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Influence of Pre-treatment of *Withania somnifera* Root Extract and Cow Urine on Hemato-biochemical Parameters in Acetaminophen-induced Toxicity in Swiss Albino Male Mice

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ABSTRACT

Background: The present study was undertaken to investigate protective activity of *W. somnifera* root extract (W.S.R.E.), cow urine (C.U.) and their combination against acetaminophen (APAP) induced toxicity in male mice. APAP also known as paracetamol, is a well-known drug used for its analgesic and antipyretic effects**.** However, outside of the therapeutic window, the toxicity may result. W.S. and C.U. are from indigenous sources of plant and animal origins, respectively with several therapeutic activities.

Methods: Sixty adult swiss albino male mice were randomly divided into six groups, comprising of ten mice in each group. Group I (control group) received 2% gum acacia suspension for 14 days orally and on day 14, 0.9% NaCl (@300 mg/kg b.wt.) intraperitoneally was administered after 30 min of prior treatment of various agents. Group II, III, IV, V and VI received 2% gum acacia, silymarin (@25 mg/kg b.wt.), *W.S.R.E.* (@100 mg/kg b.wt.), C.U. (@7.8 mL/kg b.wt.) and *W.S.R.E.*(@100 mg/kg b.wt.) and C.U. (@7.8 mL/kg b.wt.) co-treatment orally for 14 days and on day 14, APAP (@300 mg/kg b.wt.) intraperitoneally was administered after 30 min of prior treatment of various agents as mentioned. On 15th day, the animals were sacrificed and samples were collected to study various hematobiochemical and growth related parameters.

Result: The treatment of acetaminophen caused significant decrease in haemoglobin, total erythrocyte count whereas increase in total leucocyte and prothrombine time. There were significant ($p \leq 0.05$) decreases in plasma total protein, albumin and globulin values in group II (APAP), as compared to control (group I). Treatment with W.S. +C.U attenuates these alterations. W.S.R.E., C.U. and their combination pre-treatment mildly restored the changes to normal observed following APAP exposure in mice. However, the results of co-treatment group were more pronounced as compared to individual treatment groups. Thus, it was concluded that treatment with W.S.R.E. and C.U. curtailed the toxic effect of APAP, however, co-administration of both potentiated the protective effect.

Key words: Acetaminophen, Cow urine, Haematology, *Withania somnifera*.

INTRODUCTION

Liver is the vital organ of the body. The health status of liver is the healthy index for animals. A number of chemical agents and drugs are experimentally used in a routine manner to produce cellular as well as metabolic liver damage (Gosavi *et al*., 2012; Adil *et al*., 2014) to study the liver toxicity. Acetaminophen (APAP; Paracetamol-PCM) is a commonly prescribed analgesic and antipyretic drug producing dose dependent hepatotoxicity. It is metabolized in the liver mainly by glucuronidation and sulfation and at overdose get converted into a highly reactive toxic metabolite N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P450 (CYP) (Rumark, 2002). NAPQI causes depletion and exhaustion of cellular glutathione (GSH) initially (Mitchell *et al.,* 2012) followed by covalently binding with cellular proteins of plasma membrane and mitochondria (Jollow *et al.,*1973).

In the developing countries like India, exploring the indigenous sources such as drugs of plants and animals'origin as an alternative therapeutic strategy have more significance to get new therapeutically active compounds due to their easy accessibllity, safety and cost efficacy over the conventional therapies. Many medicinal plants are rich sources of natural antioxidants that can protect from oxidative stress, thus playing an important role in chemo prevention of diseases. *Withania somnifera* (L.) Dunal, (W.S.) also known as Ashwagandha is a plant of Solanaceae family (Mirjalili *et al*., 2009), which has been used for centuries in *Ayurvedic* medicine for its therapeutic potential. It has been proved that it possesses adaptogenic, antibiotic, aboritifacient, aphrodisiac, astringent, anti-inflammatory, diuretic, sedative, immunostimulatory and

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antioxidant properties (John, 2014). It is used alone or in combination with other herbs in the Indian system of medicine to treat numerous ailments like Osteoarthritis, Parkinsonism, Alzheimer' disease, anxiety (Khojah and Hafez, 2020).

The indigenous cow, scientifically called as *Bos indicus* or *Zebu* cattle, inhabitant of the Indian subcontinent is a most veneered and valuable animal in religious scriptures. It is described in '*Sushruta samhita*','*Ashtanga sangraha'* text as an effective medicinal substance or secretion of animal origin with innumerable therapeutic properties (Kekuda *et al.,* 2010). Despite the wide spread use of cow urine and *Withania somnifera* for various conditions, there is a paucity of data with regards to their efficacy as a protective agent against acetaminophen-induced toxicity. Therefore, the aim of present investigation was to evaluate the protective potential of *Withania somnifera,* cow urine and their co-treatment against APAP-induced toxicity in laboratory animals by using various hemato-biochemical parameters, body weight and organ weight changes.

MATERIALS AND METHODS

The experiment was carried out at college of Veterinary Sciences, LUVAS, Hisar from the period between August-September 2021.

Chemicals

Acetaminophen and Silymarin were purchased from M/s Sigma-Aldrich Chemicals Pvt. Ltd. Bangalore, India. RBC and WBC diluting fluid, Gum Acacia were purchased from M/s SRL diagnostics, Mumbai, India.

Preparation of extract

The roots of *Withania somnifera* were collected from Aromatic and Under-Utilized Plant Section, Department of Plant Breeding, CCSHAU, Hisar. 50 per cent (%) Aqua-methanolic extracts of *Withania somnifera* roots was prepared as per the method suggested by Rosenthaler (1930).

Collection of cow urine

The urine samples were collected from non-pregnant disease free Sahiwal cows aged between 1.5-2 years, reared under standard management practices at Livestock Farm Complex, LUVAS, Hisar. Fresh naturally voided early morning midstream urine was collected in sterile glass container. The collected urine samples of cows were first filtered through Whatmann paper no.1 to get rid of debris and then filtered with syringe filter 0.22 µM and stored at temperature 4°C before use.

Animals

Sixty adult male Swiss Albino mice weighing 20-24 gm were procured from Disease Free Small Animal House (DFSAH), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar and housed in polyacrylic cages in the departmental animal house (Dept. Of Veterinary Pharmacology and Toxicology, COVS, LUVAS). Animals were provided with standard feed and water *ad libitum.* Mice were acclimatized to laboratory conditions for 7 days before start of the experiment. Animal house temperature varied between 22-27°C with a natural light-dark cycle and humidity (50–60%) throughout the study. The prior approval of Institutional Animal Ethics Committee (IAEC) was obtained vide memo no. VCC/IAEC/630-51, dated 25.03.2021 for the use of experimental animals in this study.

Experimental design

The mice were randomly divided into six groups, comprising ten mice in each group. Group I was vehicle control group which received 2% gum acacia suspension for 14 days orally. Group II received 2% gum acacia for 14 days orally and on day 14, APAP (@300 mg/kg b.wt.) intraperitoneally was administered after 30 min of treatment. Group III, IV, V and VI received silymarin (@25 mg/kg b.wt.), *W. somnifera* root extract (@100 mg/kg b.wt.), cow urine (@7.8 mL/kg b.wt.), *W. somnifera* root extract(@100 mg/kg b.wt.) and cow urine (@7.8 mL/kg b.wt.) co-treatment orally for 14 days and on day 14, APAP (@300 mg/kg b.wt.) intraperitoneally was administered after 30 min of prior treatment of various agents as mentioned. Selection of the doses of W.*Somenifera* root extract and cow urine were as per Paul *et al*. (2021) and Gururaja *et al.* (2009a) respectively.

Sampling

On 15th day, all the animals were sacrificed 24 hr after the APAP treatment. After anaesthetizing animals with sodium thiopentone, blood and tissue samples were collected and processed accordingly.

Haematological studies

Hematological studies were carried out on the day of blood collection. Haemoglobin (Hb) estimation was done using Sahli's haemoglobinometer, as described by Oser (1976). The red blood cells (RBCs) and white blood cells (WBCs) were counted according to the procedure given by Schalm (1965). For differential leukocytes count (DLC) blood smears were prepared from the fresh blood and cells were counted under oil immersion lens (Ash and Orihel, 1991). Prothrombin time test (PTT) was counted as per method suggested by Benjamin (2005).

Biochemical studies

The biochemical parameters like plasma total protein, albumin, globulin, albumin-globulin ratio (A:G) and total triglycerides were studied using blood plasma at the end of the experiment using the standard diagnostic kits (ERBA) by semi auto-analyzer (Erba XL-ZOO model) as per the manufacturer's instruction.

Body weight

Body weights of mice of all the groups were taken initially on day 0 and then on 7th and 14th day of the experiment.

Relative body weight

The relative body weight was calculated as per following formula:

Relative body weight =

$$\frac{\text{Final body weight (g) - Initial body weight (g)}}{\text{Initial body weight (g)}} \times 100$$

Relative organ weight

After recording the gross lesions from the sacrificed mice, the organs *viz.* heart, liver, spleen and kidney were removed carefully. The organs were trimmed of extraneous tissues and weighed over electronic digital balance and then relative organ weight factors were calculated as per following formula:

Relative organ weight =

$$\frac{\text{Absolute organ weight (g)}}{\text{Total body weight (g)}} \times 100$$

Statistical analysis

Results are expressed as mean ± SEM. The statistical analysis was performed by Graph Pad Prism 9.0 Software version for Windows (San Diego, CA, USA). The differences among the groups were compared by one-way analysis of variance (ANOVA) followed by post hoc Tukey's test. $p \leq 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Absolute body weight and percent change in body weight

The data recorded at weekly interval regarding effect of different treatments on absolute and per cent change in body weight (g) in mice are shown in Fig 1 and 2, respectively. In the study, all the six groups of mice showed a gradual increase in weight gain (ranges between 23.30±0.64 to 30.4±0.60 g). No marked variation was observed in the absolute body weight of mice of all different groups throughout the entire experiment. However, on 14th day of the experiment a significant ($P \leq 0.05$) increase in percent change in body weight (23.06 ±1.82 g) was observed in the group VI (W.S.+ C.U.+ APAP) as compared to control (15.86±1.53 g) and APAP treated groups (15.02±1.00 g).

Relative organ weight

In the present study, there was significant ($P \leq 0.05$) increase in relative liver weight in acetaminophen treated group II (APAP) (6.90±0.46 g/100g b.wt.) as compared to control (group I) (4.78±0.36 g/100g b.wt). It might be due to accumulation of lipid triglycerides due to formation of fatty liver and congestion. Too much fat deposition in liver can cause liver inflammation also (Kashif *et al.,* 2017). In group III (Sily. + APAP) (4.81±0.24 g/100g b.wt) and group VI (W.S.+ C.U.+ APAP) (4.86±0.13 g/100g b.wt) there were significant ($p \leq 0.05$) decrease in relative liver weight as compared to group II (APAP) (6.90±0.46 g/100g b.wt.). There were no significant ($p \leq 0.05$) changes observed in relative heart,

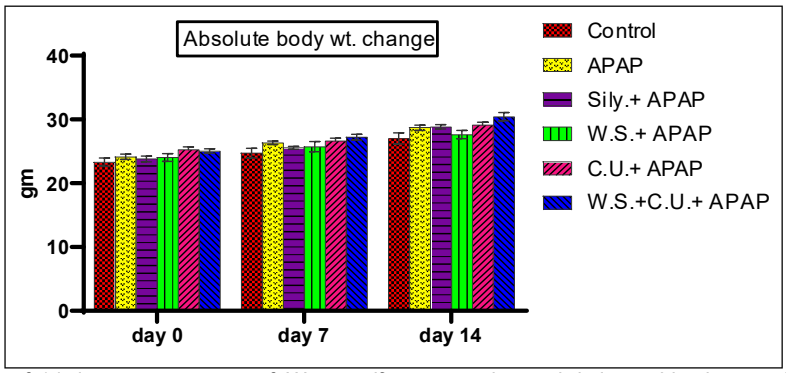

Fig 1: Effect of 14 days pre-treatment of *W. somnifera*, cow urine and their combination on absolute body weight of male mice with acetaminophen-induced toxicity.

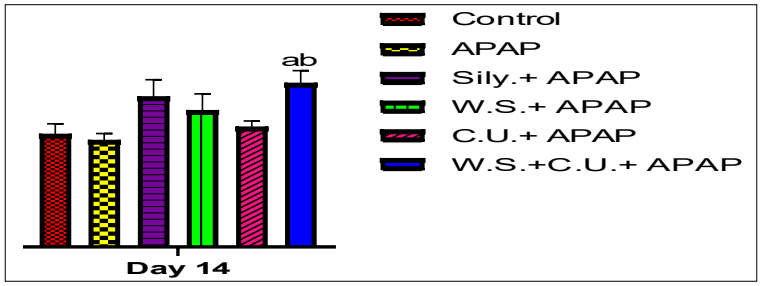

Fig 2: Effect of 14 days pre-treatment of *W. somnifera*, cow urine and their combination on percent change in body weight of male mice with acetaminophen-induced toxicity.

spleen and kidneys weight among different groups of various treatments (Table 1).

Haematological parameters

There was significant (P≤0.05) decrease in RBCs and Hb level of mice in group II (APAP) as compared group I (control). There were significant (p≤0.05) improvement in the values of Hb and RBCs in group III (Sily. + APAP), IV (W.S. + APAP) and VI (W.S.+ C.U.+ APAP) as compared to group II (APAP) (Table 2). These findings are in accordance with the study of Bhaumik and Sharma (2002), who also reported a significant decrease in Hb concentration and TEC in rabbits induced with single intravenous injection of paracetamol @ 400mg/kg b.wt. The Hbconcentration generally provides an accurate reflection of the extent to which the circulating red cell mass is reduced. The inability of the damaged hepatic parenchyma to synthesize erythropoietin reduced feed intake and reduced absorption and metabolism of nutrients may be responsible for these types of changes. Kumar, (2018) also observed ameliorative effects of powdered *W. somnifera* roots in chlorpyrifos intoxication in white leghorn cockerels by improving Hb and TEC values. Similarly, improvement of TEC and Hb values after administration of cow urine has been observed by Bapu, (2001) who found the presence of hormones *i.e.* erythropoietin and traces of iron in cow urine which stimulates the bone marrow.

In the present study, there was significant (P≤0.05) increase in TLC values of mice in group II (APAP) as compared group I (control). There were significant (p≤0.05) reduction in the TLC in group III (Sily. + APAP), IV (W.S. + APAP), V (C.U. + APAP) and VI (W.S.+ C.U.+ APAP) as compared to group II (APAP) animals (Table 3). There was significant (p≤0.05) increase in the neutrophil values in the mice of group II (APAP) as compared to group I (control). There were significant (p≤ 0.05) reduction in the neutrophil values in group III (Sily. + APAP), IV (W.S. + APAP), V (C.U. + APAP) and VI (W.S.+ C.U.+ APAP) as compared to group II (APAP) (Table 4). There was significant (p≤ 0.05) decrease in the lymphocyte values in the mice of group II (APAP) as compared to group (I). There were significant (p≤0.05) increase in the lymphocyte values in group III (Sily. + APAP), IV (W.S. + APAP), V (C.U. + APAP) and VI (W.S.+ C.U.+ APAP) as compared to group II (APAP). There were no significant changes observed in the monocyte, basophil and eosinophil count among different group of various treatments (Table 4). Increased TLC values have also been reported by Jain *et al*. (2012) in experimental animals. In their study, neutrophilia and lymphocytopenia were prominent in differential leukocyte count in all the animals subjected to hepatopathy. This might be due to stress coupled with inflammatory changes in body tissue, which is responsible for phagocytosis of toxic substances and neutrophilia was induced by tissue demand for phagocytic function (Duncun

Table 1: Effect of 14 days pre-treatment of *W. Somnifera*, Cow Urine and their combination on relative organ weight of male mice with Acetaminophen-induced toxicity.

Treatment	Relative organ wt. (g/100 g b.wt.)				
	Liver	Heart	Spleen	L. Kidney	R. Kidney
Control	4.78±0.36	0.54±0.05	0.60±0.05	0.64±0.03	0.65±0.04
APAP	6.90^{a}±0.46	0.55±0.02	0.55±0.05	0.58±0.03	0.59±0.02
Sily. + APAP	4.81^{b}±0.24	0.54±0.02	0.47±0.03	0.65±0.04	0.65±0.04
W.S. + APAP	5.63±0.65	0.51±0.03	0.53±0.03	0.73±0.07	0.72±0.08
C.U. + APAP	5.34±0.38	0.51±0.02	0.60±0.03	0.67±0.03	0.69±0.03
W.S.+C.U.+APAP	4.86^{b}±0.13	0.48±0.05	0.48±0.01	0.58±0.01	0.57±0.01

Values are expressed as Mean ± SE, n=5.

Means bearing a, b superscripts differ significantly (p≤0.05) vs control, APAP respectively.

APAP: Acetaminophen; Sily: Silymarin; W.S.: *Withania somnifera*; C.U.: Cow urine.

Table 2: Effect of 14 dayspre-treatment of *W. Somnifera*, Cow Urine and their combination on the Hb (g/dl) and TEC (10^6/mm^3) of male mice with Acetaminophen-induced toxicity.

Treatment	Hb(g/dl)	TEC (10^6/mm^3)
Control	13.52±0.92	8.8±0.34
APAP	10.54^{a}±0.32	6.26^{a}±0.09
Sily.+APAP	13.4^{b}±0.43	7.86^{ab}±0.09
W.S.+APAP	13.06^{b}±0.34	7.58^{ab}±0.13
C.U.+APAP	12.66±0.54	7.88^{ab}±0.08
W.S.+C.U.+APAP	13.48^{b}±0.24	7.98^{ab}±0.11

Values are expressed as Mean ± SE, n=5.

Means bearing a, b superscripts differ significantly (p≤0.05) vs control, APAP, respectively.

APAP: Acetaminophen; Sily: Silymarin; W.S.: *Withania somnifera*; C.U.: Cow urine.

et al., 1988). According to James *et al.,* (2006) paracetamol overdose is known to be associated with inflammation, marked by an increase in the inflammatory cytokines *i.e.* tumor necrosis-α (TNF-α), interleukin-la and interleukin-1b *etc*. which are important for the inflammatory process. *W. Somnifera* and cow urine reduces TLC and neutrophil count values of might be due to anti-inflammatory properties of cow urine (Gururaja *et al.,* 2009b; Rachna and Sreepada, 2019) and *W. somnifera* (Gupta and Singh, 2014; Devkar *et al*., 2016).

There were significant ($p \leq 0.05$) increase in the prothrombin time in group II (APAP), III (Sily. + APAP), IV (W.S. + APAP), V (C.U. + APAP) and VI (W.S.+ C.U.+ APAP) as compared to control (group I). There were significant ($p \leq 0.05$) reduction in the prothrombin time in group III (Sily. + APAP), IV (W.S. + APAP), V (C.U. + APAP) and VI (W.S.+ C.U.+ APAP) as compared to group II (APAP) (Table 3). The liver plays a key role in the blood coagulation process. Liver damage is commonly associated with variable impairment of haemostasis because almost all the clotting factors and their inhibitors are produced in the liver (Amitrano *et al.,* 2002) and damage of hepatic cells might have led to reduced production of these factors which in turn resulted in delayed clotting of blood. Animals treated with *W. Somnifera* and cow urine are found to have decreased prothrombintime, reason might be having antioxidants which reduces the hepatic injuries resulted in more protein synthesis (Devkar *et al.,* 2016).

Biochemical parameters

There were significant ($p \leq 0.05$) decrease in plasma total protein values in group II (APAP), III (Sily. + APAP), IV (W.S. + APAP), V (C.U. + APAP) and VI (W.S.+ C.U.+ APAP) as compared to control (group I). These levels were significantly ($p \leq 0.05$) restored in group III (Sily. + APAP) and VI (W.S.+ C.U.+ APAP) as compared to group II (APAP) animals (Table 5). There were significant ($p \leq 0.05$) decreases in plasma albumin and globulin values in group II (APAP) as compared to control (group I). The animals of groups of IV, V and VI has shown increased values of plasma albumin and globulin levels as compared to group II, although these changes were non-significant. There were no significant changes in albumin/globulin ratio among different group of various treatments as compared to control group (Table 5). The liver is the main site for synthesis of most plasma protein of body. Decrease in total protein, albumin and globulin levels may be indicative for the development of disorder in protein synthesis and metabolism (Dai *et al.,* 2006). In present study, decreased levels of plasma total protein, albumin and globulin in acetaminophen treated group were in accordance with the results in which significant decrease in serum protein, albumin and globulin was also reported in paracetamol-induced hepatoxicity in sheep (Bhaumik,1995), rabbits (Bhaumik and Sharma, 2002) and Wistar rats (Saheed

Table 3: Effect of 14 days pre-treatment of *W. Somnifera*, Cow Urine and their combination on theTLC ($10^3/mm^3$) and Prothrombin time (Sec) of male mice with Acetaminophen-induced toxicity.

Treatment	TLC ($10^3/mm^3$)	Prothrombin time (sec)
Control	5.52±0.28	13.00±1.00
APAP	7.18^{a}±0.13	42.60^{a}±1.6
Sily. + APAP	5.32^{b}±0.15	19.40^{ab}±0.92
W.S. + APAP	5.8^{b}±0.14	26.08^{abc}±0.86
C.U. + APAP	5.28^{b}±0.15	22.60^{ab}±1.36
W.S. + C.U.+APAP	5.12^{b}±0.11	21.00^{abd}±1.41

Values are expressed as Mean ± SE, n=5.

Means bearing a,b,c and d superscripts differ significantly($p \leq 0.05$) vs control, APAP, Sily. + APAP, W.S.+ APAP, respectively.

APAP: Acetaminophen; Sily: Silymarin; W.S.: *Withania somnifera*; C.U.: Cow Urine.

Table 4: Effect of 14 days pre-treatment of *W. Somnifera*, Cow Urine and their combination on the DLC (%) of male mice with Acetaminophen-induced toxicity.

Treatment	DLC (%)				
	Lymphocyte	Neutrophil	Monocyte	Basophil	Eosinophil
Control	79.20±1.46	19.40±1.50	1.40±0.24	0	0
APAP	71.60^{a}±2.35	26.60^{a}±2.37	1.40±0.24	0.40 ± 0.24	0
Sily. + APAP	78.60^{b}±1.43	18.80^{b}±1.35	2.40±0.24	0.20 ± 0.20	0
W.S. + APAP	78.40^{b}±1.43	19.00^{b}±1.48	2.40±0.24	0.20 ± 0.20	0
C.U. + APAP	79.20^{b}±0.86	18.40^{b}±0.87	2.40±0.40	0	0
W.S. + C.U. + APAP	78.80^{b}±1.15	19.00^{b}±1.00	2.20±0.20	0	0

Values are expressed as mean ± SE, n=5.

Means bearing a, b superscripts differ significantly ($p \leq 0.05$) vs control, APAP respectively.

APAP: Acetaminophen; Sily: Silymarin; W.S.: *Withania somnifera*; C.U.: Cow Urine.

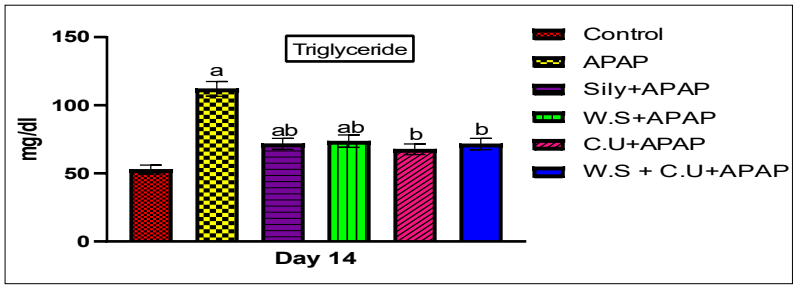

Fig 3: Effect of 14 days pre-treatment of *W. somnifera*, cow urine and their combination on the Triglycerides (mg/dl) of male mice with acetaminophen-induced toxicity.

Table 5: Effect of 14 days pre-treatment of *W. Somnifera*, Cow urine and their combination on the total protein (g/dl), Albumin (g/dl), Globulin (g/dl) and A:G Ratio of male mice with Acetaminophen-induced toxicity.

Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A:G ratio
Control	7.03±0.17	4.14±0.15	2.89±0.14	1.43±0.10
APAP	5.02^{a}±0.24	2.96^{a}±0.18	2.06a±0.18	1.44±0.17
Sily. + APAP	6.15^{ab}±0.20	3.87^{b}±0.17	2.27±0.11	1.70±0.13
W.S. + APAP	5.67^{a}±0.16	3.28±0.25	2.39±0.22	1.38±0.24
C.U. + APAP	5.77^{a}±0.20	3.41±0.21	2.36±0.21	1.44±0.22
W.S.+ C.U.+APAP	6.06^{ab}±0.15	3.55±0.19	2.45±0.06	1.45±0.11

Values are expressed as Mean ± SE, n=5

Means bearing a, b superscripts differ significantly (p≤0.05) vs control, APAP respectively.

APAP: Acetaminophen; Sily: Silymarin; W.S.: *Withania somnifera*; C.U.: Cow Urine.

et al., 2016). This alteration in protein patterns observed might be due to binding of NAPQI metabolite to albumin and formation of protein adducts (Malik *et al.,* 2013). This indicates poor liver function or impaired synthesis due to liver cell damage. The level was significantly (P≤0.05) increased in group VI (W.S.+ C.U.+ APAP) as compared to group II (APAP). These findings are similar with earlier studies in which *W. somnifera* reversed the effect of acetaminophen toxicity and thus protein level was brought towards normal (Sabina *et al.,* 2013). Gupta *et al.,* (2004) who reported the increase in the mean value of total protein and albumin in rats treated with whole cow urine due to its anabolic effect.

A significant (p≤0.05) increase in the levels of triglycerides in group II (APAP), III (Sily. + APAP) and IV (W.S. + APAP) as compared to control (group I) were noticed (Fig 3). There were significant (p≤0.05) decline in the values of group III (Sily. + APAP), IV (W.S. + APAP), V (C.U. + APAP) and VI (W.S.+ C.U.+ APAP) as compared to group II (APAP) (Fig 3). The major endogenous site for synthesis of plasma triglycerides is liver. The pattern of increase in plasma triglycerides concentration in present study are in close similarity to the findings of Sharma *et al*., (2013) in goats and Bhaumik (1995) in sheep following paracetamol intoxication.

The results of this study revealed that APAP induced mild to moderate toxicity as evident by changes in various hematobiochemical and growth related parameters in intoxicated mice. On the other hand, treatment of these mice with *W. somnifera* and cow urine attenuates these alterations. So, this study suggested that *W. somnifera* and cow urine might play a protective role in APAP induced toxicity.

CONCLUSION

In Conclusion, hepatoprotective effects of W. Somnifera root extract (W.S.R.E.), cow urine (C.U.) and their combination against acetaminophen (APAP) induced toxicity are presented through multiple ways. Treatment with W.S. + C.U attenuates drug induced alterations in various hemato-biochemical parameters. W.S.R.E., C.U. and their combination pre-treatment mildly restored the changes to normal observed following APAP exposure in mice. However, the results of co-treatment group were more pronounced as compared to individual treatment groups. Thus, it was concluded that treatment with W.S.R.E. and C.U. curtailed the toxic effect of APAP, however, co-administration of both potentiated the protective effect.

ACKNOWLEDGEMENT

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Conflict of Interest

All authors declared that there is no conflict of interest among them.

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