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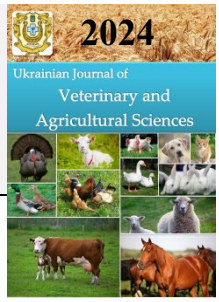

Efficacy of thankuni and grapes in arsenicosis-affected rat

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Contents

Abstract

This study was undertaken to observe the effects of Thankuni and grapes on hematological and biochemical parameters against arsenicosis in Long Evans rats. Sixty male rats were selected to perform the study. The experimental rats were randomly divided into five different groups, with n = 12 in each group. Animals in group T_0 were given normal feed and water and kept as control. Rats of group T_1 were given arsenic trioxide @ 100 mg/L of drinking water orally. Rats of group T_2 were given arsenic trioxide @ 100 mg/L drinking water and Thankuni @ 1gm/kg feed. Group T_3 was given arsenic trioxide @ 100 mg/L drinking water with grapes @ 10 mg /kg body weight thrown DW. Group T_4 was given arsenic trioxide, Thankuni, and grapes with the same dose for up to 45 days, respectively. Four randomly selected rats from each group (T_0, T_1, T_2, T_3, and T_4) were sacrificed at 15-day intervals to determine body weight, hematological and biochemical parameters. At the end of the experiment, the result showed that the minimum weight gain group was T_1 whereas the maximum weight gain was found in the T_3 and T_4 groups. The weight gain of the T_2 group is better than T_1. Reduction of TEC and Hb values were significant ($P < 0.01$) in the T_1 group. Whereas in the rest of the groups, the reduction of TEC and Hb was less than in arsenic-treated groups. In conclusion, Thankuni and grapes have a significant effect on body weight and hematological and biochemical parameters.

Keywords: Arsenicosis; Thankuni; grapes; rat.

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1. Introduction

Arsenic is currently one of the most critical environmental global contaminants and toxicants, particularly in developing countries. Arsenic is a potent cardiovascular toxicant associated with numerous biomarkers of cardiovascular diseases in exposed human populations. The toxic effects of arsenic are mediated by mitochondrial dysfunction and related to arsenic's effect on oxidative stress. Therefore, we investigated the effectiveness of antioxidants against arsenic-induced cardiovascular dysfunction (Pace et al., 2017). Arsenic binds to sulfhydryl groups on proteins, modulates protein metabolism, and generates reactive oxygen species (ROS) (Mehta & Hundal, 2016). The free radical-mediated theory becomes more acceptable to explain arsenic-induced damage. Oxidative stress is acceptable as one of the mechanisms of arsenicosis, which can be mediated by excess production of reactive oxygen species (ROS) (Li et al., 2016). For that reason, nutritional antioxidants in diseases related to oxidative stress have gained immense interest in recent years because they could be used as suitable preventive/therapeutic agents (Pineda et al., 2013).

Arsenic can be found in organic and inorganic forms in water, food, air, and soil. The most important inorganic arsenic compounds are arsenic trioxide, sodium arsenite, arsenic trichloride, arsenic acid, arsenites (trivalent forms), and calcium arsenates (pentavalent forms). Common organic arsenic forms are arsanilic acid monomethylarsinate (MMA), dimethylarsinic acid (DMA) (Delnomdedieu et al., 1995), and arsenobetaine. Of these compounds, the inorganic forms of arsenic exhibit the highest toxicity level (Saha, 2003). The safety limit of arsenic accepted by the Bangladesh Government is 0.05 mg/liter for drinking water (Fazal et al., 2001). The World Health Organization limit for drink-

ing water is 0.01 mg/liter, and for foodstuffs is 2 mg/liter on a fresh weight basis (Robinson et al., 2003).

Humans are exposed to arsenic predominantly through contaminated drinking water, whereas inhalation and skin absorption are minor routes of exposure (Shi et al., 2004). Chronic arsenic exposure through drinking water to humans leads to carcinogenesis of almost all organs, skin diseases (viz. hyper-pigmentation, hyperkeratosis) leading to cancers of skin and epithelial tissues; hepatic, renal, cardiovascular, respiratory, central nervous system, gastrointestinal, reproductive complications, and children's intellectual impairment; thereby increasing morbidity and mortality (Kapaj et al., 2006; Mazumder, 2008; Khatun et al., 2020).

The toxic effects of arsenic in the human body and their conventional management have been well studied and reviewed previously (Abdul et al., 2015). But there is no comprehensive account of the studies on alternative options for counteracting arsenic toxicity. Chronic arsenic exposure is associated with many human health conditions, including skin lesions and cancers of the liver, lung, bladder, and skin (Uddin & Huda, 2011; Shathy et al., 2020; Basher et al., 2023). Arsenicosis presents with significant changes in the Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), serum creatinine, urea, uric acid levels and various hematological parameters like TEC, TLC, Hb, blood sugar level in the Swiss albino rats (Ashraf et al., 2010). The clinical features that invariably relate to the gastrointestinal system are nausea, vomiting, colicky abdominal pain and profuse watery diarrhea (Burrows & Tyrl, 2013) and also arsenic can enter into the blood chain (Ulman et al., 1998). Recently arsenic intoxication in experimental animals has been found to be associated with hepatic tumors (Waalkes et al., 2003), Spermatogenesis (Shukla & Pandey, 1984), inhibition of testicular steroidogenic function (Sarkar et al., 1991), and severe metabolic disorders such as diabetes in humans (Tseng et al., 2002). The natural source of human exposure to arsenic occurs through the consumption of drinking water sourced from groundwater that contains dissolved inorganic arsenic (Nandi et al., 2006). Consequently, population in areas where the water supply is contaminated with arsenic (i.e.; areas of Taiwan, China, and parts of Africa) tend to have significantly higher mortality rates from conditions related to arsenic poisoning, such as bladder, lung, liver, kidney, skin, and colon cancers (Azcue & Nriagu, 1995; Meliker et al., 2007; Asaolu & Asaolu, 2010).

Thankuni (*Centella asiatica*) is an herbaceous, frost-tender perennial plant in the flowering family Apiaceae (Ploenkutham, 2018). It is native to the wetlands in Asia and it is used as a culinary vegetable and medicinal herb (Ploenkutham, 2018). Centella grows in temperate and tropical swampy areas in many world regions (Ploenkutham, 2018). Thankuni extract improves the medicinal value and these properties have been ascribed to the active principles viz., Asiaticoside, madecassic acid and madecassocide. These are pentacyclic triterpenes, found to display venous insufficiency, and various vein and wound healing properties (Kant et al., 2019). Thankuni is essential for numerous intrinsic processes. A study of arsenic toxicity and long-term treatment with thankuni in animals could establish unequivocal decisions about the impacts of arsenic.

Resveratrol is the beneficial compound found in red wine that is associated with life extension and some of the health benefits in wine. It is produced in grapes as a defense against toxins, and biological activity, cardio protective effects, antidiabetic and antioxidant effects (Sharma et al., 2018).

Grape Seed Extract (GSE), which is rich in polyphenols, has been demonstrated to possess potent antioxidant properties and is a safe and effective antioxidant compound. Previous studies have shown that the antioxidant activity of GSE is greater than that of vitamins C and E and β-carotene (Bagchi et al., 2000). GSE may exert its antioxidant and anti-inflammatory effects by scavenging oxygen free radicals, inhibiting lipid per oxidation and the formation of inflammatory cytokines, altering cell membrane receptors and intracellular signaling pathway proteins and modulating gene expression (Li et al., 2001; Kris-Etherton et al., 2004). In a previous study, we demonstrated that GSE may inhibit rat liver injury (Pan et al., 2011).

Bangladesh is available for arsenic toxicity only on tube-well water; however, data on the specific treatment for the prevention of arsenic toxicity in both humans and animals is little. Therefore, data on the effective prevention of arsenic toxicity with thankuni and grapes and their comparative efficacy will be the expected new findings, especially for Bangladesh and the world.

2. Materials and methods

Experimental animals

A total of 60 male rats of 6 weeks of age were purchased from the International Centre for Diarrheal Disease Research in Bangladesh (ICDDRB). The animals were housed in compartmented rectangular metallic cage under standard laboratory conditions (12 h light: 12 h dark, 25 ± 2^0C and humidity 60 ± 5%). The rats were acclimatized for 15 days in the laboratory before the experiment started.

Preparation of house

At first, the room and the wire cages were washed by sweeping and washing with tap water using a hose pipe connected to a tap. The room was disinfected with a phenolic disinfectant. The room was allowed to dry by leaving an electric fan and bulb switched on. Proper ventilation was provided.

Body weight (BW)

The rats were weighed first on Day 0 (Day 0 = immediate previous day of starting treatment) after grouping and marking. Then again on, Day 15, Day 30, and finally on Day 45. All results were recorded.

Clinical signs

Experimental rats were closely observed after feeding arsenic trioxide and Thankuni daily at three times (morning, afternoon, and evening), looking for the appearance of any toxic signs in them. The observations were conducted throughout the entire experimental period (from Day 1 to Day 45), and the findings were recorded.

Experimental trial

The experimental trial was conducted for 45 days. The rats in Group T_0 were maintained with only normal pellet feed and water ad libitum as control. The rats in Group T_1 were treated with arsenic trioxide at a dose of 100 mg/L in drinking water. The rats in Group T_2 were treated with arsenic trioxide at 100 mg/L in drinking water daily and Thankuni simultaneously, at a dose of 1 gm/kg feed. The Thankuni used in this experiment was collected from the local market. The rats in Group T_3 were treated with arsenic trioxide at 100 mg/L in drinking water daily and grapes

from a local supermarket, simultaneously, at a dose of 10 mg/kg per bodyweight. The animals of Group T_4 were treated with arsenic trioxide at 100 mg/L in drinking water daily and grapes at a dose of 10 mg/kg body weight and Thankuni simultaneously at a dose of 1gm/kg feed. All treatments were given for 45 days.

Sampling

After administering the treatment for 15 days, four rats from each group were anesthetized using chloroform anesthesia, and they were then sacrificed. Blood samples from each rat (about six milliliters) were collected directly from cardiac punctures, using disposable plastic syringe. The blood from each rat was then transferred into two tubes to determine biochemical parameters, and hematological tests. For the biochemical test, four milliliters (4 ml) of blood sample were taken into pre-marked centrifuge glass test tubes immediately after collection. The collected blood was kept at room temperature to allow it to clot properly, then stored in a refrigerator overnight. The serum was separated the next morning by centrifugation and the supernatant serum was taken into pre-marked Eppendorf tubes. The harvested serum was kept at -20 ºC until used. One milliliter (1 ml) of blood was taken into EDTA-coated tubes to run hematological and arsenic concentration tests. All lungs, livers, and kidneys were collected aseptically, washed with physiologic saline, and kept in pre-marked zipper polythene bag. Blood samples for hematological investigation were preserved at 4 ºC temperature. The blood samples were taken first on Day 15, then on Day 30, and lastly on Day 45.

Biochemical tests

Sera was thawed on the laboratory bench, and the SGOT and, SGPT activity, as well as the serum creatinine were determined by using Thankuni (Deneke & Rittersdorf, 1984; Deneke et al., 1985).

Statistical analysis

The collected data were statistically analyzed as per Steel and Torrie (1980), using the Completely Randomized Design (CRD). An analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were performed on SPSS 20 software to determine the difference among the treatments.

3. Results and discussion

Body weight (BW) of the rats

The body weight (BW) of the experimental rats in all groups were taken at fifteen days intervals, on Day 0, Day 15, Day 30, and Day 45. Table 1 shows that the body weight gain was the highest (298.40 ± 3.72) in T_2 group rats at 45 days, but the lowest body weight gain (97.60 ± 2.63) was found in the arsenic treated T_1 group at 45 days. The body weight gain in T_0, T_3 and T_4 were (256.80 ± 5.13), (283.80 ± 7.17), and (274.80 ± 2.84), respectively, which were better than in the arsenic treated T_1 group. The initial body weight of the groups was not significant ($P > 0.05$), but then in 15, 30, and 45 days, the mean value of body weight was significant ($P < 0.01$).

The body weight of the treated group increased with their age, but in the T_1 group it decreased compared to other groups. In the present study, arsenic reduced body weight as the age increased. The highest body weight gain was found in the T_2 group, where Thankuni were treated with arsenic. This suggests that Thankuni acts against arsenic by decreasing body weight. Sharma et al. (2007) reported that reduced body weight was observed in an arsenic treated group of Swiss albino mice, and it also significantly ($P < 0.01$) decreased the body weight of rats (Sharma et al., 2007; Wu et al., 2008).

Table 1

Effects of Thankuni and grapes on the body weight of arsenic-fed rats

Treatment	T_o	T_1	T_2	T_3	T_4	P. value
Initial	78.20 ± 3.28	82.60 ± 2.50	88.46 ± 3.32	84.20 ± 4.32	85.00 ± 2.78	NS
15 days	171.00 ± 5.56 ^{b}	87.40 ± 4.02 ^{a}	181.80 ± 5.21 ^{bc}	188.40 ± 4.97 ^{c}	195.00 ± 5.17 ^{c}	**
30 days	220.20 ± 4.32 ^{b}	94.80 ± 2.63 ^{a}	261.20 ± 2.92 ^{d}	255.40 ± 2.04 ^{d}	239.40 ± 4.74 ^{c}	**
45 days	256.80 ± 5.13 ^{b}	97.60 ± 2.63 ^{a}	298.40 ± 3.72 ^{d}	283.80 ± 7.17 ^{c}	274.80 ± 2.84 ^{c}	**

Note: within each row, figures with the same or without superscripts do not differ significantly as per the DMRT. The data were calculated at a 99 % level of significance ($P < 0.01$)

Hematological parameter

Total Erythrocyte Count (TEC)

In Table 2, Total Erythrocyte Count (TEC) values were the highest (8.54 ± 0.15) in the T_4 group at 45 days, where Thankuni and grapes were used against arsenic toxicity, but the lowest value (6.34 ± 0.26) was found in the T_1 group, where only arsenic was given. The TEC values at 15 days (7.12 ± 0.03) and 45 days were significant at ($P < 0.01$), and the values found at 30 days (7.64 ± 0.27) were also significant at ($P < 0.05$).

Table 2

Effects of Thankuni and grapes on Total Erythrocyte Count (TEC) values of arsenic-fed rats

Treatment	T_0	T_1	T_2	T_3	T_4	P. value
15 Days	6.40 ± 0.13 ^{a}	6.20 ± 0.04 ^{a}	6.48 ± 0.13 ^{a}	6.43 ± 0.13 ^{a}	7.12 ± 0.03 ^{b}	**
30 Days	6.48 ± 0.18 ^{ab}	6.23 ± 0.04 ^{a}	6.98 ± 0.18 ^{b}	6.98 ± 0.13 ^{b}	7.64 ± 0.27 ^{c}	*
45 Days	6.71 ± 0.25 ^{ab}	6.34 ± 0.26 ^{a}	7.34 ± 0.26 ^{c}	7.55 ± 0.17 ^{c}	8.54 ± 0.15 ^{d}	**

Note: within each row, figures with the same, or without superscripts do not differ significantly as per the DMRT. The data was calculated at a 99 % level of significance ($P < 0.01$)

Total Leukocyte Count (TLC):

In Table 3, total leukocyte counts on day 30 were found to be the highest (10.86 ± 0.004) in the control group rats, and lowest in the T_4 group rats, where Thankuni and grapes were administered. The differences were statistically significant among all groups of rats ($P < 0.01$). Hence, it can be deduced that Thankuni and grapes decrease the TLC level.

Hemoglobin (Hb):

The highest Hb concentration (15.26 ± 0.76) was found in the T_4 group at 30 days, and the lowest concentration was found in the T_0 group (Table 4). The difference among the values of Hb concentration at 30 days was statistically significant ($P < 0.01$), and the difference among the Hb concentration values at 15 and 45 days was statistically significant ($P < 0.05$). It might be concluded that Thankuni and grapes might slightly increase the values of Hb against arsenic toxicity in rats.

Erythrocyte Sedimentation Rate (ESR):

The highest ESR value was observed in the T_4 group, and the lowest value was observed in the control group (Table 5). The differences between the mean values of different groups were found to be significant ($P < 0.05$). It was found that hemoglobin and hematocrit values were reduced in arsenic toxicities in rats as observed in the present study, but in other groups, it was increased. The cause of the change in hematological values might be due to the toxic effect of arsenic on the hematopoietic system which is responsible for such alterations in hematological parameters. However, it is assumed that the toxic effects of arsenic trioxide on bone marrow may be responsible for erythrocytopenia (Islam et al., 2009).

Packed Cell Volume (PCV):

The PCV values decreased in the arsenic-treated group. The highest values were found in the T_4 group, and the lowest values were found in the arsenic treated T_1 group (Table 6). The differences between the mean values of the different groups were found to be non-significant. The cause of the change in hematological values might be due to the toxic effect of arsenic on the hematopoietic system, which is responsible for such alterations in hematological parameters.

Table 3

Effects of Thankuni and grapes on Total Leukocyte Count (TLC) values of arsenic-fed rats

Treatment	T_0	T_1	T_2	T_3	T_4	P. value
15 Days	9.56 ± 0.003^{d}	9.09 ± 0.005^{a}	9.41 ± 0.005^{b}	9.37 ± 0.030^{b}	9.51 ± 0.006^{c}	**
30 Days	10.86 ± 0.004^{e}	10.24 ± 0.005^{a}	10.42 ± 0.005^{c}	10.27 ± 0.005^{b}	10.76 ± 0.006^{d}	**
45 Days	10.82 ± 0.003^{c}	9.79 ± 0.008^{a}	9.87 ± 0.005^{ab}	9.88 ± 0.010^{b}	10.80 ± 0.058^{c}	**

Note: within each row, figures with the same, or without superscripts do not differ significantly as per the DMRT. The data was calculated at a 99 % level of significance ($P < 0.01$)

Table 4

Effects of Thankuni and grapes on Hemoglobin concentration (Hb) (gm/dl) values of arsenic-fed rats

Treatment	T_0	T_1	T_2	T_3	T_4	P. Value
15 Days	13.50 ± 0.65^{b}	11.13 ± 0.43^{a}	14.00 ± 0.41^{b}	13.13 ± 0.31^{b}	12.88 ± 0.43^{b}	*
30 Days	11.03 ± 0.89^{b}	8.45 ± 0.65^{a}	15.26 ± 0.76^{c}	14.98 ± 0.72^{c}	15.25 ± 0.78^{c}	**
45 Days	15.13 ± 0.97^{b}	7.75 ± 1.78^{a}	15.28 ± 0.83^{b}	15.05 ± 1.05^{b}	17.20 ± 1.54^{b}	*

Note: within each row, figures with the same, or without superscripts do not differ significantly as per the DMRT. The data was calculated at a 99 % level of significance ($P < 0.01$)

Table 5

Effects of Thankuni and grapes on Erythrocyte Sedimentation Rate (ESR) (gm/dl) values of arsenic-fed rats

Treatment	T_0	T_1	T_2	T_3	T_4	P. value
15 Days	43.88 ± 2.78^{b}	39.75 ± 1.03^{ab}	38.38 ± 0.85^{a}	36.25 ± 0.85^{a}	36.50 ± 0.65^{a}	*
30 Days	42.00 ± 3.58^{b}	31.00 ± 1.47^{a}	45.50 ± 2.10^{b}	45.13 ± 2.56^{b}	41.50 ± 1.32^{b}	*
45 Days	42.25 ± 2.81^{b}	24.25 ± 2.17^{a}	47.00 ± 3.45^{b}	49.63 ± 3.90^{b}	50.25 ± 7.16^{b}	*

Note: within each row, figures with the same or without superscripts do not differ significantly as per the DMRT. The data was calculated at a 99 % level of significance ($P < 0.01$)

Table 6

Effects of Thankuni and grapes on Packed Cell Volume (PCV) (gm/dl) values of arsenic-fed rats

Treatment	T_0	T_1	T_2	T_3	T_4	P. value
15 Days	13.67 ± 1.33	9.00 ± 0.58	12.00 ± 1.53	15.00 ± 2.52	16.00 ± 3.21	NS
30 Days	12.00 ± 0.58	11.00 ± 0.58	13.33 ± 0.88	15.67 ± 0.67	19.00 ± 2.08	NS
45 Days	18.33 ± 3.84	9.00 ± 0.58	15.33 ± 0.33	21.67 ± 6.01	20.00 ± 2.89	NS

Note: within a row, figures with the same or without superscripts do not differ significantly as per DMRT. The data were calculated at a 99 % level of significance ($P < 0.01$)

Biochemical parameters

Serum Glutamate Oxaloacetate Transaminase activity (SGOT)

The highest values of SGOT were observed in the control group (T_0), while the lowest values were observed in the T_4 group (Table 7). There were significant differences within the groups during the two days (30 and 45) of measurement ($P < 0.01$) and significant differences within the groups on day 15 ($P < 0.05$). It appears that while Thankuni alone has some effect in lowering the SGOT values in re-

sponse to prolonged administration of arsenic, the combination of Thankuni and grapes produced a more significant reduction in SGOT level comparable to the control group ($P < 0.01$). However, this finding disagreed with the previous findings that SGOT was reduced by as alone (Mahaffey et al., 1981; Ashraf et al., 2010). In Thankuni-treated (T_2), grapes-treated (T_3), and Thankuni plus grapes-treated (T_4) experimental arsenicosis groups, significantly decreased values of arsenic were recorded ($P < 0.01$).

Table 7
Effects of Thankuni and grapes on SGOT values of arsenic-fed rats

Treatment	T_0	T_1	T_2	T_3	T_4	P. value
15 Days	115.00 ± 2.89^{c}	110.00 ± 289^{bc}	100.00 ± 2.52^{a}	106.67 ± 4.41^{abc}	102.00 ± 0.58^{ab}	*
30 Days	109.00 ± 3.79^{bc}	117.00 ± 8.50^{c}	97.33 ± 1.45^{ab}	98.67 ± 1.86^{ab}	88.00 ± 1.53^{a}	**
45 Days	109.67 ± 3.18^{b}	141.67 ± 6.23^{c}	106.67 ± 1.67^{b}	105.00 ± 2.89^{b}	84.33 ± 2.33^{a}	**

Note: within a row, figures with the same or without superscripts do not differ significantly as per DMRT. The data was calculated at a 99 % level of significance ($P < 0.01$)

Serum Glutamate Pyruvate Transaminase activity (SGPT)

Continuous administration of arsenic to Long-Evans rats caused a significant increase in the blood SGPT level. The highest values of SGPT were observed in the T_1 group, where the rats were treated with only arsenic. There were insignificant differences within the groups during days 15 and 30, but this difference became statistically significant ($P < 0.01$) by day 45. The lowest values of SGPT were observed in the T_4 group, where the combination of Thankuni and grapes was administered against arsenic toxicity. In 15 days and 30 days, blood SGPT levels were increased however it is not statistically significant ($P > 0.05$). In 45 days, the level of blood SGPT decreased at a statistically significant ($P < 0.01$) (Table 8). Overall, SGPT values have a decreasing trend with the progress of time in all groups, which agreed with the previous research findings (Islam, 2008). It may be concluded that prolonged treatment with Thankuni and grapes may reduce the blood SGPT level.

Table 8
Effects of Thankuni and grapes on SGPT values of arsenic-fed rats

Treatment	T_0	T_1	T_2	T_3	T_4	P. value
15 Days	71.67 ± 0.89	77.00 ± 1.53	64.33 ± 7.17	72.67 ± 1.45	79.00 ± 1.52	NS
30 Days	73.00 ± 0.58	76.00 ± 3.51	68.67 ± 8.95	64.33 ± 2.33	70.67 ± 0.67	NS
45 Days	74.00 ± 0.00^{b}	89.33 ± 4.84^{c}	68.67 ± 7.36^{b}	42.33 ± 0.89^{a}	45.67 ± 2.33^{a}	**

Note: Within a row, figures with the same or without superscripts do not differ significantly as per DMRT. The data was calculated at a 99 % level of significance ($P < 0.01$).
Figures indicate the Mean ± SE (standard error); NS means not significant.
** = Significant at $P < 0.01$ level of probability
*= Significant at $P < 0.05$ level of probability.

Serum creatinine

Serum creatinine with the highest values was found in the T_3 group at 45 days, and the lowest values were observed in the control groups. The differences were found to be significant ($P < 0.05$) on day 15. The differences between the mean values from the 30 and 45-day groups were found to be significant ($P < 0.01$). On day 30, the lowest mean values were observed in the control group. The highest mean values were observed in the T_3 group rats, and the differences were statistically significant ($P < 0.01$). On day 45, the lowest mean values were observed in the T_2 group, and the highest mean values were observed in the T_3 group rats. These differences were statistically significant ($P < 0.01$). The differences between groups T_2 and T_3 were statistically significant ($P < 0.01$). However, the contents increased in the T_1, T_3, and T_4 groups, but decreased in the T_2 group on day 30, compared to day 45. On day 45, the values of serum creatinine were the highest in the T_3 group rats and lowest in the T_2 group. The differences were observed to be significant ($P < 0.01$) on day 45 (Table 9). There was a significant difference in serum creatinine level observed between the control group and all other treatment group rats throughout the whole study period, which depicts dissimilar results with previous studies (Nurun Nabi et al., 2005) involving human beings. The study showed that the patients with arsenicosis had significantly lower levels of serum creatinine compared to the control and it was also observed that there was a relationship between arsenic level and the degree of chronic renal insufficiency in men (Zheng et al., 2015). There were no significant rises in the serum creatinine levels of arsenic-treated mice (Mitchell et al., 2000; Islam et al., 2009).

Table 9
Effects of Thankuni and grapes on Serum creatinine values of arsenic-fed rats

Treatment	T_0	T_1	T_2	T_3	T_4	P. value
15 Days	0.51 ± 0.005^{a}	0.62 ± 0.003^{c}	0.52 ± 0.005^{a}	0.59 ± 0.042^{c}	0.53 ± 0.003^{a}	*
30 Days	0.52 ± 0.003^{a}	0.65 ± 0.005^{c}	0.54 ± 0.008^{b}	0.64 ± 0.005^{c}	0.52 ± 0.006^{a}	**
45 days	0.52 ± 0.006^{a}	0.67 ± 0.003^{c}	0.51 ± 0.003^{a}	0.69 ± 0.012^{c}	0.57 ± 0.023^{b}	**

Note: within a row, figures with the same or without superscripts do not differ significantly as per DMRT. The data was calculated at a 99 % significance level ($P < 0.01$)

4. Conclusions

A study of arsenic toxicity and long-term treatment with Thankuni and grapes in animals could be able to establish unequivocal decisions about the impacts of arsenic, Thankuni, and grapes on biochemical parameters, as well as provide strong evidence of the efficacy of Thankuni treatment for arsenic toxicities. Treatment with Thankuni and grapes might increase body weight. Thankuni and grapes alone can reduce the effects of arsenic toxicity, but if both are used in combination, it may be more effective. Arsenic toxicity has adverse effects on hematological and biochemical parameters in rats. Thankuni and grapes have a protective effect in improving these parameters. Combined Thankuni and grapes treatment is more effective for arsenic toxicity. This study suggested that Thankuni and grapes have significantly reduced the arsenic concentration of inorganic arsenic toxicity in rats. Further investigation in this line may make more clear evidence to use Thankuni as a treatment for arsenic toxicity.

Conflict of interest

The authors declare that there is no conflict of interest.

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