

## Original article

Safety assessment of *Cinnamomum zeylanicum* Blume oil in male and female wistar rats

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## Abstract

The aim of the present study was to evaluate safety of cinnamon oil (*Cinnamomum zeylanicum* Blume) in male and female wistar rats. Forty wistar rats divided into eight groups, each group contains 5 males and 5 females. Group I and V served as vehicle control for male and female, respectively. Cinnamon oil was administered orally at dose of 50, 100 and 200 mg/kg body weight, once daily for 28 days in male rats of Groups II, III and IV as well as in female rats of Groups VI, VII and VIII, respectively. There was no significant difference was observed in body weight and feed consumption of II, III and IV as compare to Group I (male control), as well as female rats of Groups VI, VII and VIII as compared to Group V (female control). No significant changes have been observed in hematology parameters like Hb, RBCs, PCV, TLCs, MCV, MCH and MCHC, as well as no significant changes observed in serum creatinine, BUN, bilirubin, AST, ALT, total cholesterol, total protein and albumin in cinnamon oil treated male rats of Groups II, III and IV and in female rats of Groups VI, VII and VIII as compared to male and female control rats, respectively at the end of experiment. Histopathology of kidney, liver, spleen and heart from cinnamon oil treated male and female rats did not show any marked gross or histopathological changes. Results of the present study suggested that cinnamon oil was found safe, following repeated oral administration @ 50, 100 and 200 mg/kg b.wt., once daily for 28 days in male and female wistar rats.

**Key words:** *Cinnamomum zeylanicum* Blume, safety assessment, cinnamon oil, wistar rats

## 1. Introduction

Essential oils (EOs) also called volatile or ethereal oils are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation, enfleurage or extraction but the method of steam distillation is most commonly used for commercial production of EOs. As estimated, 3000 EOs are known, of which about 300 are commercially important, destined chiefly for the flavors and fragrances market (Van de Braak and Leijten, 1999). Researchers all over the world are trying to characterize a range of biological properties of essential oils which includes antimicrobial, antiviral, antimutagenic, anticancer, antioxidant, anti-inflammatory, immunomodulatory and antiprotozoal activities (Bakkali *et al.*, 2008). Herbal derived substances provide as an alternative rational means for the treatment of many diseases in human and animals (Manoharachary and Nagaraju, 2016; Udupa Nayanabhirama, 2016). In recent reported study, selected medicinal

plants from the surroundings of Junagadh district were screening for their phytochemical potential and result showed that few plants have a high level of phenolic or flavonoid compounds which may be a good source of natural antioxidant agents with great therapeutic importance (Bhatt *et al.*, 2019).

Cinnamon oil is extracted from *Cinnamomum zeylanicum* Blume. It is also called Cinnamon in English, Dalchini in Hindi, Taj in Gujarati (Indian spices board). Cinnamon is a native of Sri Lanka and tropical Asia. In India, it is found in southern part of the country. The genus *Cinnamomum* comprises of about 250 species, out of which 20 occur in India. The most important volatile oils from cinnamon are from *C. zeylanicum* bark and leaf oils, *C. cassia* (Cassia oil) and *C. camphora*. The chemical composition of cinnamon oils varies, depending on several factors that include the part of the plant used, age of trees, growing season and location, and extraction methods (Wong *et al.* 2014; Chakraborty *et al.* 2015). Different parts of the cinnamon plant have different primary constituents. Cinnamaldehyde is majorly found in bark oil, eugenol in leaf oil, and camphor in root-bark oil (Wijesekera, 1978). Cinnamon bark contains up to 4% of essential oil, consisting primarily of cinnamaldehyde (60%-75%), eugenol (1%-10%), cinnamyl acetate (1%-5%),  $\beta$ -caryophyllene (1%-4%), linalool (1%-3%), and 1.8-cineole (1%-2%) (WHO, 1999), whereas the main constituents found in the leaves of *C. zeylanicum* are eugenol (79.75%), *trans*-cinnamaldehyde (16.25%), and linalool (0.14%) (Wang *et al.*, 2009).

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Cinnamon has been used as a spice for thousands of years. In Ayurvedic medicine, cinnamon bark has been used as an antiemetic, anti-diarrheal, anti-flatulent and general stimulant (Hsieh, 2000). Several studies have reported pharmacotherapeutic activity of cinnamon and its essential oils. Krishnamoorthy and Rema (2003) reported that cinnamon bark oil is used frequently in the food, pharmaceutical and perfume industries. Cinnamon is also known to be a carminative, expectorant, and anti-diarrheal, and useful for bronchitis, itching, and urinary disease (Leela, 2008). Recent pharmacological studies have shown that besides its role as a spice, cinnamon or its essential oils can be used as a hypoglycemic and cholesterol-lowering (Khan *et al.*, 2003), wound healing (Kamath *et al.*, 2003), anti-inflammatory (Tung *et al.*, 2008; Prajapati *et al.*, 2019a; Prajapati *et al.*, 2019b), risk-reducing agent for colon cancer (Wondrak *et al.*, 2010), anti-coagulant properties (Husain and Ali, 2013) and antibacterials (Matan *et al.*, 2006; Prajapati *et al.*, 2018). There are limited reports on the safety evaluation of cinnamon oil in rats till date. Hence, the present study was undertaken to evaluate the effects of repeated oral administration of cinnamon oil at three different doses, daily for 28 days in male and female rats.

## 2. Materials and Methods

### 2.1 Experimental animals

The study was conducted on adult healthy male and female wistar rats. 20 male rats (370 to 420 g) and 20 female rats (220 to 260 g) of 8-10 weeks of age were procured from Cadila Health Care Ltd. (R & D Centre), Ahmedabad, Gujarat. All the protocols as per the committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines on the care and use of laboratory animals were followed and approved by the Institutional Animal Ethics Committee (Project No. IAEC/280/VPT/2018) of Veterinary College, AAU, Anand. Rats were kept under constant observation during entire period of study. The animals were housed in standard polypropylene cages and maintained under controlled room temperature  $22 \pm 2^\circ\text{C}$  and humidity  $55 \pm 5\%$  with 12 h light and 12 h dark cycle. All the rats were fed normal pellet diet and deionized water was provided *ad libitum* throughout the course of the experiment. All the rats were kept under acclimatization for 5 days prior to grouping and initiation of experiment. Rats were kept under constant observation during entire period of study. All necessary managemental procedures were adopted to keep the rats free from stress.

### 2.2 Drugs and chemicals

Cinnamon essential oil (Natural, Functional grade) was purchased from Sigma-Aldrich. All other reagents used of analytical grade like biochemical kits for estimation of serum biochemical parameters were purchased from Coral Clinical System (Goa, India).

### 2.3 Experimental design

Forty rats were divided into eight groups, each group contains 5 males and 5 females. Group I was served as the male control and Group V served as female control group. Cinnamon oil was administered orally at dose of 50, 100 and 200 mg/kg body weight once daily for 28 days to male rats of Groups II, III and IV and female rats of Groups VI, VII and VIII, respectively. The oil was administered daily orally to rats directly into stomach by using rat oral feeding gavage for 28 days.

### 2.4 Body weight and feed consumption

Body weight and feed consumption of all rats were measured weekly interval for 28 days.

### 2.5 Hematological estimation

On termination of experiment, blood samples were collected from retro-orbital plexuses under light anesthesia with the help of capillary tube. Blood samples collected in test tubes with  $\text{K}_3\text{EDTA}$  were utilized for estimation of various hematological parameters (Hb, RBCs, PCV, TLCs, MCV, MCH and MCHC) by hematology auto analyzer (Mindray, BC-2800 Vet, Garnerville, New York).

### 2.6 Biochemical estimation

Blood was harvested and kept at room temperature to collect serum. Serum biochemical parameters like serum creatinine, blood urea nitrogen (BUN), serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), total cholesterol (TC), total Bilirubin, total protein and total albumin were estimated by using auto serum chemistry analyser (Mindray BS-120, Mumbai, India).

### 2.7 Histopathology

After opening the carcass, gross lesions were recorded and collected tissues like kidney, liver, spleen and heart were fixed in 10 % formalin. The formalin fixed tissues were processed by paraffin wax embedding method of tissue sectioning. Sections from the tissues were cut at 5-6 microns thickness with automatic section cutting machine (Leica, Germany) and were stained with haematoxylin and eosin (H & E) stains.

### 2.8 Statistical analysis

All the data have been presented as mean  $\pm$  SE. Statistical comparisons of the means were made using one-way analysis of variance (ANOVA) using software SPSS (Version 25). Significant differences ( $p < 0.05$ ) between different experimental groups were analyzed by Duncan's test.

## 3. Results

### 3.1 Effects on clinical signs and mortality

The animals of all the groups were observed daily for clinical signs and mortality. There were no adverse signs of toxicity as well as mortality observed in all male and female rats that received different doses of cinnamon oil during entire study period.

### 3.2 Effect on body weight and feed consumption

There were no significant differences observed in body weight of male and female rats of different treatment groups on day 7, 14, 21 and 28 at dose of 50, 100 and 200 mg/kg as compared to rats of male and female control groups are shown in Table 1 and also graphically depicted in Figure 1. Similarly, no significant difference were observed in feed consumption of male and female rats of different treatment groups on 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> week at dose of 50, 100 and 200 mg/kg as compared to rats of male and female control groups are presented in Table 2 and also graphically depicted in Figure 2.

**Table 1:** Effect of repeated oral administration of cinnamon oil for 28 days on body weight (g) of male (I-IV) and female (V-VIII) wistar rats (Mean  $\pm$  SE; n=5)

Group	Treatment	0 day	7 day	14 day	21 day	28 day
I	Control (M)	370 $\pm$ 7.07	382 $\pm$ 8.60	385 $\pm$ 5.48	385 $\pm$ 5.48	394 $\pm$ 5.10
II	CZ-50 (M)	388 $\pm$ 7.35	380 $\pm$ 8.94	384 $\pm$ 9.27	392 $\pm$ 4.90	398 $\pm$ 3.74
III	CZ-100 (M)	379 $\pm$ 7.31	384 $\pm$ 7.48	400 $\pm$ 3.16	408 $\pm$ 5.83	414 $\pm$ 5.79
IV	CZ-200 (M)	369 $\pm$ 12.39	372 $\pm$ 12.41	390 $\pm$ 11.83	402 $\pm$ 12.81	414 $\pm$ 10.30
V	Control (F)	239 $\pm$ 3.32	241 $\pm$ 2.45	246 $\pm$ 3.67	248 $\pm$ 8.00	251 $\pm$ 7.14
VI	CZ-50 (F)	244 $\pm$ 6.78	246 $\pm$ 6	256 $\pm$ 8.72	250 $\pm$ 9.49	252 $\pm$ 10.32
VII	CZ-100 (F)	242 $\pm$ 4.90	226 $\pm$ 6.78	236 $\pm$ 4.00	225 $\pm$ 8.66	242 $\pm$ 7.68
VIII	CZ-200 (F)	256 $\pm$ 12.08	246 $\pm$ 15.68	238 $\pm$ 18.55	246 $\pm$ 16.00	238 $\pm$ 15.62

Mean values did not differ significantly between groups ( $p > 0.05$ ).

CZ-50 (M/F) = *Cinnamomum zeylanicum* oil @ 50 mg/kg b.wt. for male/female rats

CZ-100 (M/F) = *Cinnamomum zeylanicum* oil @ 100 mg/kg b.wt. male/female rats

CZ-200 (M/F) = *Cinnamomum zeylanicum* oil @ 200 mg/kg b.wt. male/female rats

**Table 2:** Effect of repeated oral administration of cinnamon oil for 28 days on feed consumption (g) of male (I-IV) and female (V-VIII) wistar rats (Mean  $\pm$  SE; n=5)

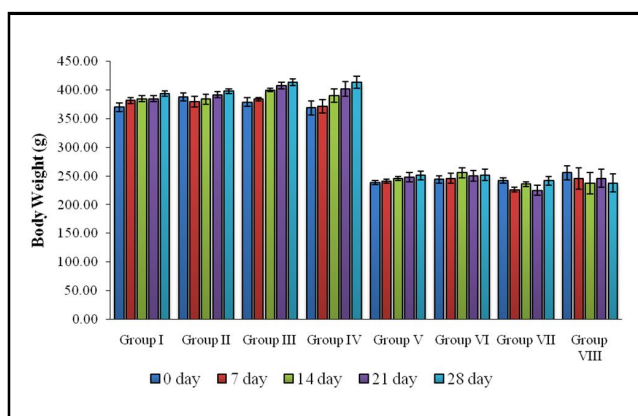
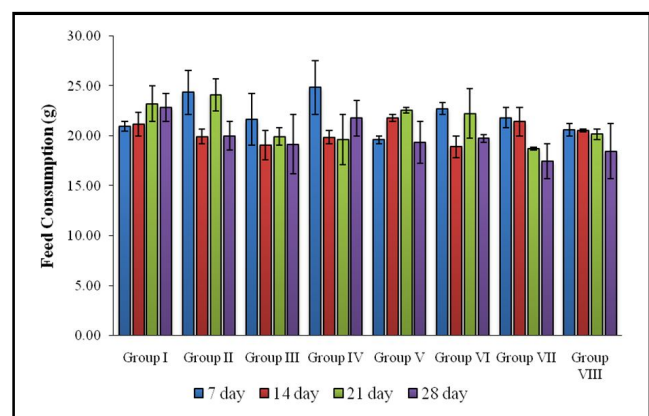
Group	Treatment	7 day	14 day	21 day	28 day
I	Control (M)	20.95 $\pm$ 0.48	21.19 $\pm$ 1.19	23.21 $\pm$ 1.79	22.86 $\pm$ 1.43
II	CZ-50 (M)	24.38 $\pm$ 2.19	19.95 $\pm$ 0.76	24.10 $\pm$ 1.62	20.00 $\pm$ 1.43
III	CZ-100 (M)	21.67 $\pm$ 2.62	19.10 $\pm$ 1.48	19.95 $\pm$ 0.90	19.17 $\pm$ 2.98
IV	CZ-200 (M)	24.88 $\pm$ 2.69	19.88 $\pm$ 0.69	19.67 $\pm$ 2.52	21.79 $\pm$ 1.79
V	Control (F)	19.62 $\pm$ 0.38	21.79 $\pm$ 0.36	22.57 $\pm$ 0.29	19.33 $\pm$ 2.10
VI	CZ-50 (F)	22.74 $\pm$ 0.60	18.93 $\pm$ 1.07	22.26 $\pm$ 2.45	19.76 $\pm$ 0.38
VII	CZ-100 (F)	21.83 $\pm$ 1.02	21.43 $\pm$ 1.43	18.71 $\pm$ 0.14	17.48 $\pm$ 1.76
VIII	CZ-200 (F)	20.62 $\pm$ 0.62	20.57 $\pm$ 0.14	20.19 $\pm$ 0.52	18.48 $\pm$ 2.76

Mean values did not differ significantly between groups ( $p > 0.05$ ).

CZ-50 (M/F) = *Cinnamomum zeylanicum* oil @ 50 mg/kg b.wt. for male/female rats

CZ-100 (M/F) = *Cinnamomum zeylanicum* oil @ 100 mg/kg b.wt. male/female rats

CZ-200 (M/F) = *Cinnamomum zeylanicum* oil @ 200 mg/kg b.wt. male/female rats

**Figure 1:** Effect of repeated oral administration of cinnamon oil for 28 days on body weight of male (I-IV) and female (V-VIII) rats (Mean  $\pm$  SE; n=5).**Figure 2:** Effect of repeated oral administration of Cinnamon oil for 28 days on feed consumption of male (I-IV) and female (V-VIII) rats (Mean  $\pm$  SE; n=5).

### 3.3 Effect on hematological parameters

No significant changes have been observed in Hb, RBCs, PCV, TLCs, MCV, MCH and MCHC levels in cinnamon oil treated male rats (II, III and IV) and female rats (VI, VII and VIII) as compared to control rats (I and V, respectively) at the end of experiment on 28<sup>th</sup> day as depicted in Table 3.

### 3.4 Effect on serum biochemical parameters

No significant changes have been observed in serum creatinine level, blood urea nitrogen (BUN), serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), total cholesterol (TC), total bilirubin, total protein and total albumin in cinnamon oil treated male rats (II, III and IV) and female rats (VI, VII and VIII) as compared to control rats (I and V, respectively) at the end of experiment on 28<sup>th</sup> day as depicted in Table 4.

**Table 3:** The effect of repeated oral administration of cinnamon oil for 28 days on Hb, RBC, PCV, TLC, MCV, MCH and MCHC of rats (Mean  $\pm$  SE; n=5)

Group	Treatment	Hb (g/dl)	RBCs ( $10^6/\mu\text{l}$ )	PCV (%)	TLCs ( $10^3/\mu\text{l}$ )	MCV (fl)	MCH (pg)	MCHC (g/dl)
I	Control (M)	14.92 $\pm$ 1.05	9.12 $\pm$ 0.41	43.98 $\pm$ 0.67	9.50 $\pm$ 0.64	48.57 $\pm$ 2.05	16.54 $\pm$ 1.40	34.06 $\pm$ 2.76
II	CZ-50 (M)	16.34 $\pm$ 0.63	9.59 $\pm$ 0.48	42.08 $\pm$ 1.48	9.53 $\pm$ 0.77	44.06 $\pm$ 1.18	17.31 $\pm$ 1.46	39.2 $\pm$ 2.79
III	CZ-100 (M)	16.9 $\pm$ 0.75	9.45 $\pm$ 0.37	44.70 $\pm$ 2.12	9.30 $\pm$ 0.95	47.76 $\pm$ 3.37	18.07 $\pm$ 1.34	37.97 $\pm$ 1.52
IV	CZ-200 (M)	16.00 $\pm$ 0.77	9.71 $\pm$ 0.11	43.80 $\pm$ 1.40	9.08 $\pm$ 0.77	45.08 $\pm$ 1.29	16.44 $\pm$ 0.62	36.55 $\pm$ 1.58
V	Control (F)	15.26 $\pm$ 0.40	8.78 $\pm$ 0.58	42.95 $\pm$ 2.11	9.00 $\pm$ 0.39	49.83 $\pm$ 4.38	17.70 $\pm$ 1.27	36.07 $\pm$ 2.79
VI	CZ-50 (F)	16.30 $\pm$ 0.33	9.62 $\pm$ 0.40	44.97 $\pm$ 1.64	9.08 $\pm$ 0.90	47.09 $\pm$ 2.52	17.04 $\pm$ 0.62	36.38 $\pm$ 1.08
VII	CZ-100 (F)	16.20 $\pm$ 0.43	8.92 $\pm$ 0.26	43.26 $\pm$ 1.26	7.44 $\pm$ 0.82	48.67 $\pm$ 2.07	18.17 $\pm$ 0.14	37.59 $\pm$ 1.55
VIII	CZ-200 (F)	15.86 $\pm$ 0.41	8.71 $\pm$ 0.25	43.96 $\pm$ 2.01	7.64 $\pm$ 0.64	50.78 $\pm$ 3.29	18.26 $\pm$ 0.59	36.43 $\pm$ 2.18

Mean values did not differ significantly between groups ( $p > 0.05$ ) for any of the parameters.

CZ-50 (M/F) = *Cinnamomum zeylanicum* oil @ 50 mg/kg b.wt. for male/female rats

CZ-100 (M/F) = *Cinnamomum zeylanicum* oil @ 100 mg/kg b.wt. male/female rats

CZ-200 (M/F) = *Cinnamomum zeylanicum* oil @ 200 mg/kg b.wt. male/female rats

**Table 4:** The effect of repeated oral administration of cinnamon oil for 28 days on creatinine and BUN, AST, ALT, TC, Bilirubin, Total protein and Albumin of rats (Mean  $\pm$  SE; n=5)

Group	Treatment	Creatinine (mg/dl)	BUN (mg/dl)	AST (U/l)	ALT (U/l)	TC (mg/dl)	Bilirubin (mg/dl)	Total protein (g/dl)	Albumin (g/dl)
I	Control (M)	0.65 $\pm$ 0.06	17.79 $\pm$ 2.20	66.42 $\pm$ 3.30	145.75 $\pm$ 11.89	91.27 $\pm$ 5.29	0.67 $\pm$ 0.05	5.99 $\pm$ 0.20	4.23 $\pm$ 0.12
II	CZ-50 (M)	0.62 $\pm$ 0.02	17.82 $\pm$ 1.19	66.25 $\pm$ 2.67	145.88 $\pm$ 6.81	91.13 $\pm$ 5.99	0.67 $\pm$ 0.02	5.92 $\pm$ 0.13	4.25 $\pm$ 0.12
III	CZ-100 (M)	0.62 $\pm$ 0.02	17.43 $\pm$ 1.27	66.46 $\pm$ 5.65	145.98 $\pm$ 10.05	97.61 $\pm$ 7.82	0.66 $\pm$ 0.03	5.94 $\pm$ 0.12	4.35 $\pm$ 0.05
IV	CZ-200 (M)	0.61 $\pm$ 0.05	19.61 $\pm$ 1.03	66.29 $\pm$ 2.99	144.95 $\pm$ 9.13	102.45 $\pm$ 4.09	0.67 $\pm$ 0.05	5.94 $\pm$ 0.16	4.37 $\pm$ 0.15
V	Control (F)	0.57 $\pm$ 0.03	18.68 $\pm$ 1.47	63.75 $\pm$ 9.59	127.17 $\pm$ 4.26	83.63 $\pm$ 4.72	0.85 $\pm$ 0.05	6.33 $\pm$ 0.12	4.20 $\pm$ 0.24
VI	CZ-50 (F)	0.60 $\pm$ 0.04	19.32 $\pm$ 1.17	47.09 $\pm$ 7.01	17.04 $\pm$ 4.79	36.38 $\pm$ 9.54	0.8 $\pm$ 0.08	6.33 $\pm$ 0.12	4.46 $\pm$ 0.08
VII	CZ-100 (F)	0.54 $\pm$ 0.04	17.46 $\pm$ 0.69	48.67 $\pm$ 6.79	18.17 $\pm$ 10.08	37.59 $\pm$ 5.28	0.82 $\pm$ 0.07	6.31 $\pm$ 0.13	4.45 $\pm$ 0.01
VIII	CZ-200 (F)	0.56 $\pm$ 0.03	17.36 $\pm$ 1.19	50.78 $\pm$ 5.75	18.26 $\pm$ 14.77	36.43 $\pm$ 5.92	0.84 $\pm$ 0.09	6.33 $\pm$ 0.15	4.22 $\pm$ 0.20

Mean values did not differ significantly between groups ( $p > 0.05$ ) for any of the parameters.

CZ-50 (M/F) = *Cinnamomum zeylanicum* oil @ 50 mg/kg b.wt. for male/female rats

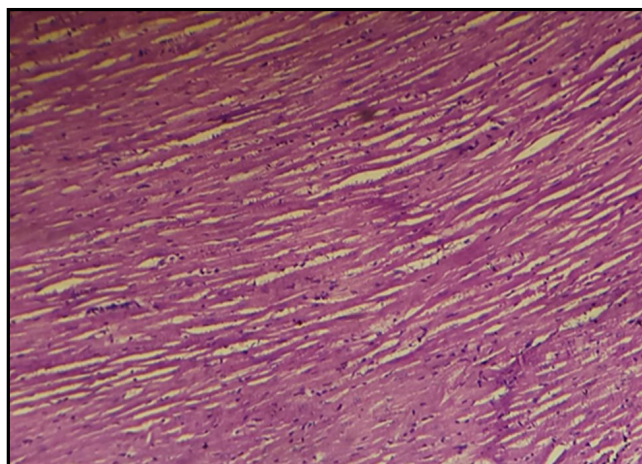
CZ-100 (M/F) = *Cinnamomum zeylanicum* oil @ 100 mg/kg b.wt. male/female rats

CZ-200 (M/F) = *Cinnamomum zeylanicum* oil @ 200 mg/kg b.wt. male/female rats

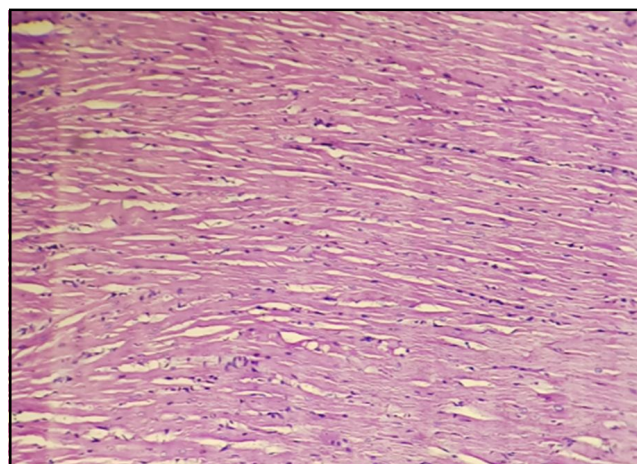
### 3.5 Histopathology

All the male and female rats were sacrificed at the end of experiment and subjected to post mortem examination. Organs (kidney, liver, spleen and heart) were collected for gross and histopathological examination. In the present study, histopathology of organs like

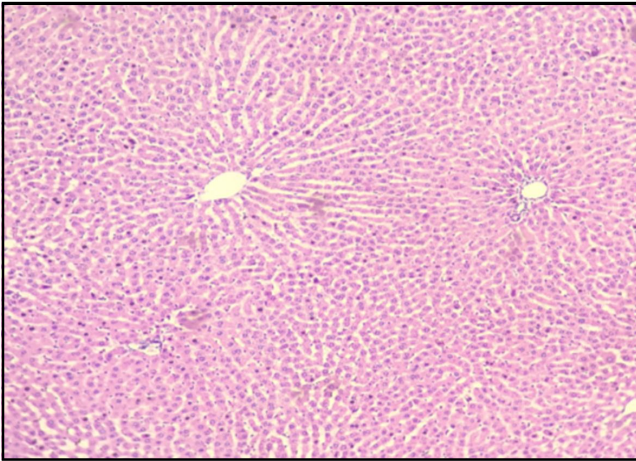
kidney, liver, spleen and heart from control rats (Group I) did not reveal any gross or microscopic changes. Likewise, histopathology of kidney, liver, spleen and heart from cinnamon oil treated male rats (II, III and IV) and female rats (VI, VII and VIII) did not show any marked gross or histopathological changes as compared to vehicle control rats as depicted in Figures 3 to 10.



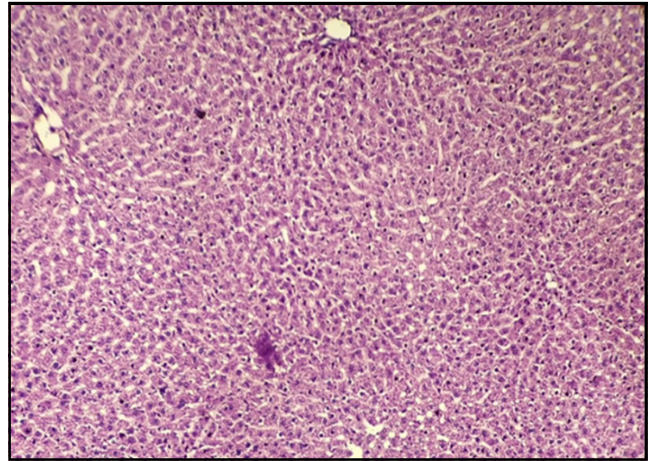
**Figure 3:** Section of heart from male control rats (group I) showing normal architecture (H & E stain X 120).



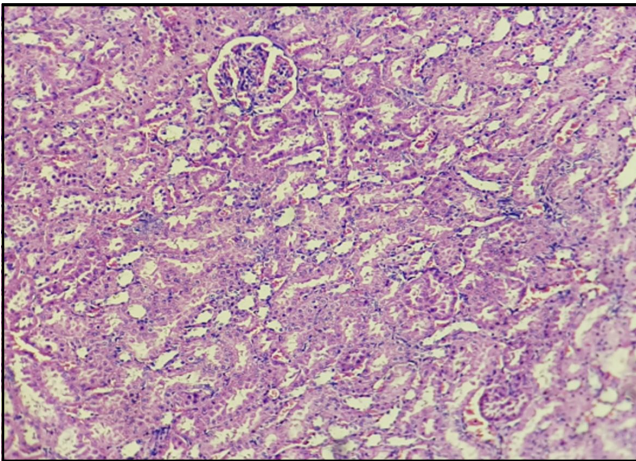
**Figure 4:** Section of heart from cinnamon oil treated male rats of group III (200 mg/kg) showing no pathological alterations in architecture (H & E stain X 120).



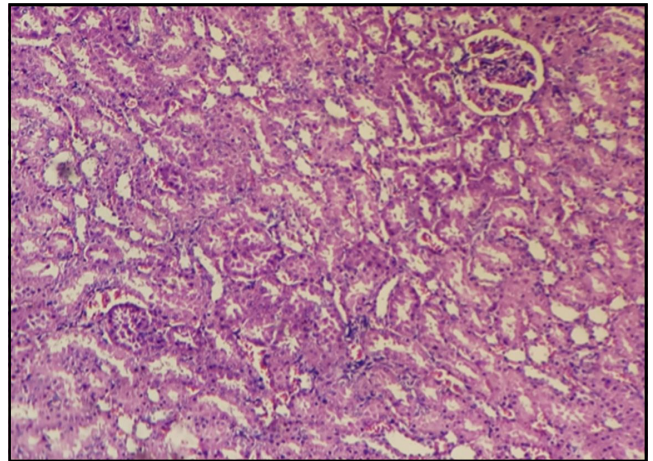
**Figure 5:** Section of liver from female control rats (group V) showing normal architecture (H & E stain X 120).



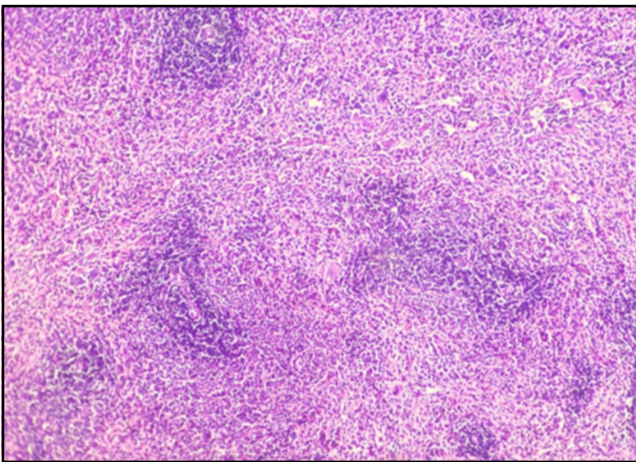
**Figure 6:** Section of liver from cinnamon oil treated female rats of group VIII (200 mg/kg) showing no pathological alterations in architecture (H & E stain X 120).



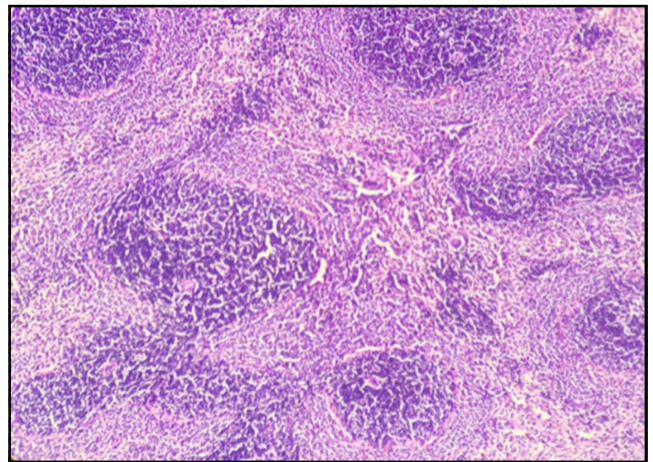
**Figure 7:** Section of kidney from male control rats (group I) showing normal architecture (H & E stain X 120).



**Figure 8:** Section of kidney from cinnamon oil treated male rats of group III (200 mg/kg) showing no pathological alterations in architecture (H & E stain X 120).



**Figure 9:** Section of spleen from female control rats (group V) showing normal architecture (H & E stain X 120).



**Figure 10:** Section of spleen from cinnamon oil treated female rats of group VIII (200 mg/kg) showing no pathological alterations in architecture (H & E stain X 120).

#### 4. Discussion

In the present study, no clinical signs or mortality were observed in all cinnamon oil treated male and female rats. Similarly, Ahmad *et al.* (2015) reported no any adverse effects on behavior, mortality, water intake at all concentrations of cinnamon bark aqueous extract treated rats. Yun *et al.* (2018) also evaluated sub-chronic toxicity of cinnamon extract (*Cinnamomum cassia*) and they observed no adverse clinical signs and mortality at highest dose (1000 mg/kg b.wt.) in rats. Likewise, Humbal *et al.* (2019) also reported no any observed clinical signs or mortality following repeated oral administration of clove oil @ 50, 100 and 200 mg/kg, daily once for 28 days male and female rats.

In our study, no significant change in body weight and feed consumption were observed in cinnamon oil treated rats from 0 to 28<sup>th</sup> day of study. Similar result was found by Shah *et al.* (1998) in chronic oral toxicity (100 mg/kg/day for 90 days) study of ethanolic extract on *Cinnamomum zeylanicum* Nees bark in mice. Ahmad *et al.* (2013) evaluated the safety of methanol extract of *Cinnamomum burmannii* by acute (single dose) and sub-chronic (repeated doses) oral administration to Sprague-Dawley rats. Results showed that no significant differences were observed in body weight of rats in the treatment groups as compared to the control group. Ahmad *et al.* (2015) evaluated toxicological effects of cinnamon bark aqueous extract at three doses 0.1, 0.5 and 2.0 g/kg in rats. They reported non-significant difference in the body weight and feed consumption of treated rats. Similarly, in an another 13-week repeat-dose oral toxicity study of cinnamon extract (*Cinnamomum cassia*) in rats revealed that body weights of rats were normal even after receiving cinnamon extract up to 2000 mg/kg treated rats (Yun *et al.*, 2018). Similarly, Humbal *et al.* (2019) reported no significant change in body weight and feed consumption were observed in clove oil treated @ 50, 100 and 200 mg/kg, daily once for 28 days in male and female rats as compared to control rats, respectively.

In the present study, findings showed no significant changes in Hb, RBCs, PCV, TLCs, MCV, MCH and MCHC levels in cinnamon oil treated rats. Similarly, Ahmad *et al.* (2013) evaluated the effects of repeated doses (sub-chronic) oral administration of methanol extract of *Cinnamomum burmannii* on haematological parameters in Sprague-Dawley rats. Results showed no significant differences in any of the hematological parameters of rats given cinnamon oil as compared to control rats. Similarly, Ahmad *et al.* (2015) reported effects of cinnamon bark aqueous extract on haematology in rats. They reported non-significant changes in red blood cell counts (RBCs), haemoglobin concentration (Hb), pack cell volume (PCV), total white blood cell counts (WBCs), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and differential leukocyte counts (DLCs) in rats treated with cinnamon bark aqueous extract at the dose of 0.1, 0.5 and 2 g/kg b.wt. for 28 days. Yun *et al.* (2018) also reported that cinnamon extract caused no toxicological significance in hematopoiesis and leukopoiesis associated parameters like PCV, Hb, WBCs, RBCs, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC) in male and female rats. Similarly, no significant changes have been observed in Hb, RBCs, PCV, TLCs, MCV, MCH and MCHC in clove oil treated (50, 100 and 200 mg/kg, daily once for 28 days) male and female rats at the end of experiment as compared to control rats (Humbal *et al.*, 2019).

In the present study, findings showed no significant changes in serum creatinine level, blood urea nitrogen (BUN), serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), total cholesterol (TC), total bilirubin, total protein and total albumin in cinnamon oil treated rats. Similar result was found by Shah *et al.* (1998) when they evaluated acute oral toxicity of ethanolic extract of *Cinnamomum zeylanicum* Nees bark in mice. Acute dosages were 0.5, 1.0 and 3 g/kg (Single dose) while the chronic dosage was 100 mg/kg/day for 90 days. Results showed that creatinine, BUN, bilirubin, alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) had no significant difference in any of the parameters tested between the control and the treated groups. Ahmad *et al.* (2013) evaluated the safety of methanol extract of *Cinnamomum burmannii* by acute and sub-chronic oral administration to Sprague-Dawley rats. Analysis of biochemical parameters like creatinine, BUN, bilirubin, alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) showed no significant difference in any of the parameters tested between the control and the treated groups of either sex of the rats. El-sayed *et al.* (2017) reported effect of dietary supplementation of cinnamon powder on growth performance, interleukin-6 and serum biochemistry in albino rats. In this study, no alteration in the levels of total proteins and albumin was found. Yun *et al.* (2018) investigated *in vivo* safety of cinnamon extract (*Cinnamomum cassia*). Serum biochemical analysis revealed that total bilirubin, BUN, ALT and albumin levels in rats did not show any significant changes. Likewise in support of the present study, Humbal *et al.* (2019) reported effects of repeated oral administration of *Syzygium aromaticum* oil (clove oil) at three different dose (50, 100 and 200 mg/kg, once daily) for 28 days on serum biochemical parameters. The results revealed no significant change have been observed in serum creatinine, BUN, total bilirubin, AST, ALT, total cholesterol, total protein and albumin in clove oil treated male and female rats as compared to control rats at the end of experiment.

The findings of the present study showed no defined pathological lesions in histoarchitecture of kidney, liver, spleen and heart in cinnamon oil treated groups. During necropsy, no appreciable gross changes were observed in kidney, liver, spleen and heart of any experimental rats. Ahmad *et al.* (2013) evaluated the effects of methanol extract of *Cinnamomum burmannii* by sub-chronic (repeated doses) oral administration on histopathology in Sprague-Dawley rats. They reported no lesions or pathological changes attributable to treatment with methanol extract of *Cinnamomum burmannii* in the organs of either sex of the treated rats as compared to their respective normal rats. Similarly, in support to our findings, Yun *et al.* (2018) investigated 13-week repeat-dose oral toxicity study of cinnamon extract (*Cinnamomum cassia*) revealed that there were no histopathological changes. In gross visual examination, cinnamon extract treated rats did not cause any macroscopic pathology in organs of rats compared to control rats. Humbal *et al.* (2019) reported no any marked gross or histopathological alteration in organs like kidney, liver, spleen and heart in clove oil treated male and female rats.

#### 5. Conclusion

The present study revealed that cinnamon oil was found safe following repeated oral administration @ 50, 100 and 200 mg/kg body weight once daily for 28 days in male and female wistar rats, based on results of non-significant alteration in body weight, feed consumption, haemato-biochemical parameters and histopathological examination.

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## Conflict of interest

The authors declare that there are no conflicts of interest in the course of conducting the research. All the authors had final decision regarding the manuscript and decision to submit the findings for publication.

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