Prevention of Acetaminophen Induced Hepatorenal Toxicity in Mice with Fruits of *Terminalia chebula* (Myrobalan)

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ABSTRACT

Protective role of fruits of *Terminalia chebula (*myrobalan) at three dose levels (200,150 &100mg/kg bw) against acetaminophen (paracetamol) induced hepato and nephrotoxicity with single sublethal dose (300mg/kg bw) has been assessed. Parameters of study are glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), billirubin, alkaline phosphatase (ALP) as liver function tests, creatinine and urea as kidney function tests and histology of liver and kidney for pathology. *T.chebula* could well antagonize acetaminophen induced hepatorenal toxicity in dose dependent manner. However, myrobalan could not afford protection against lethal dose of acetaminophen. Probable protective role is discussed in detail on the basis of known properties of different constituents of fruits of *Terminalia chebula*.

Keywords: *Terminalia chebula*, acetaminophen/paracetamol, liver-kidney, mice, antioxidants.

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Introduction

During recent past few herbal compounds have been screened for their ability to reduce and/or nullify acetaminophen induced hepatotoxicity[1]. Over dosage of acetaminophen mainly causes dose dependent, fatal hepatic necrosis[2] however renal tubular necrosis and hypoglycemic coma may also occur[3].

Myrobalan, fruits of *Terminalia chebula* (Combretaceae) has been found to protect mammalian liver from toxicity caused by sub-chronic administration of rifampicin, isoniazid and pyrazinamide in combination[4], it could also protect kidneys too against nephrotoxins including nickel chloride[5], ferric nitrilotriacetic acid (Fe- NTA)[6]. Present study is undertaken to find out influence of myrobalan on acetaminophen induced hepatorenal toxicity in mice.

Matherials and Methods

Animal Model

Isogenic healthy male swiss albino mice of 20-25 gms. were obtained from Biological Production Division, Government Veterinary College, how MP. They were maintained on standard food and tap water *ad-libitum*. They were housed in animal house in propylene cages.

Herbal drug

Dried intact fruits of *T. chebula* were purchased from local herbal shop and were gently backed in a stainless steel container. After cooling they were ground in electrical grinder to get fine powder. Known quantity of fruits of *Terminalia chebula* (Myrobalan) powder was thoroughly mixed in known amount of distilled water using mortar and pestle which was filtered by ordinary filter paper. This clear aqueous filtrate was orally administered to mice using blunt, bent thick (No. 18) needle fitted on a syringe. The dose of drug was selected from described values in the literature on herbal drug.

Experiment I: Protection at lethal dose

Preliminary experiments were performed on mice to estimate the protective effect of this herbal compound against single lethal dose of paracetamol (1g/kg). Animals were divided into two groups of 10 animals each. One group was treated orally with the test drug *Terminalia chebula* at maximum dose (4gm/kg bw) and followed after 1 hour by intraperitoneal injection of paracetamol.

Another group was administered distilled water instead of drug. The mortality was observed 24 hour after paracetamol administration in both groups. Percentage protection against lethal effect of paracetamol was calculated.

Experiment II : Hepatoprotective and Nephroprotective study: Hepatic and renal injury was induced in mice by subcutaneous administration of single sublethal dose (300mg/kg/bw) of paracetamol injection. Details are shown in Table:1.

Biochemical Observations On day 9, 48 hours after paracetamol administration blood sample from each animal of each group was taken directly from heart under mild chloroform anesthesia. Biochemical parameters GOT, GPT and billirubin, AP as liver function tests and creatinine and urea as kidney function tests are evaluated using ready to use available kits made by standard companies (i.e. BEACON diagnostics Pvt. Ltd., AGAPPE diagnostics, ACCUREX biochemical Pvt. Ltd., VITAL diagnostic (P) (Ltd.) in a recognized pathological clinic.

Histopathological Observation

Also, on 9th day pieces of liver and kidney from each animal were fixed in Bouins fluid for routine histopathology. Hematoxylin-eosine stained sections were observed for histopathology.

Statistical Analysis:

Experiments were done thrice. The data were subjected to Students 'T' test at 5% level of significance.

Results

Lethality test: (Experiment I) : All mice of second group (acetaminophen exposed) died showing 100% mortality. In another group, which had received *Terminalia chebula* prior to acetaminophen challenge showed 20% survival. This is shown in Table 2. This indicates that *Terminalia chebula* could afford very little protection against lethal dose of acetaminophen.

Hepato-nephroprotection:

(Experiment-II)

Histological Observations

Self explanatory figures and captions are given in plate I and II.

Physiological Observations (Table:3)

Levels of enzymes remained unaffected among mice that were pretreated with highest dose (200 mg/kg bw) of *Terminalia chebula* before paracetamol challenge. Paracetamol injection caused sharp rise in the serum levels of all GOT, GPT, ALP, Bilirubin & creatinine and urea indicating severe liver & kidney injury. Lower dose (150 mg/kg bw) of *Terminalia chebula* could keep level of serum enzymes significantly lower than that the values obtained in Group II (Paracetamol exposed). Lowest dose (100 mg/kg bw) *Terminalia chebula* provided partial protection against paracetamol induced hepatotoxicity but not towards renal toxicity. Histological findings and physiological observations corroborate each other.

Discussion

Toxicity of paracetamol in mice is an established fact[7]. Several earlier reports in human and in animal studies have cemented this fact[2,8,9]. Due to this reason paracetamol is used as experimental toxin to induce liver and kidney damage in experimental studies.

Pretreatment i.e. prophylactic administration of aqueous suspension of powdered fruits of *Terminalia chebula* (Myrobalan) at three different doses for 07 days to mice could provide appreciable protection against acetaminophen (paracetamol) challenge at sub lethal experiments. Possibly myrobalan a natural antioxidant might have mainly strengthened endogenous antioxidant defense in the liver and kidneys of mice, however, it could have also exerted protective role via other routes as it contains several components[10]. Individual plant components like sulfhydryl and flavonoid compounds, gallic acid, ellagic acid, mucic acid, citric acid, reducing sugars and tannins can modulate effects of many genotoxicants[11], myrobalan possesses many such compounds[10], which can be held responsible for reducing cytotoxic effects of acetaminophen in mice hepatorenal tissues.

Ellagic acid present in myrobalan could have also lowered acetaminophen-induced hepatotoxicity in mice in the present experiments as it has been reported to do so against acetaminophen in mice[12]. Being a strong antioxidant, ellagic acid attenuates the damaging effect of H_2O_2 , scavenge superoxide anion and hydroxyl anion. Ellagic acid can also act on drug-metabolizing enzymes and prevent the formation of toxic metabolites[13].

Cytotoxicity of N- acetyl-p-benzoquinone imine (NAPQI), metabolite of acetaminophen in cultured rat hepatocytes could be fully prevented by the addition of N-acetyl cysteine, GSH, or ascorbate[14]. Ascorbate present in myrobalan[15]could have afforded protection against acetaminophen in the present study. Exogenous administration of N-acetyl-L-cysteine could restore experimentally depleted GSH level in liver and Kidneys of rat within 60 and 120 minutes respectively[16].

07 day prior i.e. prophylactic administration of myrobalan to mice in the present study could have also caused enhanced GSH contents of liver and kindneys (and probably in other tissues too) because myrobalan contains many amino acids including those precursors of GSH[17,16]. About 4% sulphated ashes are present in myrobalan[10] which can be utilized for GSH synthesis.

Myrobalan administration could have restored acetaminophen-induced alteration in renal glutathione content, activities of glutathione-s-transferase, glutathione reductase, glutathione peroxidase and lipid peroxidation, hydrogen peroxide generation and serum creatinine level as myrobalan has been found to do so against nickel chloride-induced oxidative stress in the kidneys of rat[18].

Chebulic acid, a component of myrobalan could have maintained correct ratio of GSSH, oxidized form of glutathione (GSH) to the total GSH (GSH-GSSH) as it has been shown to do so following tertiary butyl hydro peroxide exposure to isolated hepatocytes[19].

Myrobalan pretreatment can maintain optimal level of GSH and cellular protective enzymes, reduce H_2O_2 content and histoarchitecture of kidneys as it could do against Fe NTA i.e. ferric nitrilotriacetic acid[20].

Myrobalan has been found to scavenge DPPH, hydroxyl and superoxide radicals *in-vitro*[21,22] later property has been further confirmed by electron spin resonance (ESR) sepctrometry[23]. Aqueous extract of myrobalan could inhibit gamma radiation induced lipid peroxidation in rat liver microsomes and could also restore superoxide dismutase in liver mitochondria[24,15.] Aqueous extract of myrobalan cold also exert antioxidant effect *in-vivo* and *in-vitro* in rat against terbutyl hydroperoxide toxicity[25]. Myrobalan has been found even to protect mitochondria in streptozotocin-induced diabetic rats[26]. Such antioxidant activity and mitochondria protecting ability of myrobalan can be held responsible for preventing hepatorenal toxicity of acetaminophen which is also mediated through H_2O_2 and mitochondria[27,28].

Aqueous extract of myrobalan has been found to be antimutagenic as it could inhibit gamma radiation induced DNA breaks in plasmid pBR 322[24,15]. Prophylactic administration of myrobalan prior to whole body irradiation of mice could reduce peroxidation of membrane lipids and DNA damage. Extract of myrobalan could also protect DNA damage in human lymphocytes *in-vitro*[29]. Antioxidant and membrane stabilizing activities of myrobalan has been held responsible for its hepatoprotective potential[4] . Myrobalan has been found to increase life span of HEK-N/F cells by 40% due of shortening of age dependent shortning of telomeric DNA after t-BuOOH and VVB exposures[30].

Antioxidant property of myrobalan has also been held responsible for its antigenotoxic potential against lead nitrate, acetaminophen, aluminum chloride and cadmium choride[31-34].

Myrobalan could even revert lead-induced mitostatic effect in the root tip cells of *Allium cepa*[35]. It is interesting to mention here that root cells also have their own detoxification system similar to that of animal cells and involve many peroxidases, SOD, and catalase[36]. Such preventive action of myrobalan can be held responsible for regeneration of hepatocytes and renal tubular cells even in the presence of acetaminophen. It is concluded that myrobalan reduces hepatorenal toxicity of acetaminophen in mice.

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Plate:I Histology of Mice Liver HE 150 X

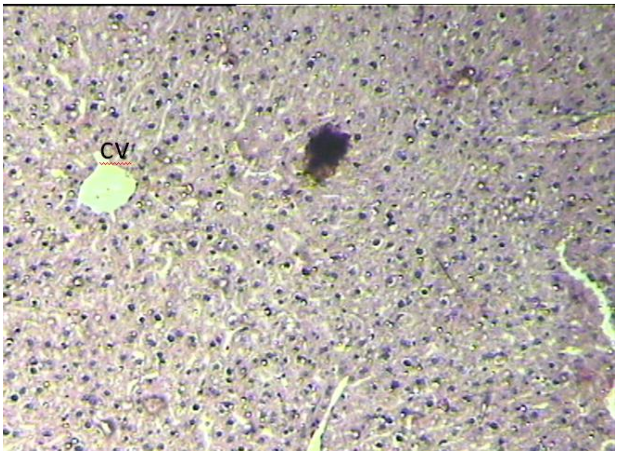

Fig.1: Showing normal histology of liver of control group of mice. Radiating chords of hepatocytes arounds central vein (cv) indicate well organized histoarchitecture. No inclusion and no infiltration.

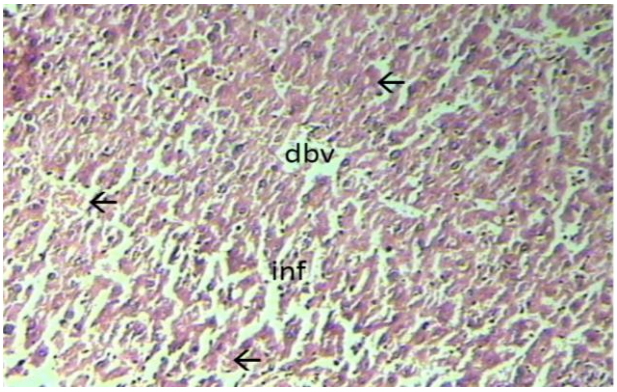

Fig.2: Showing severe disorganization of mice liver at 48 hr after single injection of acetaminophen (300 mg/kg bw i.p.). Damaged hepatocytes are seen as eosinophilic spots (←). Damaged & collapsed blood vessel (dbv) is seen with rough margins and infiltration(inf).

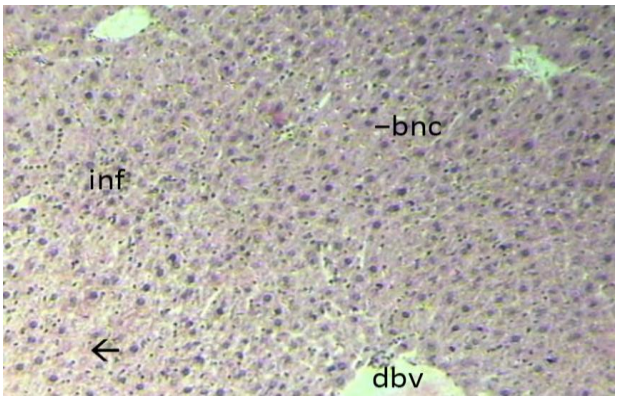

Fig.3: Showing liver of mice at 48 hr after single dose of acetaminophen (300 mg/kg bw i.p.) after 7 days pretreatment with fruit of *Terminalia chebula* at highest dose (200 mg/kg bw) showing both normal hepatocytes as well as disorganized ones. Mild infiltration and mild damaged blood vessel is seen. Binucleated cells (bnc) are evident. Histoarchitecture is quite better than what is seen in figure 2. Drug could appreciably reduce paracetamol toxicity.

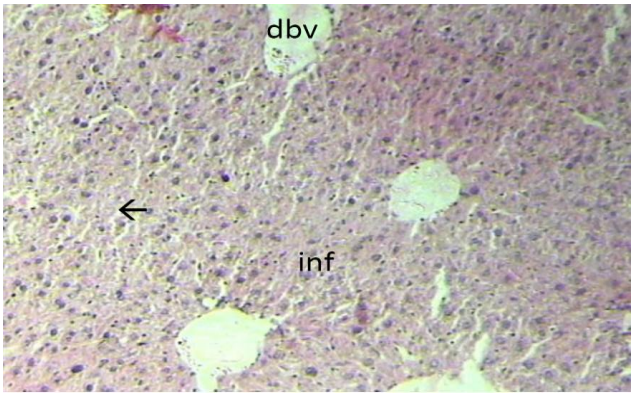

Fig.4: Showing liver of mice at 48 hr after single dose of acetaminophen (300 mg/kg bw i.p.) after 7 days pretreatment with fruit of *Terminalia chebula* at lower test dose (150 mg/kg bw). Infiltration from damaged blood vessel is evident. Damaged hepatocytes(←) are seen. Damage is more pronounced than what is seen in earlier figure. Still drug could reduce acetaminophen toxicity as better histology is seen than figure 2. Drug could afford partial protection.

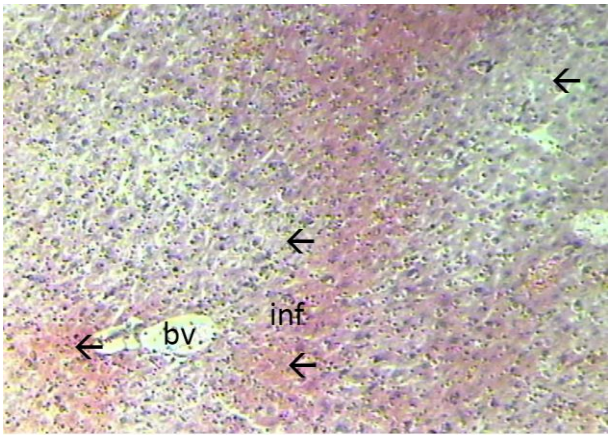

Fig.5; Showing liver of mice at 48 hr after single dose of acetaminophen with (300 mg/kg bw i.p.) after 7 days pretreatment with fruit of *Terminalia chebula* at lowest test dose (100 mg/kg bw). Severe infiltration from damaged blood vessel is evident. Liver tissue consist of damaged hepatocytes (←). Drug could still combat against toxicity of paracetamol at lowest dose as still liver tissue is seen in better condition than figue 2.

Plate: II Histology of Mice Kidney HE 150 X

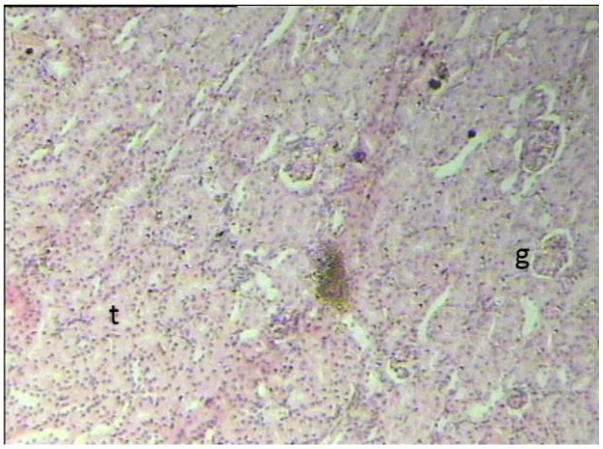

Fig.1: Showing normal histology of kidney of control group of mice with well organized glomeruli (g) and tubules (t).

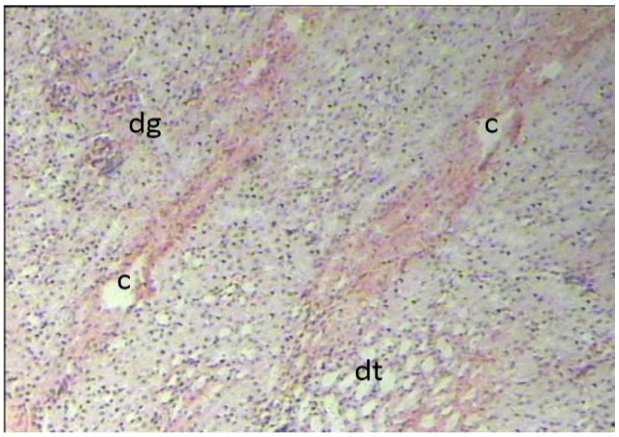

Fig.2: Showing severe disorganization of mice kidney tissue at 48hr after single injection of acetaminophen (300 mg/kg bw i.p.). Damaged glomeruli (dg) & dilated tubules (dt) are seen. Dead tubules i. e. cast (c) are also seen.

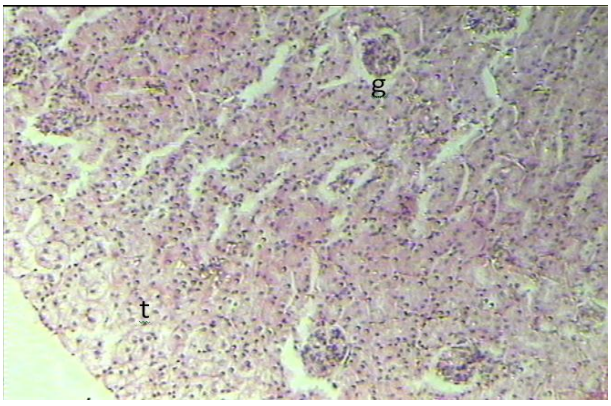

Fig.3: Showing mice kidney at 48 hr after single injection of acetaminophen (300 mg/kg bw i.p.) after 7 days pretreatment with fruit of *Terminalia chebula* at highest test dose (200 mg/kg bw). Showing control like histoarchitecture. Drug could afford protection appreciably.

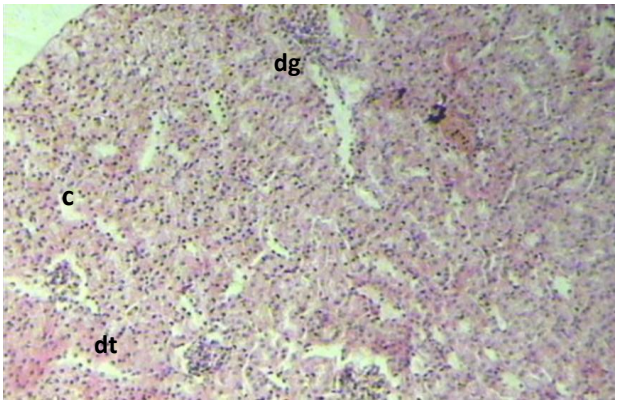

Fig.4: Showing mice kidney at 48 hr after single injection of acetaminophen (300 mg/kg bw i.p.) after 7 days pretreatment with fruit of *Terminalia chebula* at lower test dose (150 mg/kg bw). Mild tubular dilation (dt) with mild disorganized glomeruli (dg) are seen but still better histology is seen than figure 2. Drug afford partial protection.

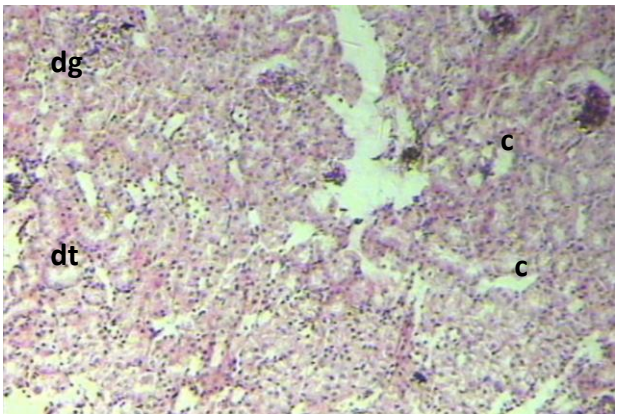

Fig.5: Showing mice kidney at 48 hr after single injection with acetaminophen (300 mg/kg bw i.p.) after 7 days pretreatment with fruit of *Terminalia chebula* at lowest test dose (100 mg/kg bw). Severe disorganization i.e.tubular dilation(dt) much disorganized glomeruli (dg) and few casts (c) are seen. This figure resembles with figure 2. Drug could not afford protection.

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Table1: Experimental Design.

Group I	*Control group:* Mice were pretreated orally with distilled water daily for 7 days followed by single s.c. injection of benzyl alcohol 2 hr after last treatment (volume equal to that of injection of paracetamol used) on 7th day in group II.
Group II	*Paracetamol treated group:* Mice were pretreated orally with distilled water orally for 7 days followed by single s.c.injection of sublethal dose (300mg/kg/bw) of paracetamol on 7th day, 2 hr. after last treatment.
Group III IV ,V	*Drug pretreated and Paracetamol challenged groups:* Mice were pretreated with the fruit of *Terminalia chebula* in distilled water at three doses (200,150,100mg/kg bw) orally daily for 7 days followed by single s.c. injection of paracetamol sublethal dose (300mg/kg bw) on 7th day 2 hr after last treatment.

Table 2. Protection against lethal dose of acetaminophen by *Terminalia chebula*

S.No.	Group	Total number of mice used	Mortality out of 10	Percentage protection
1.	Acetaminphen treated group	10	10 (100%)	0%
2.	Acetaminphen challenge to myrobalan treated group	10	08(80%)	20%

Table 3: Effects of pretreatment with different doses of fruit of *Terminalia chebula* against acetaminophen induced changes in the serum levels of enzymes in mice (n=6; Mean±SEM)

S.NO.	GROUPS	LIVER FUNCTION TESTS				KIDNEY FUNCTION TESTS	
		AST (U/L)	ALT (U/L)	ALP (U/L)	BILIRUBIN (MG/DL)	CREATININE (MG/DL)	UREA (MG/DL)
1.	Group I (Controls)	62.40±0.86	53.75±0.74	123.71±1.31	0.33±0.02	0.44±0.07	53.76±0.85
2.	Group II (Acetaminophen challenged at 300 mg/kg bw) % change vs control	130.85[a]±1.33 [109.69%↑]	154.30[a]±1.91 [187.06%↑]	172.91[a]±1.81 [39.77%↑]	0.85[a]±0.04 [157.57%↑]	1.20[a]±0.10 [172.72%↑]	104.88[a]±1.18 [95.08%↑]
3.	Group III (Pretreated with higher dose 200 mg/kg bw of *T.chebula* & challenged with acetaminophen at 300 mg/kg bw) % change vs control % difference from group II	65.27[b]±1.20 [04.59%↑]NS [50.11%↓]	57.33[b]±2.01 [06.66%↑]NS [62.84%↓]	126.11[b]±2.14 [01.94%↑]NS [27.06%↓]	0.41[b]±0.03 [24.24%↑]NS [51.76%↓]	0.49[b]±0.09 [11.36%↑]NS [59.16%↓]	56.14[b]±1.15 [04.42%↑]NS [46.47%↓]
4.	Group IV (Pretreated with lower dose 150 mg/kg bw of *T.chebula* & challenged with acetaminophen at 300 mg/kg bw) % change vs control % difference from group II	86.12[a,b]±1.48 [38.01%↑] [34.18%↓]	83.77[a,b]±1.89 [55.85%↑] [45.70%↓]	142.08[a,b]±2.21 [14.84%↑] [17.83%↓]	0.56[a,b]±0.02 [69.69%↑] [34.11%↓]	0.65 [a,b]±0.04 [47.72%↑] 45.83%↓]	72.09[a,b]±1.23 [34.09%↑] [31.26%↓]
5.	Group V (Pretreated with lowest dose 100 mg/kg bw of *T.chebula* & challenged with acetaminophen at 300 mg/kg bw) % change vs control % difference from group II	110.09[a,b]±1.25 [76.42%↑] [15.86%↓]	104.56[a,b]±2.14 [94.53%↑] [32.23%↓]	155.78[a,b]±1.98 [25.92%↑] [09.90%↓]	0.63[a,b]±0.02 [90.90%↑] [25.88%↓]	1.15[a]±0.07 [161.36%↑] [04.16%↓]NS	101.54[a]±1.10 [88.87%↑] [03.18%↓]NS

'a'= Significant Group I vs all groups; ↑ =Rise

'b'= Significant Group II vs Group III, Group IV, Group V; ↓= Decline , NS= Non significan