a nicotine-induced convulsion. Administration of a 125 mg/kg dose intraperitoneally produced hypothermia, antipyreticosis, and a prolongation of hex barbital-induced anesthesia [26].

In Acute Exposure study Newzealand White rabbits were dosed dermally with cinnamic aldehyde at 0.59, 0.83, 1.00, 1.23 & 1.50 mL/kg. The test substance was kept in contact with the skin for 24 hours. The animals were observed daily for signs of mortality, toxicity and pharmacological effects. Skin reactions were scored on days 1, 7 and 14. Body weights were recorded pretest and at termination. All animals were examined for gross pathology. The LD50 and 95% confidence limits are: 1.2 (0.9 - 1.6) mL/kg of the body weight. Number of deaths at each dose level: 0.59 mL/kg = 0 dead/2 treated; 0.83 mL/kg = 2 dead/4 treated; 1.00 mL/kg = 1 dead/4 treated; 1.23 mL/kg = 1 dead/4 treated; 1.50 mL/kg = 4 dead/4 treated. Deaths occurred by day 3, and were preceded with pre-death physical signs of few feces, lethargy, ataxia and rales. Necropsy of the deaths revealed abnormalities of the lungs, liver, kidneys, treated skin and GI tract, as well as brown staining of the anogenital area and yellow staining of the nose/mouth area. Survivors: signs of diarrhea, few feces, emaciation, ataxia and limited mobility due to severe skin reaction, abnormalities of skin and intestines [36].

In a 12-week feeding study, groups of five male and five female weanling rats were maintained on diets containing only cinnamaldehyde at levels resulting in daily intakes of 58, 114 or 227 mg/kg. No adverse effects were observed (at any of the dietary levels of cinnamaldehyde) on appearance, behavior, growth, food consumption, efficiency of food utilization, presence of sugar or albumin in the urine, blood hemoglobin, liver and kidney weights, or gross pathology [26].

Groups of 10 male and 10 female rats were maintained for 16 weeks on diets containing cinnamaldehyde at levels of 0, 1000, 2500 and 10000 ppm (approximately equivalent to 50, 125 and 500 mg/kg/day). Neither body weight gains, hematology, nor examination of the major organs revealed significant differences between the test and control animals at levels of either 1000 or 2500 ppm. None of the above parameters revealed adverse effects following a dietary intake of 10 000 ppm for 16 weeks, with the exception of a "slight hepatic cell swelling" and a "slight hyperkeratosis of squamous portion" of the stomach, noted upon microscopic examination [26].

Some rats were administered the test substance by gavages for 2 week at doses of 0, 235, 470, 940, 1880 & 3750 mg/kg/day. Complete autopsy was performed on all animals that died, and at the termination of the study on all treated and control animals. Body wt and the organ wt of the liver, right kidney and the spleen were determined. Toxic response effects by dose level. All rats dosed at 1880 and 3750 mg/kg/day died or were killed when moribund during the first 7 days of dosing. Microscopic lesions included a minimal to moderate for stomach hyperplasia in males at doses of 470 mg/kg/day and higher. There were no consistent differences in organ wt or organ wt: body wt ratios between surviving

treated or controls. Clinical signs and gross lesions were absent in surviving rats. Test substance at dose 470 mg/kg/day and above produces for stomach hyperplasia and was lethal at doses of 1880 and above [36].

In Subchronic Exposure mice were administered the test substance by gavages for 3 weeks /at doses of 656, 1310, 2620, 5250 and 10,500 mg/kg/day .Complete autopsy was performed on all animals that died, and at the termination of the study on all treated and control animals. Body wt and the organ wt. of the liver, right kidney and the spleen were determined. All mice gavaged at doses of 5250 and 10,500 mg/kg/day, as well as all female mice and three male mice dosed with 2620 mg/kg/day died within first 2 days. No clinical signs or gross or microscopic lesions were visible in these mice. The only microscopic lesions observed in surviving mice were a minimal to mild for stomach hyperplasia and a minimal kidney nephropathy at doses of 1310 mg/kg/day and higher. Test substance at doses of 1310 mg/kg/day and above produced for stomach hyperplasia and was lethal at dose of 5250 and above [36].

In Subchronic exposure group of rats were fed a diet containing 0, 0.625, 1.25, 2.5, 5.0 or 10% cinnamaldehyde microcapsules for 14 days. A complete autopsy was performed on all animals that died, and at the termination of the study on all treated and control animals. Body wt and the organ wt. of the liver, right kidney and the spleen were determined. Marked dose related depression in body wt gain, slight decrease in spleen: body wt ratio for male rats in 10% group, dose dependent decrease in food consumption. Gross lesions in both sexes were limited to a reduction in the size of reproductive organs and secondary sex glands (seminal vesicles, prostates of males and ovaries of females). Hyperplasia of the fore stomach was observed. Treatment of rats with microencapsulated cinnamaldehyde resulted in marked dose-dependent depression of body weight, hypo-plastic changes in reproductive organs & accessory sex glands and hyperplasia of the for stomach mucosa [36].

In Subchronic Exposure group of rats were fed a diet containing 0, 0.625, 1.25, 2.5, 5.0 or 10 % cinnamaldehyde microcapsules for 21 days. A complete autopsy was performed on all animals that died, and at the termination of the study on all treated and control animals. Body wt and the organ wt. of the liver, right kidney and the spleen were determined. Dose-related decrease in body wt, decrease in absolute liver and kidney wt., hyperplasia of the for stomach epithelium at highest dose (10 %) characterized by a focal thickening of the stratified squamous epithelium, accompanied by hyperkeratosis Treatment of mice with Microencapsulated cinnamaldehyde resulted in dose-dependent depression of body weight and hyperplasia of the for stomach epithelium at highest dose (10 %) characterized by a focal thickening of the stratified squamous epithelium, accompanied by hyperkeratosis [36].

In a 24-week screening test, groups of 15 male and 15 female A/He mice received in the first eight weeks of the test period, a total dose of 0.8 or 4.0 g/kg bw of cinnamaldehyde in 24 thrice-weekly ip injections. The higher dose had previously been calculated to be the maximum tolerated dose. There was no increase in the incidence of tumors of the lung, liver, kidney, spleen, thymus, intestine, or salivary or endocrine glands. Survival was reduced in high-dose males to 60 % and in high-dose females to 75 %, but survival in the low-dose groups was unaffected [26].

Cinnamaldehyde was evaluated for developmental toxicity in a proposed new short-term in vivo animal bioassay. In this assay, pregnant mice are dosed with the test agent in mid-pregnancy and allowed to go to term. Observations are then made on litter size as well as the birth weight, neonatal growth, and survival of pups as indicators of developmental toxicity. Forty-nine pregnant CD-1 mice were given 1200 mg/kg/day cinnamaldehyde in corn oil by gavage on days 6-13 of gestation and were allowed to deliver. No toxic effect was observed in the dams or in their offspring for the parameters assayed [37].

Cinnamaldehyde was administered by gavages to Sprague-Dawley rats on days 7-17 of pregnancy at doses of 5, 25 or 250 mg/kg/day ... Actual dose received by dose level and sex: 0, 5, 25 or 250 mg/kg/day ... No signs of maternal toxicity, decreased weight gain between day 7 & 20 with decrease in food intake. Offspring toxicity F1 and F2: Increased incidence of poor cranial ossification decreased ossification of tympanic bulla at 25 or 250 mg/kg/day, increased incidence of dilated pelvis/reduced papilla in kidney, increased incidence of reduced cranial ossification, dilated ureter. One case of facial malformation & few cases of hypoplastic/dysplastic kidney. Authors' abstract states "significant increases of the incidences of dilated pelvis/reduced papilla in the kidney, dilated ureters >2 abnormal stern brae per fetus were detected in the 2-mg/kg group." However no such dose group (2-mg/kg) is reported in either the methodology or the Results section. The changes in treated groups might have been influenced by the greater litter size in the higher dose groups [36].

## 2.10 Genotoxicity

In Sprague-Dawley rats, a single oral dose equal to 1/2 LD50 did not induce DNA fragmentation in liver and gastric mucosa as evaluated by the alkaline elution technique, increased the frequency of micro nucleated hepatocytes but not of bone marrow micro nucleated polychromatic erythrocytes, and gave rise to a significantly higher incidence of total nuclear anomalies but not of micro nucleated cells in for stomach mucosa. In Swiss mice, the equitoxic dose of cinnamaldehyde caused the same clastogenic effect in the liver, while a negative response was observed in both bone marrow and for stomach mucosa. Finally, in rats initiated with N-nitrosodiethylamine the administration of 500 mg/kg/day cinnamaldehyde for 14 successive days produced a modest but statistically significant increase of the average diameter and area of gamma-glutamyltranspeptidase-positive foci that, together with changes observed in other parameters, might be considered indicative of a potential promoting activity. Taken as a whole, these findings confirm that high doses of cinnamaldehyde may induce genetic alterations at the chromosomal level, and suggest that the liver is the preferential target of its undesirable effects [38].

Vanillin (VAN) and cinnamaldehyde (CIN) are dietary anti-mutagens that, when added to assay plates, reduced the spontaneous mutant frequency in *Salmonella typhimurium* strain TA104 (hisG428, rfa, uvrB, pKM101) by 50 % ... Relative to untreated cells, treatment with either VAN or CIN produced a significant reduction in mutations at GC sites, whereas neither compound produced a significant reduction in mutations at AT sites. Anti-mutagenesis experiments in hisG428 strains of *Salmonella* with varying DNA repair backgrounds showed that VAN and CIN require SOS repair genes to produce an anti-mutagenic effect against spontaneous mutagenesis. Studies evaluating the effect of VAN and CIN on growth rate showed that neither compound suppressed growth relative to untreated cells [39].

Male Albino Sprague-Dawley rats were given a single oral dose of cinnamaldehyde in concentrations of 550, 1100 or 1650 mg/kg and male Swiss mice were given a single oral dose of 850, 1200 or 2550 mg/kg cinnamaldehyde. The frequency of micronucleated polychromatic erythrocytes was evaluated in marrow, hepatocytes and gastric mucosa. For both rats and mice no increase in the frequency of MNPCE in bone marrow 48 hrs after administration of cinnamaldehyde; it induced a dose-dependent clastogenic effect in the liver; significantly higher incidence of total nuclear anomalies but not of micronucleated cells in for stomach mucosa were seen in the rats. High doses of cinnamaldehyde may produce a clastogenic effect [36].

### **2.11 Non-Human Toxicity Values**

A. LD50 Rat oral 3400 mg/kg [40].

B. LD50 Mouse oral 200 mg/kg [41].

C. LD50 Guinea pig oral 1600 mg/kg [42].

D. LD50 Mouse oral 3,400 mg/kg [36].

### **2.12 National Toxicology Program Reports**

Groups of 50 male and 50 female mice were fed diets containing 1,000, 2,100, or 4,100 ppm microencapsulated trans-cinnamaldehyde for 2 years. Additional groups of 50 male and 50 female mice received untreated feed (untreated controls). Dietary concentrations of 1,000, 2,100, or 4,100 ppm delivered average daily doses of approximately 125, 270, or 550 mg/kg to males and females. Survival of males in the 2,100 ppm group was less than that of the vehicle control group. Mean body weights of 2,100 and 4,100 ppm males and females were generally less than those of the vehicle controls throughout the study, and mean body weights of 1,000 ppm males were less after week 74. There were no neoplasms that were attributed to exposure to trans-cinnamaldehyde [43]. Groups of 10 male and 10 female B6C3F1 mice were fed diets containing 4,100, 8,200, 16,500, or 33,000 ppm microencapsulated trans-cinnamaldehyde for 3 months. Additional groups of 10 male and 10 female mice received untreated feed (untreated controls) or feed containing placebo microcapsules (vehicle controls). One vehicle control male, one 4,100 ppm male, and one 33,000 ppm male died during the first week of the study due to inanition that resulted from difficulty with the feeder. Five 16,500 ppm and eight 33,000 ppm male mice died during weeks 2 and 3 due to unpalatability of the dosed feed. Mean body weights of all exposed groups of males and of females exposed to 8,200 ppm or greater were significantly less than those of the vehicle controls. The incidence of squamous epithelial hyperplasia of the fore stomach mucosa in 33,000 ppm females was significantly increased, and olfactory epithelial degeneration of the nasal cavity occurred in 16,500 and 33,000 ppm males and females [43].

## Material and Method

### 3.1 Material Used

#### 3.1.1 Experimental Animals

Male and female albino rats where are four to five weeks old, weighing  $100\pm 10$  g obtained from the national institute of research and aromatic plants. The rats were kept in cages where they are housed under barrier-maintained conditions of 12 hr dark/light cycles. Rats were fed pellet diet for laboratory rats which prepared from wheat, dried meat, salt and water.



Figure 3.1 Albino Rat

#### 3.1.2 Device



Figure3.2Mindary BS-200 Instruments

### 3.2 Methods

The Cinnamon bark sample was collected from shop in the central market of Khartoum then it was dried and crushed until it became a soft powder .The powder was mixed with food to made dose level of 500,1200 and 1900 mg/kg body weight day as food pellets . The rats were separated to four group each group contain five rats. The first control group which did not receive Cinnamaldehyde. Second group received 500 mg/kg/day (ED50), third group received 1200 mg/kg/day (TD50) and the last group received 1900 mg/kg/day (LD50) of Cinnamaldehyde with food.

#### 3.2.2 Sample collection

Sufficient amount of blood samples were collected in light green –toper tube (contain lithium heparin anticoagulant), the blood was mixed by gently. Plasma was separated by centrifugation 300 RPM for 3 times. Then the following tests were performed using Mindary BS-200 instrument according to the following method:

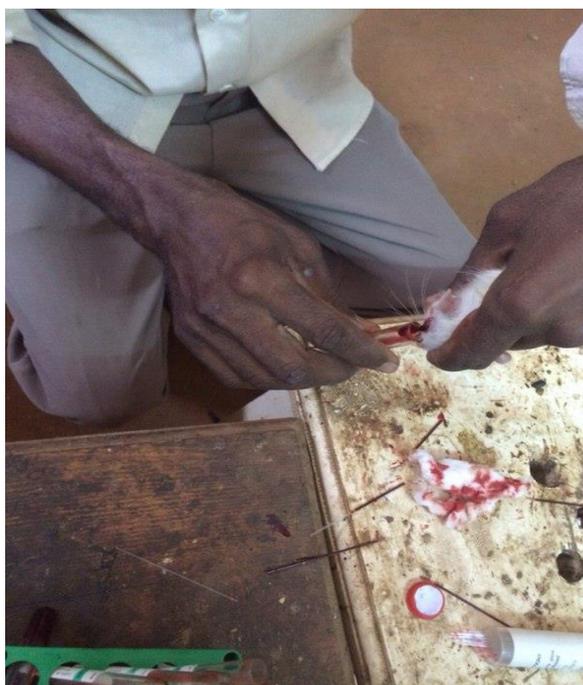


Figure 3.3 Sample Collection

#### 1-GOT

##### Method:

UV-assay according to IFCC (International federation of clinical chemistry and laboratory medicine) without pyridoxal phosphate activation.

#### 2. ALP:

Method: International federation of clinical chemistry and laboratory medicine (IFCC)

**3-Urea:****Method:**

Urease-glutamate Dehydrogenase, UV method

**4-Creatinine:****Method:**

Modified Jaffé method

**3.3 Statistical analysis method**

Completely randomized design had been implemented to conduct the experiment with # (how may) treatments replicated 3 or for times each. The data was collected and analyzed. The analysis of variance statistical methods using SAS statistical package version 9.1

**4.1 Results**

Table 4.1 Statistical Analysis results comparing different groups with control group:

<b>parameter</b> <b>Group</b>	<b>GOT</b> <b>Mean ± STD</b>	<b>ALP</b> <b>Mean ± STD</b>	<b>Urea</b> <b>Mean ± STD</b>	<b>Creatinine</b> <b>Mean ± STD</b>
<b>Control</b>	129.4 ± 23.564 NS	639.8 ± 131.220 NS	38.8 ± 4.32 NS	0.62 ± 0.130 NS
<b>TD50</b>	134.4 ± 38.894 NS	1169.4 ± 567.626 S	40.4 ± 3.13 S	0.68 ± 0.083 NS
<b>LD50</b>	83 ± 75.792 NS	1702.6 ± 537.186 HS	47.6 ± 4.615 HS	0.74 ± 0.114 NS
<b>ED50</b>	76 ± 49.685 NS	836 ± 251.441 NS	35.75 ± 3.947 NS	0.65 ± 0.129 NS

[NS =  $P \geq 0.05$ , HS =  $P \leq 0.03$ , S =  $P \leq 0.05$ ]

**Key:**

NS= not significant

HS= highly significant    S= significant

**Table 4.2** P values of different serum parameters:

Parameters	P values
ALP	0.0073
GOT	0.2139
Urea	0.0032
Creatinine	0.4280

**Table 4.3** Doses of Cinnamaldehyde received by different groups of Rats:

Group	Dose
Control	Did not received dose
ED50	500 mg/kg/day
TD50	1200 mg/kg/day
LD50	1900 /kg/day

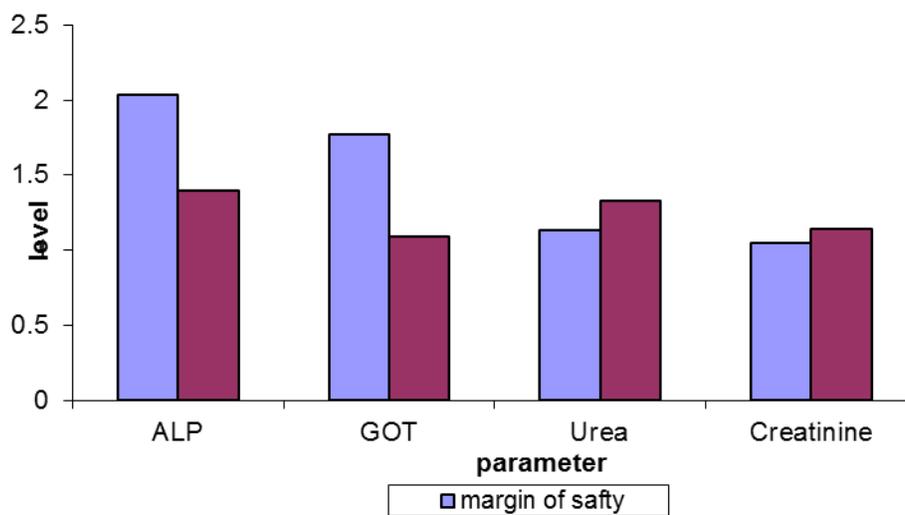
**Table 4.4** Margin of Safety and Lethality of different serum parameter

Parameter	Margin of safety	Lethality
ALP	2.037	1.399
GOT	1.768	1.092
Urea	1.13	1.331
Ceriatinine	1.046	1.138

**Where**

1. Margin of safety =  $TD_{50}/ED_{50}$

2. Lethality =  $LD_{50}/ED_{50}$

**Figure 4.1** Cinnamaldehyde Margin of Safety and Lethality

## 4.2 Discussion

A previous study showed that there was no death in the rats that were receiving microcapsulated cinnamaldehyde. Rats receiving cinnamaldehyde in food show a dose related decrease in their body weight [43]. In this study; certain behavioral changes were observed manifested by a decrease in the activity and body weight in the group that received ED50, while LD50 group showed the same symptoms with more severity and two members were comatose [37]. Other groups (control and ED50) did not show any behavioral changes. No animal death occurred in all groups that received cinnamaldehyde at different doses in their food and this makes it a relatively safe food additive, but still chronicity testing is essential.

One study carried on white newzealand rabbits, which were given intradermal doses with cinnamaldehyde and observed daily for signs of mortality, toxicity and weight variations. Some rabbits died due to abnormalities in the lungs, liver and kidneys [36]. In this study the statistical analyses indicated that the group who received the lethal dose LD50 showed a significant increase in ALP levels as an indicator of hepatotoxicity.

The group that received the toxic dose also showed a significant increase in ALP levels which was less than that showed by LD50 [26]. ED50 group did not show a significant increase in ALP like LD50 and ED50 groups. High ALP levels may indicate that either the liver has been damaged or there is a condition causing an increase in bone cell activity, this effect may be dose related [27].

Regarding GOT enzyme levels, all groups that received the cinnamaldehyde in different doses (ED50, TD50 and LD50) did not show significant changes in the enzyme levels [26].

Also there was an increase in the urea levels of the LD50 group, While TD50 group showed significant increase but smaller than that showed by LD50 and no significant increase was observed in ED50 group. These results indicate that the nephrotoxicity is dose related. [36]

The increase in urea level may indicate heart problems, urinary insufficiency and stress [44]. The entire groups (ED50, TD50 and LD50) did not show a significant change in serum creatinine levels [44].

## 5.1 Conclusion

Cinnamaldehyde is well known as a food flavor worldwide with high human consumption. In conclusion, the results showed that cinnamaldehyde was found to be nephrotoxic, hepatotoxic and may be cardiotoxic in a dose related pattern there should be awareness and rational use of cinnamon bark within margins of safety and lethality showed in this research.

## 5.2 Recommendation

- Further studies are recommended using oral cinnamaldehyde dose in food and as solution to compare toxic effects produced by the two different routes of administration.
- cardiotoxicity and antiobesity effects should also be studied regarding the use of cinnamaldehyde food flavor.
- The duration of the study should be increased to assess the long term use of cinnamaldehyde whether it can cause liver and kidney damage.
- Further histopathological and genetic testing can be performed to determine if there are morphological or genetic structural changes in the hepatocytes and nephrones.

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