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Role of Nano-curcumin on carbon tetrachloride (CCl_4) induced hepatotoxicity in rats

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Abstract
To investigate the hepatoprotective effect of nanocurcumin on carbon tetrachloride induced hepatotoxicity, 48 rats were divided into eight groups consisting six animals each. Hepato-toxicity was induced using CCl_4 at the dose of 2 ml/kg body weight intraperitoneally twice weekly. Nanocurcumin prepared by evaporative-precipitation of nanosuspension was administered at the dose of 40, 80 and 160 mg/kg body weight orally daily for 28 days. The protective effect of nanocurcumin was compared with curcumin and turmeric (160 mg/kg body weight). Silymarin was used as standard. Results revealed that CCl_4 elevated LDH and liver enzymes (AST, ALT, ALP) activities, bilirubin and uric acid blood levels, and decreased total protein, albumin, and globulin. Nanocurcumin significantly decreased liver enzymes (AST, ALT, ALP) and LDH activities, bilirubin and uric acid, and increased levels of total protein, albumin, and globulin in a dose-dependent manner. It elucidated the hepatoprotective effect of nanocurcumin on carbon tetrachloride induced hepatic-toxicity.

Keywords: Nanocurcumin, curcumin, carbon tetrachloride (CCl_4), hepatotoxicity, hepatoprotective, silymarin

Introduction
Curcumin, the active ingredient of turmeric (*Curcuma longa*) has been vested by nature with wide spectrum of beneficial properties which include anti-inflammatory, antioxidant, chemo-preventive and chemotherapeutic activities. It is a free radical scavenger and hydrogen donor which exhibits both pro and antioxidant activities. It is also endowed with the capability to binds with metals, more particularly with iron and copper and can function as an ion chelator. Curcuma, a genus in the plant family of Zingiberacea, is the biological source for curcuminoids, including curcumin. The yellow pigmented fraction of *Curcuma longa* contains curcuminoids, which are chemically related to its principal ingredient, curcumin. The three main curcuminoids isolated from turmeric are curcumin, demethoxy curcumin, and bisdemethoxy curcumin. Curcumin is one of these plant derived compound which is found to have many therapeutic advantages along with the hepatoprotective effect.
Despite of all these advantages, applications of curcumin are limited in clinical trials because of its poor solubility and low oral bioavailability. Today, nano preparations have emerged as a platform for the efficient delivery of drug to overcome these types of problems.
Nanocurcumin is a modified form of curcumin in which the particles of curcumin are transformed into nanoparticles that are more soluble and deliverable in the body. These particles have been shown to be more targeted to the tissue of interest that leads to better drug delivery and faster treatment without any wastage or side effects. Nanocurcumin has been identified for its potential to treat several types of cancers toxicity caused by heavy metals and as an immuno-stimulating agent for parasitic diseases and presently, various studies have been conducted on nanocurcumin to investigate the preventive and therapeutic effect against diethylnitrosamine (DEN) induced hepatocellular carcinoma in rats (Chuang *et al*., 2000 and Duncan *et al*., 1994) [1, 2]. But, till date to the best of our knowledge, no report of hepatoprotective effect of nanocurcumin has been studied on carbon tetrachloride induced hepatotoxicity model. So we have taken up this study with an objective to evaluate the hepatoprotective effect of nanocurcumin on carbon tetrachloride (CCl_4) induced hepato toxicity in rats.

Materials and Methods
Experimental animals
The study was performed in accordance with the guidelines for the use and care of laboratory

animals by Institutional Animal Ethical Committee (Approval No. 770/ac/CPCSEA/FVSc/AAU/IAEC/15-16/355). Experiment was performed on healthy Wistar albino rats weighing between 120-150 g. All the animals were kept in polypropylene cages in a small group of 6 rats per cage. Animals had free access to standard balanced ration and clean drinking water *ad libitum* and were maintained in a standard laboratory conditions (12:12 hour light/ dark cycle at ambient temperature ranging between (22-25 ^{0}C).

Preparation of nanocurcumin

Nano-curcumin was prepared as per the method described by Kakran *et al*. (2012) [8]: Evaporative precipitation of nanosuspension (EPN). Following this method, the nano particle of size 140.5 nm was obtained from original curcumin size of 4706.0 nm.

Administration of drugs

Each animal was weighed on a weighing balance to ascertain the dose of nanocurcumin, curcumin, turmeric, silymarin, carbon tetrachloride. Carbon tetrachloride (CCl_4) was administered with olive oil, and silymarin syrup was administered as such. Nanocurcumin, curcumin and turmeric powder was administered by dissolving in CMC.

Group (I): Served as normal control and was administered olive oil i.p., at the dose rate of 2 ml/kg body weight on the day of administration of CCl_4 to the treatment groups and CMC (1% w/v) p.o., 1 ml/100g body weight for 4 weeks.

Group (II): Served as positive control and were administered 1:2 (v/v) mixtures of CCl_4 in olive oil (2 ml/kg body weight) i.p. for 4 weeks.

Group (III): Rats were administered silymarin @ 100 mg/kg body weight p.o. daily and also administered 2 ml/kg body weight of 1:2 (v/v) mixtures of CCl_4 in olive oil by i.p. route once in a week for 4 weeks.

Group (IV): Rats were administered nanocurcumin @ 40 mg/kg body weight p.o. daily and also administered 2 ml/kg body weight of 1:2 (v/v) mixtures of CCl_4 in olive oil by i.p. route once in a week for 4 weeks.

Group (V): Rats were administered nanocurcumin @ 80 mg/kg body weight p.o. daily and also administered 2 ml/kg body weight of 1:2 (v/v) mixtures of CCl_4 in olive oil by i.p. route once in a week for 4 weeks.

Group (VI): Rats were administered nanocurcumin @ 160 mg/kg body weight p.o. daily and also administered 2 ml/kg body weight of 1:2 (v/v) mixtures of CCl_4 in olive oil by i.p. route once in a week for 4 weeks.

Group (VII): Rats were given curcumin @ 160 mg/kg body weight p.o. daily and also administered 2 ml/kg body weight of 1:2 (v/v) mixtures of CCl_4 in olive oil by i.p. route once in a week for 4 weeks.

Group (VIII): Rats were administered turmeric powder @ 160 mg/kg body weight p.o. and also administered 2 ml/kg body weight of 1:2 (v/v) mixtures of CCl_4 in olive oil i.p. route once in a week for 4 weeks.

Collection of blood

Blood was collected from each rat by puncturing retro-orbital plexus with the help of capillary tubes on 0, 7th, 14th, 21st and 28th day. Blood samples were collected into clot activator vials for serum preparation. The separated serum was used for the estimation of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), uric acid, total protein, albumin, globulin and bilirubin. The tests were carried out with the help of biochemical kits and the parameters were estimated by UV-VIS spectrophotometer.

Results and Discussion

Table 1: Effect of nano-curcumin on serum enzyme (AST) in CCl_4 induced hepatic damage in rats

Groups	Days				
	0	7	14	21	28
I	124.28±2.60	120.94±1.31	123.06±0.01^{h}	121.05±0.01^{e}	123.12±3.07^{d}
II	123.28±2.06	125.74±2.99	165.06±0.01^{a}	242.79±3.41^{a}	355.95±3.88^{a}
III	123.27±2.51	123.73±0.49	135.33±0.11^{f}	145.48±0.76^{d}	251.62±18.30^{c}
IV	123.12±3.07	124.60±0.50	160.05±0.01^{c}	198.64±1.34^{c}	295.29±17.50^{b}
V	120.94±1.32	123.31±0.75	153.06±0.01^{d}	188.63±1.25^{c}	269.55±25.11^{bc}
VI	124.75±1.71	122.95±0.40	132.05±0.01^{g}	141.86±0.95^{d}	249.54±12.78^{c}
VII	123.66±2.75	125.31±1.25	156.05±0.01^{d}	210.84±7.52^{b}	348.45±6.78^{e}
VIII	124.85±3.44	125.63±1.34	164.61±0.08^{a}	240.97±7.86^{a}	355.46±5.78^{a}

Values are expressed as Mean ± SE. Significant level is (p<0.01). The different superscript letters differ significantly.

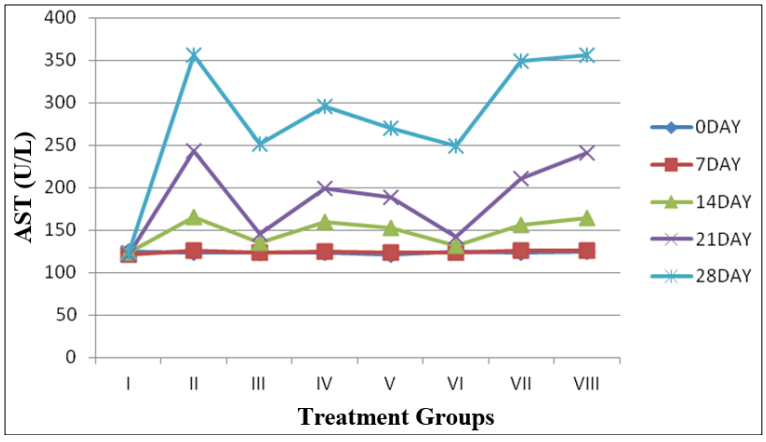

Fig 1: Graphical representation of serum aspertate transaminase (AST) of CCl_4 induced group.

Table 2: Effect of nano-curcumin on serum enzyme (ALT) in CCl_4 induced hepatic damage in rats

Groups	Days				
	0	7	14	21	28
I	123.05±2.06	122.61±1.98	124.10±2.46^{d}	123.10±2.60^{c}	124.37±1.79^{d}
II	123.68±1.73	126.83±0.82	202.93±4.90^{a}	274.23±8.95^{a}	372.57±1.19^{a}
III	122.61±1.98	125.35±1.14b	142.34±1.62^{c}	172.88±5.87^{b}	262.93±17.66^{c}
IV	124.60±3.12	124.89±0.66	161.89±2.55^{b}	185.77±11.98^{b}	303.66±15.74^{b}
V	124.10±2.46	124.66±1.12	152.52±2.41^{b}	177.97±10.44^{b}	266.33±8.72^{c}
VI	123.55±1.94	123.09±0.70	139.63±1.19^{c}	169.66±4.70^{b}	258.83±10.10^{c}
VII	124.54±2.40	126.62±0.87	200.27±2.54^{a}	271.66±8.09^{h}	371.47±5.86^{e}
VIII	124.37±1.79	126.43±1.61	201.57±5.76^{a}	273.38±8.79^{a}	372.01±1.28^{a}

Values are expressed as Mean ± SE. Significant level is (p< 0.01). The different superscript letters differ significantly.

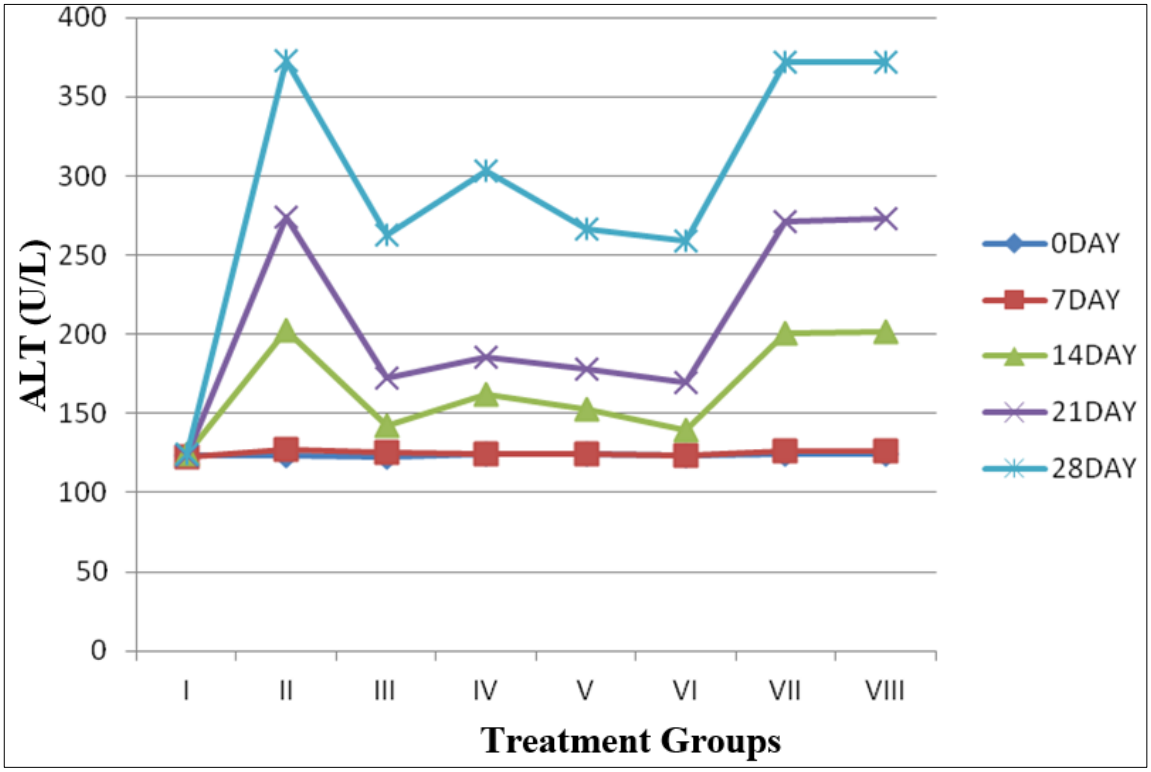

Fig 2: Graphical representation of serum alanine transaminase (ALT) of CCl_4 induced group.

Table 3: Effect of nano-curcumin on serum enzyme (ALP) in CCl_4 induced hepatic damage in rats

Groups	Days				
	0	7	14	21	28
I	124.38±2.70	124.96±2.12	125.84±2.32^{d}	125.68±2.62^{e}	123.07±0.01^{d}
II	125.02±1.20	127.81±1.08	190.73±0.77^{a}	383.77±1.77^{a}	412.40±2.48^{a}
III	125.84±2.32	125.19±1.20	154.95±8.02^{c}	147.57±1.14^{d}	199.42±4.09^{c}
IV	125.14±1.13	126.47±1.05	177.10±11.64^{ab}	283.31±18.82^{b}	230.61±17.53^{b}
V	122.51±2.25	125.93±0.91	168.35±6.40^{bc}	202.08±1.71^{c}	203.89±5.13^{c}
VI	125.68±2.62	125.11±0.55	151.80±13.33^{c}	142.53±0.96^{de}	196.88±1.82^{c}
VII	124.05±1.45	127.51±1.23	189.43±2.05^{ab}	381.35±1.52^{h}	410.42±6.73^{g}
VIII	126.92±3.27	127.29±0.77	190.16±6.07^{a}	383.14±1.75^{a}	411.95±2.60^{a}

Values are expressed as Mean ± SE. Significant level is (p<0.01). The different superscript letters differ significantly.

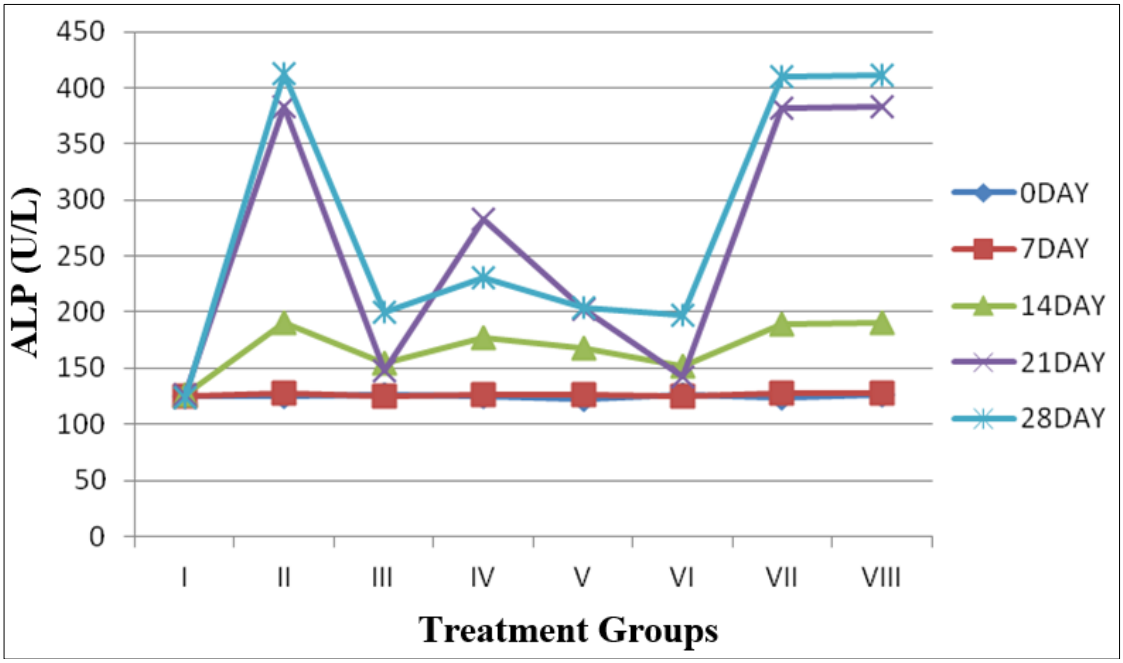

Fig 3: Graphical representation of serum alkaline phosphatase (ALP) of CCl_4 induced group

Table 4: Effect of nano-curcumin on serum bilirubin in CCl_4 induced hepatic damage in rats

Groups	Days				
	0	7	14	21	28
I	0.25±0.01	0.25±0.01	0.29±0.02^{d}	0.24±0.01^{f}	0.26±0.01^{e}
II	0.24±0.01	0.28±0.01	0.43±0.03^{a}	0.58±0.01^{a}	0.81±0.03^{a}
III	0.25±0.01	0.26±0.01	0.33±0.01^{cd}	0.32±0.01^{de}	0.38±0.02^{cd}
IV	0.23±0.01	0.28±0.01	0.37±0.01^{bc}	0.38±0.02^{c}	0.56±0.02^{b}
V	0.24±0.01	0.27±0.011	0.30±0.01^{d}	0.35±0.01^{cd}	0.42±0.02^{c}
VI	0.26±0.01	0.26±0.01	0.28±0.01^{d}	0.29±0.02^{ef}	0.33±0.01^{d}
VII	0.25±0.01	0.28±0.001	0.42±0.02^{ab}	0.52±0.02^{b}	0.80±0.02^{f}
VIII	0.25±0.01	0.28±0.02	0.42±0.02^{a}	0.55±0.03^{ab}	0.81±0.02^{a}

Values are expressed as Mean ± SE. Significant level is (p<0.01). The different superscript letters differ significantly.

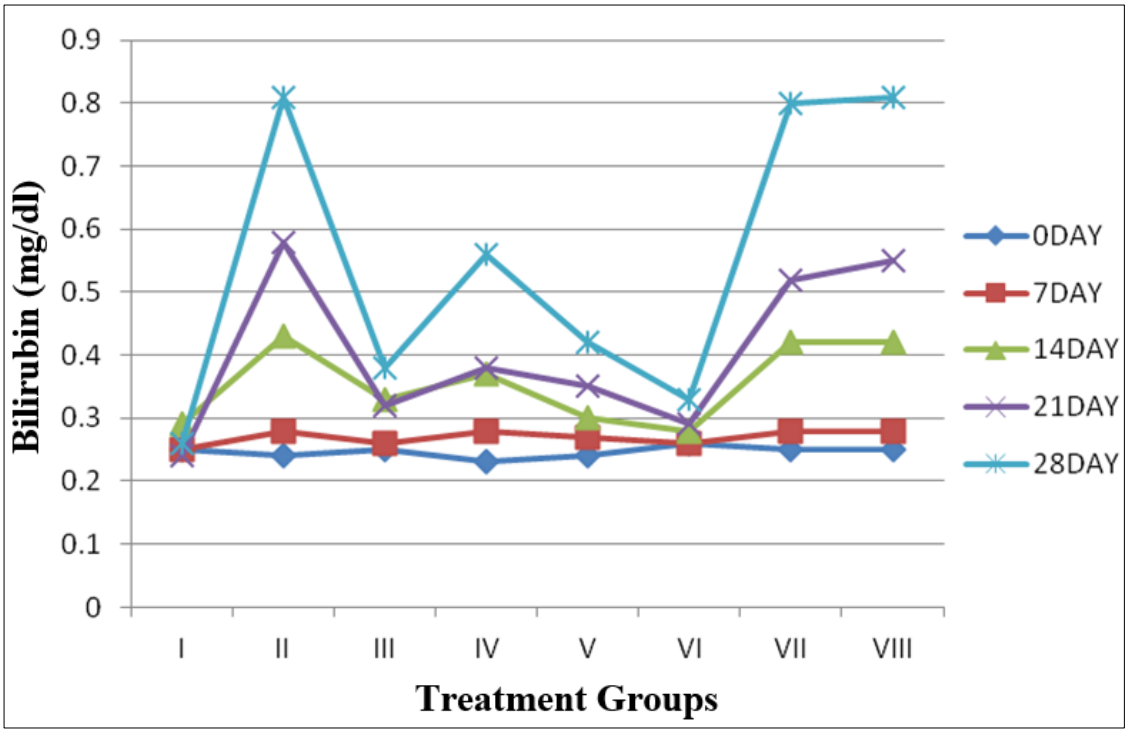

Fig 4: Graphical representation of serum bilirubin of CCl_4 induced group.

Table 5: Effect of nano-curcumin on serum total protein in CCL_4 induced hepatic damage in rats

Groups	Days				
	0	7	14	21	28
I	6.33±0.44	6.71±0.20	6.67±0.10^{a}	6.65±0.09^{a}	6.53±0.01^{a}
II	6.42±0.29	6.22±0.01	5.76±0.18^{c}	3.57±0.17^{e}	2.47±0.13^{d}
III	6.37±0.02	6.51±0.03	6.36±0.01^{a}	6.06±0.01^{c}	5.93±0.01^{b}
IV	6.50±0.44	6.34±0.01	6.30±0.01^{ab}	5.55±0.03^{d}	5.27±0.04^{c}
V	6.67±0.33	6.37±0.52	6.35±0.02^{a}	6.22±0.02^{bc}	5.88±0.01^{b}
VI	6.68±0.49	6.39±0.03	6.49±0.11^{a}	6.49±0.11^{ab}	6.34±0.01^{a}
VII	6.54±0.03	6.32±0.35	5.80±0.33^{bc}	3.83±0.15^{f}	2.64±0.11^{h}
VIII	6.47±0.42	6.23±0.35	5.78±0.32^{bc}	3.59±0.09^{e}	2.49±0.15^{d}

Values are expressed as Mean ± SE. significant level is (p<0.01). The different superscript letters differ significantly.

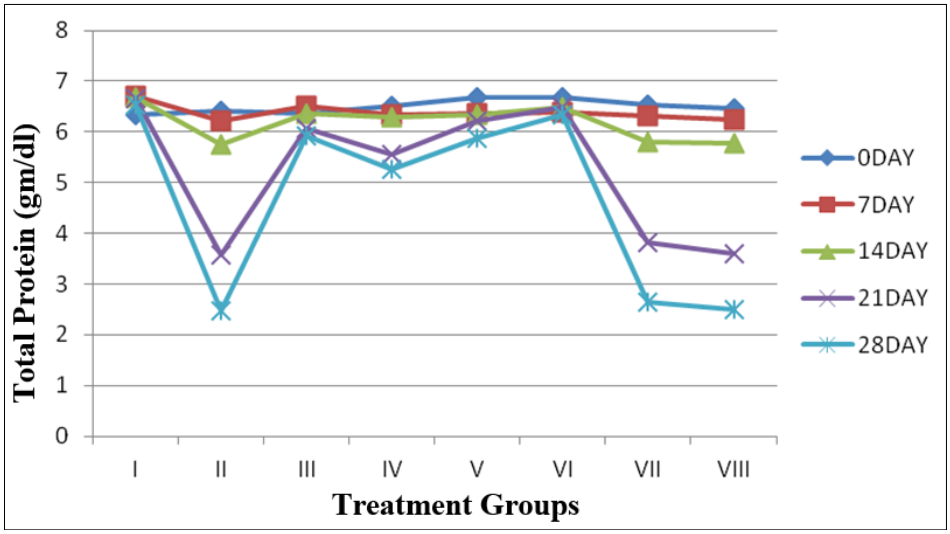

Fig 5: Graphical representation of serum total protein of CCl_4 induced group

Table 6: Effect of nano-curcumin on serum albumin in CCL_4 induced hepatic damage in rats

Groups	Days				
	0	7	14	21	28
I	4.26±0.04^{cd}	4.24±0.03^{a}	4.31±0.03^{a}	4.34±0.03^{a}	4.28±0.01^{a}
II	4.34±0.03^{bc}	3.37±0.07^{b}	2.17±0.30^{c}	0.10±0.18^{e}	0.86±0.12^{e}
III	4.25±0.03^{de}	4.21±0.03^{a}	3.98±0.17^{ab}	3.94±0.21^{b}	4.06±0.02^{ab}
IV	4.16±0.03^{e}	4.17±0.001^{a}	3.68±0.02^{b}	3.55±0.03^{c}	3.07±0.01^{c}
V	4.40±0.04^{ab}	4.20±0.01^{a}	3.89±0.02^{ab}	3.85±0.03^{bc}	3.84±0.03^{b}
VI	4.39±0.05^{ab}	4.29±0.12^{a}	4.02±0.15^{ab}	4.15±0.02^{ab}	4.22±0.20^{ab}
VII	4.44±0.03^{a}	3.39±0.12^{b}	2.46±0.41^{c}	2.28±0.18^{d}	1.73±0.28^{d}
VIII	4.19±0.01^{de}	3.37±0.09^{b}	2.19±0.21^{c}	1.21±0.13^{e}	0.96±0.18^{e}

Values are expressed as Mean ± SE. Significant level is (p< 0.01). The different superscript letters differ significantly.

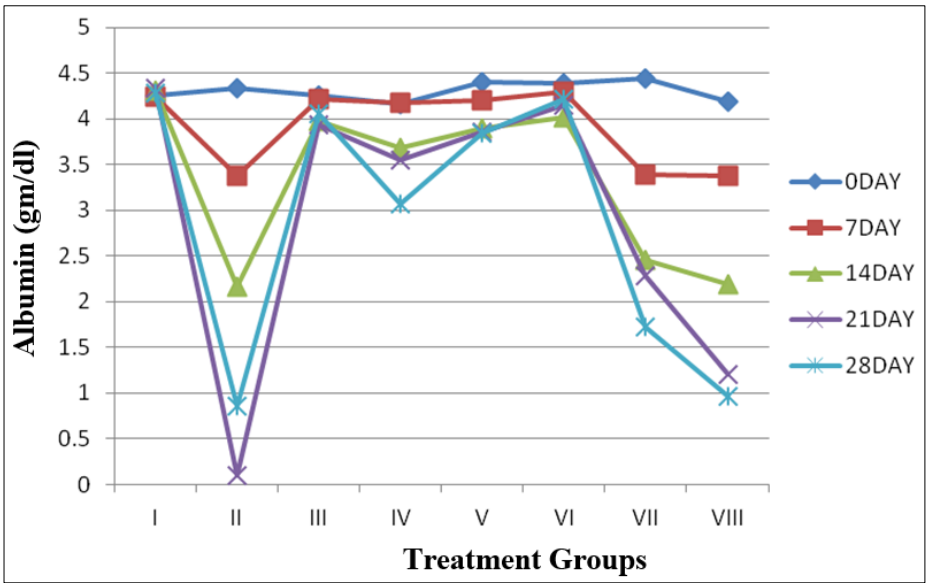

Fig 6: Graphical representation of serum albumin of CCl_4 induced group.

Table 7: Effect of nano-curcumin on serum globulin in CCL_4 induced hepatic damage in rats

Groups	Days				
	0	7	14	21	28
I	2.06±0.45	2.47±0.20	2.35±0.11^{c}	2.31±0.11^{ab}	2.25±0.01^{a}
II	2.09±0.29	2.85±0.07	3.59±0.45^{a}	2.57±0.23^{a}	1.61±0.10^{bc}
III	2.12±0.01	2.30±0.04	2.38±0.16^{c}	2.12±0.20^{ab}	1.87±0.02^{abc}
IV	2.34±0.44	2.17±0.01	2.62±0.02^{bc}	2.00±0.01^{bc}	2.21±0.05^{a}
V	2.27±0.33	2.17±0.51	2.46±0.03^{c}	2.37±0.05^{ab}	2.04±0.04^{ab}
VI	2.30±0.47	2.11±0.15	2.47±0.18^{c}	2.34±0.11^{ab}	2.12±0.20^{a}
VII	2.10±0.06	2.93±0.34	3.34±0.56^{ab}	1.55±0.30^{c}	0.91±0.29^{d}
VIII	2.27±0.42	2.86±0.36	3.59±0.21^{a}	2.37±0.12^{ab}	1.53±0.27^{c}

Values are expressed as Mean ± SE. Significant level is (p<0.01). The different superscript letters differ significantly.

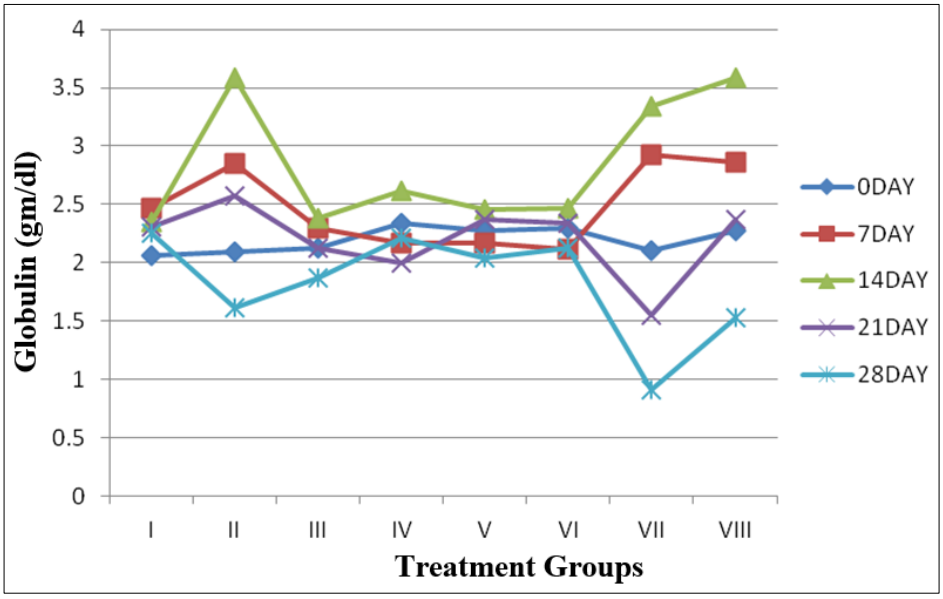

Fig 7: Graphical representation of serum globulin of CCl_4 induced group

Table 8: Effect of nano-curcumin on serum lactate dehydrogenase (LDH) in CCL_4 induced hepatic damage in rats

Groups	Days				
	0	7	14	21	28
I	131.17±2.56	130.76±1.42	131.6±0.05^{d}	130.08±1.44^{d}	130.47±3.04^{c}
II	130.08±1.44	135.89±2.46	196.32±1.40^{a}	242.36±5.93^{a}	330.50±9.75^{a}
III	132.84±1.57	133.45±1.90	143.33±2.45^{c}	171.52±3.90^{bc}	239.4±4.47^{b}
IV	130.47±3.04	132.24±3.03	155.39±3.61^{b}	183.01±2.97^{b}	255.59±9.11^{b}
V	133.32±1.43	131.49±0.99	150.29±2.10^{b}	176.40±3.18^{bc}	247.72±4.15^{b}
VI	132.18±3.74	131.31±0.48	142.19±2.27^{c}	169.52±5.29^{c}	237.75±4.33^{b}
VII	131.88±2.98	135.77±1.15	194.88±1.43^{a}	238.76±5.001^{e}	324.37±7.88^{e}
VIII	132.32±3.69	135.86±2.37	196.15±3.27^{a}	241.62±4.38^{a}	328.71±8.99^{a}

Values are expressed as Mean ± SE. Significant level is (p<0.01). The different superscript letters differ significantly.

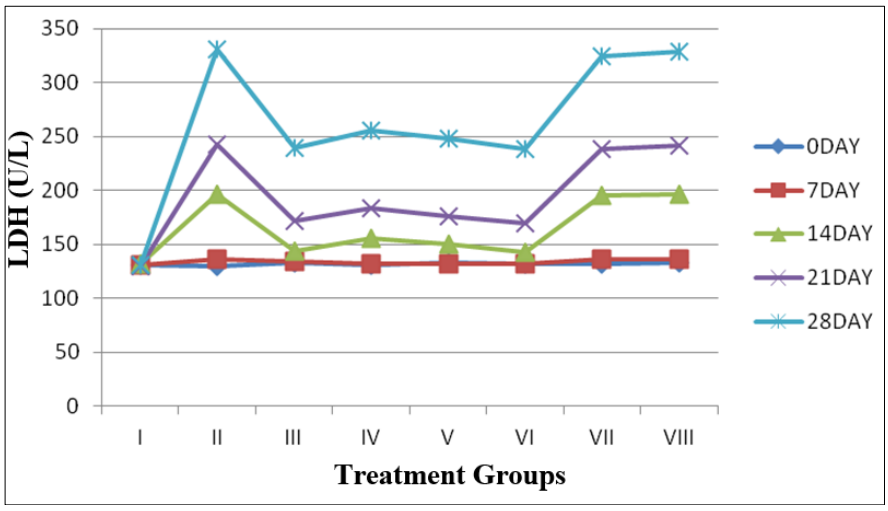

Fig 8: Graphical representation of serum lactate dehydrogenase (LDH) of CCl_4 induced group.

Table 9: Effect of nano-curcumin on serum uric acid in CCL_4 induced hepatic damage in rats

Groups	Days				
	0	7	14	21	28
I	2.24±0.01^{bc}	2.35±0.03	2.46±0.05^{d}	2.36±0.04^{d}	2.41±0.09^{e}
II	2.32±0.02^{ab}	2.45±0.05	4.19±0.21^{a}	5.91±0.24^{a}	6.77±0.53^{a}
III	2.41±0.09^{a}	2.44±0.03	3.39±0.05^{c}	3.56±0.10^{bc}	4.44±0.23^{cd}
IV	2.36±0.04^{ab}	2.43±0.01	3.61±0.04^{bc}	4.21±0.29^{b}	5.47±0.58^{bc}
V	2.15±0.03^{c}	2.42±0.03	3.55±0.03^{c}	3.81±0.04^{bc}	4.17±0.25^{d}
VI	2.34±0.02^{ab}	2.40±0.04	3.36±0.28^{c}	3.37±0.10^{c}	3.95±0.20^{d}
VII	2.36±0.07^{ab}	2.43±0.02	4.09±0.34^{ab}	5.34±0.47^{f}	6.49±0.55^{h}
VIII	2.26±0.03^{bc}	2.44±0.05	4.13±0.18^{ab}	5.85±0.24^{a}	6.62±0.56^{ab}

Values are expressed as Mean ± SE. Significant level is (p< 0.01). The different superscript letters differ significantly.

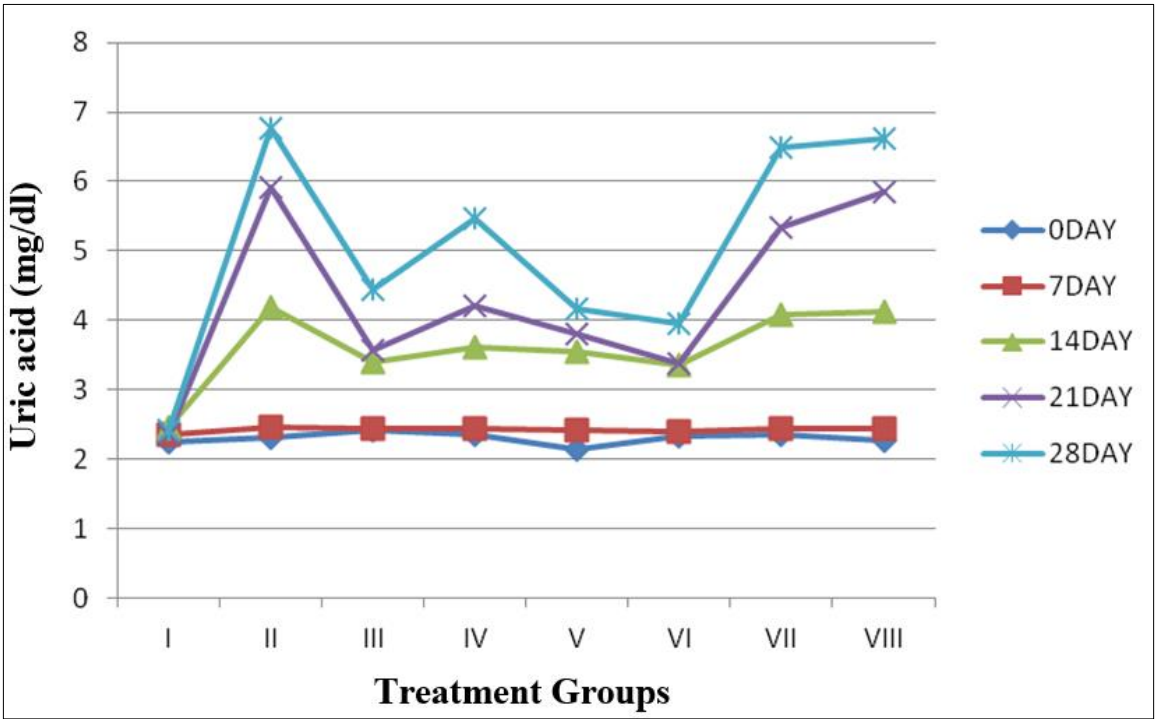

Fig 9: Graphical representation of serum uric acid of CCl_4 induced group

Aspartate transaminase (AST)

In the present study, it was observed that the administration of CCl_4 at the dose rate of 2 ml/kg body weight by intra peritoneal route over an interval of 72 hours for four consecutive weeks significantly (p< 0.01) increased the level of serum marker enzyme AST as compared to the normal control animals. This is an evidence of existence of liver injury due to toxicity. On administration of nanocurcumin to the rats with hepatic injury induced by CCl_4, recovery from injury was observed as evident from the significant (p< 0.01) decrease in the activities of serum marker enzyme at the dose rate of 40, 80, 160 mg/kg body weight of nanocurcumin. Also, in the group treated with curcumin at the dose rate of 160 mg/kg body weight, significant (p< 0.01) decrease in AST level was observed as compared to that of the CCl_4 toxicity induced group (+ve control group), but it was less effective as compared to the three doses of nanocurcumin. In the turmeric (160 mg/kg body weight) administered group the level of AST was not found to be decreased significantly. The oxidative injury caused by CCl_4 disrupted the hepatocellular plasma membrane and the enzymes normally present in the cytosol were released into the blood circulation as indicated by elevated serum enzyme levels. In the present study, the elevated level of the marker enzyme observed in CCl_4 toxicity induced rats indicates liver damage induced by hepatotoxins. Treatment with nano-curcumin ameliorated the toxic effects of CCl_4 and the above marker enzyme was restored towards the normal level. This effect might be due to the free radical scavenging activity of nano-curcumin and the results obtained in this study was in agreement with the earlier findings of Fu *et al.*, 2008; Gamal *et al.*, 2016; Hismiogullari *et al.*, 2014; Mahmoud *et al.*, 2015; Park *et al.*, 2000; Zhao *et al.*, 2014 [3, 4, 7, 11, 13, 17] who had reported the protective effect of curcumin against CCl_4 induced hepatotoxicity and concluded that curcumin lowered the activity of serum aspartate aminotransferase (AST) enzyme in rats intoxicated with CCl_4.

Alanine transaminase (ALT)

Serum hepatobilliary enzyme ALT is present in high concentration in the liver under normal conditions. When there is hepatocyte necrosis or membrane damage, the enzyme is released into the circulation, as indicated by elevated serum enzyme level. In the present study, the elevated levels of this marker enzyme in CCl_4 toxicity induced rats indicated that liver damage is induced by hepatotoxins. The high level of ALT enzyme was found in the CCl_4 toxicity induced group which was increased significantly (p< 0.01) compared to that of the normal control group. In the treatment groups i.e., the groups treated with nanocurcumin at the dose rate of 40, 80, 160 mg/kg body weight, the level of ALT was found to be decreased significantly (p< 0.01) as compared to that of the CCl_4

toxicity induced group which might be due to the free radical scavenging activity of nanocurcumin and the results obtained in this study were in accordance to the findings of previous workers (Fu *et al.*, 2008; Gamal *et al.*, 2016; Hismiogullari *et al.*, 2014; Mahmoud *et al.*, 2015; Park *et al.*, 2000; Zhao *et al.*, 2014) [3, 4, 7, 11, 13, 17].

Alkaline phosphatase (ALP)

The elevated levels of this marker enzyme observed in CCl_4 and paracetamol intoxicated rats indicated liver damage. Significant ($p< 0.01$) high level of ALP enzyme activity was found in the CCl_4 and paracetamol toxicity induced groups as compared to that of the normal control groups. In the treatment group of nanocurcumin at the dose rate of 40, 80, 160 mg/kg body weight, the level of ALP was found to be decreased significantly ($p< 0.01$) as compared to that of the respective CCl_4 and paracetamol induced hepatic damage groups. In the curcumin (160 mg/kg body weight) treated group, the ALP level was also found to be decreased significantly ($p< 0.01$) but it was less effective than the nannocurcumin and in turmeric (160 mg/kg body weight) treated group there was no significant difference in comparison to the respective CCl_4 and paracetamol induced hepatotoxicity groups. Serum ALP activity are related to the function of hepatic cell. Increase in serum level of ALP is due to the increased synthesis, in presence of increase billiary pressure (Moss and Butterworth 1974) [12]. Alkaline phosphatase is excreted normally via bile by the liver. In liver injury due to hepatotoxin, there is a defective excretion of bile by the liver which is reflected in their increased levels in serum. Effective control of nanocurcumin on serum alkaline phosphatase might be due to the improvement in the secretory mechanism of the hepatic cell. This finding was supported by the findings of Fu *et al.*, 2008; Girish and Pradhan, 2012; Park *et al.*, 2000 [3, 5, 13].

Total bilirubin

In the CCl_4 induced toxicity group the level of total bilirubin was found to be increased significantly ($p< 0.01$) as compared to that of the normal control group. On the other hand, treatment with the three different doses of 40, 80 and 160 mg/kg body weight of nanocurcumin the level of total bilirubin was found to be reduced significantly ($p< 0.01$) as compared to the CCl_4 induced hepatotoxicity group. In the curcumin (160 mg/kg body weight) treated group the level of total bilirubin was also found to be decreased significantly ($p< 0.01$) but it was less effective than that of nanocurcumin, however, in the turmeric (160 mg/kg body weight) treated group no significant difference was observed as compared to the CCl_4 induced hepatic damage group. Accumulation of bilirubin is a measure of alterations in binding, conjugation and excretory capacity of hepatocytes. Hepatocellular injury is usually accompanied by cellular swelling that can compress bile canaliculi and invariably causes some cholestasis. In this case, the components of the bile occur in abnormal concentrations in blood. Cholestasis may also develop from extra-or intra hepatic physical obstruction of biliary flow or by abnormal secretion by hepatocytes (Duncan *et al.*, 1994) [2]. Therefore, the elevated level of bilirubin is usually an indication of biliary obstruction, haemolysis, and in some cases renal failure (Thapa and Walia, 2007) [15]. Effect of nanocurcumin on total bilirubin might be due to the ability of nanocurcumin to recover cell damages by inhibiting the oxidation of polyunsaturated fatty acid of the cell membrane. The results of the present study were in agreement with the findings of (Khedr and Khedr 2014) [9].

Total protein

In the CCl_4 induced hepatic toxicity group the level of total protein was found to be reduced significantly ($p< 0.01$) compared to that of the normal control group. On the other hand, the level of total protein was found to be increased significantly ($p< 0.01$) in nanocurcumin treated groups (with the three different doses of 40, 80 and 160 mg/kg body weight), as compared to the CCl_4 induced hepatotoxicity group. In the curcumin (160 mg/kg body weight) treated group the level of total protein was also found to be increased significantly ($p< 0.01$) but it was less effective than that of nanocurcumin where as in the turmeric (160 mg/kg body weight) treated group there was no any significant difference observed as compared to the CCl_4 induced toxicity group. The reduction in the serum total protein might be due to the reduction in hepatic protein synthesis. Effect of nanocurcumin on total protein level was might be due to the ability of nanocurcumin to recover liver synthesis function. The present findings are parallel with the findings of Gamal *et al.*, 2016; Mahmoud *et al.*, 2015; Zhao *et al.*, 2014 [4, 11, 17].

Albumin

In the CCl_4 induced hepatotoxicity group the level of albumin was found to be decreased significantly ($p< 0.01$) as compared to that of the normal control group. Treatment with the three different doses of nanocurcumin such as 40, 80 and 160 mg/kg body weight, the level of albumin was found to be increased significantly ($p< 0.01$) as compared to the CCl_4 induced toxicity group. In the curcumin (160 mg/kg body weight) treated group the level of albumin was also found to be increased significantly ($p< 0.01$) but it was found to be less effective than that of nanocurcumin but in the turmeric (160 mg/kg) treated group there was no any significant difference with CCl_4 induced toxicity group. The reduction in the serum albumin might be due to the reduction in hepatic protein synthesis. Increased level of albumin in the nanocurcumin treated group proved the recovery of liver synthesis function, because it is well known that increase in albumin represents a crucial factor in restoring liver function. One of the main targets of the treatment of liver damage is the rise of albumin level. Our findings also correlates with the reports of Gamal *et al.*, 2016; Mahmoud *et al.*, 2015; Zhao *et al.*, 2014 [4, 11, 17].

Globulin

The level of globulin was found to be reduced significantly ($p< 0.01$) in the CCl_4 toxicity induced group as compared to that of the normal control group. On treatment with the three different doses of nanocurcumin such as 40, 80 and 160 mg/kg body weight, the level of globulin was found to be increased significantly ($p< 0.01$) as compared to the CCl_4 induced hepatotoxicity group. In the curcumin (160 mg/kg body weight) treated group the globulin level was also found to be increased significantly ($p< 0.01$) but it was found to be less effective than that of nanocurcumin. On the other hand in the turmeric (160 mg/kg) treated group no significant difference was observed compared to the CCl_4 induced toxicity group. The reduction in the serum globulin might be due to the reduction in the hepatic protein synthesis and decreased effect on total protein and albumin level. Increased level of globulin in the nanocurcumin treated group proved the recovery of liver synthesis function. Whereas the result of

the present study was found to be similar when hepatic damage induced by paracetamol which is correlates with the finding of Yousef *et al.*, 2010 [16].

Lactate dehydrogenase (LDH)

In the present study, the elevated level of LDH activity was observed in CCl_4 toxicity induced rats that indicated liver damage. The high level of LDH activity was found in the CCl_4 hepatotoxicity induced group which was increased significantly ($p< 0.01$) compared to that of the normal control group. In the treatment group of nanocurcumin at the dose rate of 40, 80, 160 mg/kg body weight, the level of LDH was found to be decreased significantly ($p< 0.01$) compared to that of the CCl_4 toxicity induced group which might be due to the free radical scavenging activity of nanocurcumin. Whereas the findings of the present study was found to be correlates with Hatem and Hussein, 2010; Yousef *et al.*, 2010 [6, 16] who had also reported that curcumin resulted in a significant ($p< 0.05$) improvement in LDH level toward the normal values of the control rats in paracetamol induced hepatic damage study.

Uric acid

In the CCl_4 induced toxicity group the level of total uric acid was found to be increased significantly ($p< 0.01$) compared to that of the normal control group. On the other hand treatment with the three different doses of 40, 80 and 160 mg/kg body weight of nanocurcumin the uric acid level was reduced significantly ($p< 0.01$) as compared to the CCl_4 induced hepatotoxicity group. In the curcumin (160 mg/kg body weight) treated group the uric acid level was also found to be decreased significantly ($p< 0.01$) but it was less effective than the nanocurcumin, however in the turmeric (160 mg/kg body weight) treated group there was no any significant difference observed compared to the CCl_4 induced toxicity group. Nanocurcumin reduced the elevated level of uric acid by its free radical scavenging property that simultneously ameliorate oxidative stress caused by the free radicals produced via oxidation of polyunsaturated fatty acid of the cell membrane. The results of the present study were in agreement with the findings of Khorsandi and Orazizadeh 2008; Rubaei *et al.*, 2014 [10, 14].

Conclusion

- The results of the present study demonstrated that nano-curcumin and curcumin both have protective effect on liver in carbon tetrachloride induced hepatic damage.
- The tumeric powder failed to show any significant difference in blood parameters.
- Nanocurcumin showed dose dependent hepatoprotective effect.
- From the present study it can be concluded that, nanocurcumin showed better efficacy than curcumin in respect to hepatoprotective effect.

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