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Effect of *T. indica* and *T. arjuna* on Hematological Parameters in Rats Exposed to Fluoride

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ABSTRACT

Excessive fluoride intake is found toxic to the body. It is a highly soluble salt, ubiquitously present in water and its contamination in drinking water poses a public health problem, needs immediate attention. Since oxidative damage is the main cause of fluoride toxicity, the antioxidant potential of tamarind and arjuna having many anti-oxidant phyto-chemicals, may be exploited for the amelioration of its toxicity. This study aims to evaluate the renoprotective effect of *Tamarindus indica* and *Terminalia arjuna* on hematology in rats exposed to sodium fluoride. The experimental groups included: (I) control, (II) NaF (100ppm in drinking water for 56 days), (III) NaF@ 100 ppm and extract of *T. indica* @ 200 mg/kg b.w. orally for 56 days, (IV) NaF @ 100 ppm and *T. arjuna* extract @50 mg/kg b.w. orally, (V) NaF @ 100 ppm, extract of *Tamarindus indica* @ 100 mg/kg b.w. orally and *T. arjuna* @ 25 mg/kg b.w. orally for 56 days, (VI) Ascorbic acid @ 100 mg/kg b.w. orally and NaF@ 100 ppm for 56 days. Blood samples were collected at different intervals. Haemoglobin, total leucocyte count, differential leucocyte count and total erythrocyte count were estimated. Compared to the control group, the fluoride-treated group showed significant differences in hematological parameter, including decrease in lymphocyte percentage. The group that received ascorbic acid showed significant increase in the lymphocyte percentage of the fluoride-exposed rats when compared to group II. The rats treated with *T. indica* and *T. arjuna*, separately and in combination, showed a numerical increase in lymphocyte percentage when compared to group II.

HIGHLIGHTS

- We studied the impact of fluoride induced nephrotoxicity in albino rats.
- We investigated the effects of *Tamarindus indica* and *Terminalia arjuna*, individually and in combination, on fluoride-induced nephrotoxicity in albino rats.

Keywords: *Tamarindus indica, Terminalia arjuna,* sodium fluoride, rats, blood

Fluoride contamination in drinking water is a significant public health issue in many regions across the globe. Over 25 countries are affected by fluorosis and other fluoride-related health problems. India is one of the countries most impacted by fluoride contamination, ranking first

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among the top 20 nations with populations at risk from groundwater fluoride concentrations exceeding 1.5 mg/L. Approximately 49 million people are potentially affected by fluoride exposure in India (Podgorski and Berg, 2022).

In India, 230 districts across 20 states face risks associated with high fluoride levels in drinking water. The states most severely affected include Andhra Pradesh, Gujarat, and Rajasthan, while states such as Punjab, Haryana, Madhya Pradesh, Maharashtra, Tamil Nadu, West Bengal, Uttar Pradesh, Bihar, and Assam experience moderate fluoride contamination. According to the Bureau of Indian Standards, the permissible limit for fluoride in drinking water is set at 1 ppm (Srivastava and Flora, 2020). Long-term exposure to fluoride through contaminated groundwater causes damage not only to skeletal tissue and teeth but also to other tissues like kidneys. Studies indicate that long-term fluoride accumulation in different organs causes oxidative stress through inhibition of different enzymatic systems and increased generation of free radicals. Fluoride induced oxidative stress plays an important role in the progression of a variety of renal disorders such as impairment of urine concentrating ability, diabetic nephropathy, chronic kidney disease and kidney failure. The World Health Organization is acutely aware that fluoride is a kidney toxin, recently citing the finding that approximately 100,000 individuals in the Assam region in India have been taken ill with kidney failure, stiff joints and anaemia as a result of high natural levels of fluoride in the water (WHO, 2015). Similar results have been found in other parts of India (Pain, 2017).

It is well-documented that chronic fluoride exposure decreases milk production in animals. In general, those domesticated buffalo animals who are suffering from skeletal fluorosis are physically weak and lame. In addition, chronic fluoride exposure impairs reproductive function in animals, which ultimately affects animal productivity. To know the current status of fluorosis, the estimation of fluoride in blood serum and urine is the most authentic way (Choubisa, 2022).

It is now well-established that the toxic effects of fluoride can be ameliorated by antioxidants. Although further clinical studies are warranted, phytochemicals present in plants appear to be beneficial for attenuating disease-associated symptoms via their free radical scavenging ability thus preventing oxidative stress-associated chronic diseases.

Tamarind [*Tamarindus indica L. (T. indica*)] is one of the fruit tree species that is used for traditional medicine. It is reported to possess antioxidant, antidiabetic, cardioprotective, antihyperlipidemic, antimicrobial, hepatoprotective, and laxative activity because of its rich source of essential amino acids and phytochemicals. Phytochemicals found in *T. indica* include phytosterols, saponins, polyphenols, flavonoids, fiber and ascorbic acid (Vasant and Narasimhacharya, 2012). It can also be preferred as nutritional support for malnourished patients as it is affordable and easily accessible (Kuru, 2014).

Terminalia arjuna (T. arjuna) commonly known as "Arjuna" is a medicinal plant rich in tannins and triterpenes and extensively used in Ayurvedic medicine as a cardiac tonic (Scassellati-Sforzolini *et al.,* 1999). *T. arjuna* is known to possess antioxidant, antiapoptotic, hypotensive, antiatherogenic and anti-inflammatory effects among others (Ramya *et al.,* 2017). The bark of *T. arjuna* has a very high level of flavonoids and variety of tannins thus considered to have the most important medicinal value (Amalraj and Gopi, 2017).

Literature suggests tamarind can be useful in fluoride toxicity by enhancing urinary excretion of fluoride. The mechanism by which tamarind is helpful in urinary excretion of fluoride is not well established. Since, oxidative damage is the main cause behind the toxicity of fluoride, antioxidant properties of tamarind and arjuna may be helpful in amelioration of sodium fluoride induced nephrotoxicity in rats. In this study, ameliorative efficacy of *Tamarindus indica* and *Terminalia arjuna* was evaluated in sodium fluoride induced toxicity in rats. Therefore, an attempt was made to investigate the nephrotoxicity caused by sub-chronic fluoride exposure and to assess the protective effects of *T. indica* and *T. arjuna.*

MATERIALS AND METHODS

The research was conducted in the Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Jabalpur, Madhya Pradesh.

Experimental animals

The study was conducted on apparently healthy male Wistar albino rats weighing around 140-160 g. They

were kept in polypropylene cages and maintained on a pelleted rat feed procured from Nutrivet Life-Sciences, Pune. The water was provided *ad libitum* throughout the study. Rats were maintained under standard management conditions. Before the start of the experiment, animals were kept in laboratory conditions for a period of 7 days or more for acclimatization. The experiments were carried out in accordance with the guidelines of the Institutional Animal Ethics Committee (42/IAEC/Vety./2023), College of Veterinary Science and Animal Husbandry, Jabalpur, Madhya Pradesh. All the experimental animals were kept under constant observation during the entire period of the study.

Dose selection

The 100 ppm NaF in drinking water have been used by Chourasia (2022) and Li *et al.* (2021). The dose selection for *T. indica* (200 mg/kg b.w. orally) was based on the previous reports of Khandare *et al.* (2018) and Gupta *et al*. (2013), which showed that this dose to be protective against fluoride-induced toxicity in rats. *T. arjuna* was given at the dose rate of 50 mg/kg b.w. orally based on the study conducted by Sinha *et al.* (2008). This dose prevented mouse heart from fluoride-induced cardiotoxicity. The dose of ascorbic acid (100 mg/kg b.w.) was selected based on available literature (Sinha *et al.,* 2008). Half of the dose of *T. indica* (100 mg/kg b.w. orally) and *T. arjuna* (25 mg/kg b.w. orally) was selected for amelioration in fluoride exposed rats.

Experimental design

All the animals were weighed and randomly divided into six groups having eight rats in each. The experimental groups included: (I) control, (II) NaF (100 ppm in drinking water for 56 days), (III) NaF (100 ppm) and extract of *T. indica* at the dose rate of 200 mg/kg b.w. orally for 56 days, (IV) NaF (100 ppm) and *T. arjuna* extract at the dose rate of 50 mg/kg b.w. orally, (V) NaF (100 ppm), extract of *T. indica* at the dose rate of 100 mg/kg b.w. and *T. arjuna* at the dose rate of 25 mg/kg b.w. orally for 56 days and (VI) NaF (100 ppm) and Ascorbic acid at the dose rate of 100 mg/kg b.w. orally for 56 days. The experimental protocol is presented below:

For induction of fluoride toxicity, sodium fluoride was given to rats through drinking water daily for 56 days. *T. indica* and *T. arjuna* extracts were given by oral gavage once daily. Blood samples were collected at different time intervals.

Chemicals – Sodium fluoride pure, 98% and Ascorbic acid were used in the present experiment.

Plant material

Fruits of *Tamarindus indica* and bark of *Terminalia arjuna* were collected from the Department of Plant Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya (J.N.K.V.V.), Jabalpur (M.P.).

Preparation of *Tamarindus indica* fruit pulp extract

Tamarind fruits were washed with distilled water to remove the dirt and air dried. The fruit kernels and seeds were removed and fruit pulp was separated. The pulp was dried in shed and stored in an airtight glass container. One hundred grams of pulp was mixed with 1.5 L of methanol: water (1:1) and kept at 25ºC overnight. Thereafter, it was stirred with a magnetic stirrer for one hour and filtered. The

Table 1: Experimental design of study

Group	No. of rats	Treatment (56 days)	
I	8	R.O. water (less than 0.5 ppm fluoride)	—
II	8	Sodium fluoride @ 100 ppm in drinking water	—
III	8	Sodium fluoride @ 100 ppm in drinking water	*Tamarindus indica* @ 200 mg/kg b.w. daily
IV	8	Sodium fluoride @ 100 ppm in drinking water	*Terminalia arjuna* @ 50 mg/kg b.w. daily
V	8	Sodium fluoride @ 100 ppm in drinking water	*Tamarindus indica* @ 100 mg/kg + *Terminalia arjuna* @ 25 mg/kg b.w. daily
VI	8	Sodium fluoride @ 100 ppm in drinking water	Ascorbic acid @ 100 mg/kg b.w. daily

extracted solution was subjected to solvent evaporation in a water bath. A pasty material was obtained and stored at 4ºC until use (Gupta *et al*., 2013).

Preparation of *Terminalia arjuna* bark extract

The dried bark of *T. arjuna* was powdered and extracted with ethanol: water (70:30 v/v) using cold maceration method in a conical flask. The extract was manually shaken every hour for initial six hours. Afterwards, it was kept in a shaker at 200 rpm. The extract was filtered and concentrated in a water bath at 30ºC. The extract was stored in an air tight container at -20ºC until further analysis (Cota *et al.*, 2020).

Collection of samples

Blood: Blood was collected on 0, 29th and 56th day of the study. All the rats were fasted overnight (12-14 hours) before the collection. Blood was collected in clot activator and K_3 EDTA coated vials from retro-orbital plexus and stored at -20ºC as described by Sorg and Buckner, (1964) for estimations of hematological markers.

Hematological analysis: Haemoglobin concentration (Hb), total leucocyte count (TLC), differential leucocyte count (DLC) and total erythrocyte count (TEC) were estimated using Auto haematology analyzer.

Statistical analysis

The data was analyzed using the software R studio, Package-dplyr. Data were analyzed by one way ANOVA and means were compared with Tukey's post-hoc test. Data were expressed as mean ± SE. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

In this study, the effect of *Tamarindus indica, Terminalia arjuna* alone and their combination on fluoride induced toxicity was seen in Wistar rats for 56 days.

In the present study, hematological examination of blood samples included estimating various erythrocytic and leukocytic parameters viz., hemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) on 0, 29th and 56th day.

In comparison to the control group (I), the hemoglobin levels in rats exposed to fluoride (group II) showed no significant variation. The results (mean ± SE) are presented in table 2.

Arpita and Bidyut, (2012) reported that fluoride causes changes. However, in the present study, no statistical differences were observed. Similar findings were reported by Priya *et al*. (1997) when rats were treated with 20 ppm of fluoride in the drinking water (31 days). Atmaca *et al*. (2014) also did not find any significant variation in hemoglobin when rats were exposed to 100mg/L fluoride in drinking water for 21 days.

The mean total leukocyte count (mean ± SE) values for the control and various treatment groups are presented in table 3. The values of total leukocyte count did not show any significant difference between the control (group I)

Table 2: Effect of *T. indica, T. arjuna* and Ascorbic acid administration on Hemoglobin (g/dL) concentration in NaF intoxicated rats

Group	Treatment	Day 0	Day 29	Day 56
I	R.O. water (less than 0.5 ppm fluoride)	$14.41^{Ba}\pm0.72$	$14.48^{Bab}\pm0.30$	$16.71^{Aa}\pm0.32$
II	Sodium fluoride @ 100 ppm in drinking water	$13.12^{Aa}\pm0.51$	$13.58^{Ab}\pm0.35$	$14.65^{Aa}\pm1.23$
III	Sodium fluoride @ 100 ppm in drinking water + *Tamarindus indica* @ 200 mg/kg b.w. daily	$13.41^{Ba}\pm0.46$	$14.27^{Bab}\pm0.51$	$17.55^{Aa}\pm0.16$
IV	Sodium fluoride @ 100 ppm in drinking water + *Terminalia arjuna* @ 50 mg/kg b.w. daily	$14.93^{Ba}\pm0.24$	$15.66^{ABa}\pm0.23$	$16.56^{Aa}\pm0.31$
V	Sodium fluoride @ 100 ppm in drinking water + *Tamarindus indica* @ 100 mg/kg + *Terminalia arjuna* @ 25 mg/kg b.w. daily	$13.96^{Aa}\pm0.36$	$13.97^{Ab}\pm0.34$	$15.20^{Aa}\pm1.67$
VI	Sodium fluoride @ 100 ppm in drinking water + Ascorbic acid @ 100 mg/kg b.w. daily	$14.56^{Ba}\pm0.55$	$14.86^{Bab}\pm0.38$	$17.31^{Aa}\pm0.23$

Means bearing different superscripts between rows (lower case) and columns (upper case) differ significantly ($p<0.05$).

Table 3: Effect of *T. indica, T. arjuna* and Ascorbic acid administration on Total leukocyte count ($x10^6$ /mm^3) in NaF intoxicated rats

Group	Treatment	Day 0	Day 29	Day 56
I	R.O. water (less than 0.5 ppm fluoride)	6.81^{Ba}±0.30	8.50^{Aa}±0.19	7.20^{ABab}±0.59
II	Sodium fluoride @ 100 ppm in drinking water	6.73^{Ba}±0.24	8.90^{ABa}±0.64	9.81^{Aa}±1.83
III	Sodium fluoride @ 100 ppm in drinking water + *Tamarindus indica* @ 200 mg/kg b.w. daily	7.02^{Ba}±0.28	8.92^{Aa}±0.34	7.61^{Bab}±0.28
IV	Sodium fluoride @ 100 ppm in drinking water + *Terminalia arjuna* @ 50 mg/kg b.w. daily	7.11^{Ba}±0.32	8.89^{Aa}±0.35	9.27^{Aab}±0.40
V	Sodium fluoride @ 100 ppm in drinking water + *Tamarindus indica* @ 100 mg/kg + *Terminalia arjuna* @ 25 mg/kg b.w. daily	7.25^{ABa}±0.60	8.59^{Aa}±0.20	6.09^{Bb}±0.30
VI	Sodium fluoride @ 100 ppm in drinking water + Ascorbic acid @ 100 mg/kg b.w. daily	6.85^{Aa}±0.21	6.73^{Ab}±0.51	5.98^{Ab}±0.45

Means bearing different superscripts between rows (lower case) and columns (upper case) differ significantly ($p<0.05$).

Table 4: Effect of *T. indica, T. arjuna* and Ascorbic acid administration on Total erythrocyte count ($x10^6$/µl) in NaF intoxicated rats

Group	Treatment	Day 0	Day 29	Day 56
I	R.O. water (less than 0.5 ppm fluoride)	6.10^{Ba}±0.58	5.99^{Bab}±0.38	9.15^{Aa}±0.17
II	Sodium fluoride @ 100 ppm in drinking water	5.02^{Ba}±0.41	4.67^{Bb}±0.28	7.91^{Aa}±0.59
III	Sodium fluoride @ 100 ppm in drinking water + *Tamarindus indica* @ 200 mg/kg b.w. daily	5.26^{Ba}±0.22	5.31^{Bab}±0.49	9.39^{Aa}±0.09
IV	Sodium fluoride @ 100 ppm in drinking water + *Terminalia arjuna* @ 50 mg/kg b.w. daily	5.38^{Ba}±0.33	6.35^{Ba}±0.42	9.03^{Aa}±0.22
V	Sodium fluoride @ 100 ppm in drinking water + *Tamarindus indica* @ 100 mg/kg + *Terminalia arjuna* @ 25 mg/kg b.w. daily	5.17^{Ba}±0.21	5.18^{Bab}±0.37	8.35^{Aa}±0.80
VI	Sodium fluoride @ 100 ppm in drinking water + Ascorbic acid @ 100 mg/kg b.w. daily	6.16^{Ba}±0.33	5.54^{Bab}±0.33	9.66^{Aa}±0.12

Means bearing different superscripts between rows (lower case) and columns (upper case) differ significantly ($p<0.05$).

and different treatment groups. Priya *et al*. (1997) also reported no significant difference in total leukocyte count in rats treated with 20 ppm of fluoride.

The mean total erythrocyte count (mean ± SE) values for the control and various treatment groups are presented in Table 4. Compared to control (group I), total erythrocyte count did not vary significantly in rats exposed to fluoride (group II). Similarly, previous studies have shown that fluoride at 100 ppm did not cause any significant alteration in total erythrocyte count of rats (Chourasia, 2022).

The mean lymphocyte percentage (mean ± SE) for the control and various treatment groups are presented in Table 5. On the 29th day, lymphocyte percentage was significantly decreased in rats exposed to fluoride (group II) (56.00±2.11 %) when compared control (group I) (66.88±1.00 %). Co-administration of ascorbic acid (group VI) (64.88±1.00 %) significantly increased the lymphocyte percentage of the fluoride-exposed rats on day 29th when compared to group II. The rats treated with *T. indica* and *T. arjuna* (groups III and IV) (63.15±2.18 %, 62.97±2.70 %), separately and in combination (group V) (61.25±1.63 %), showed a numerical increase in lymphocyte percentage that was consistent with groups I.

The administration of NaF orally for 28 days to Wistar rats caused dose dependent reduction in the percent of lymphocytes (Giri *et al.,* 2015). The findings related to hematological alterations may be attributed to stress induced by sodium fluoride (NaF), inhibition of Na+/K+ ATPase activity within cells and reduction in erythropoiesis, which may result from elevated fluoride levels in both serum and bone over an extended duration. Additionally, nutritional imbalances and a decrease in

Table 5: Effect of *T. indica, T. arjuna* and Ascorbic acid administration on Lymphocyte percentage (%) in NaF intoxicated rats

Group	Treatment	Day 0	Day 29	Day 56
I	R.O. water (less than 0.5 ppm fluoride)	52.48^{Ca}±0.84	66.88^{Ba}±1.00	76.90^{Aa}±2.95
II	Sodium fluoride @ 100 ppm in drinking water	52.76^{Ba}±0.72	56.00^{Bb}±2.11	69.45^{Aa}±4.28
III	Sodium fluoride @ 100 ppm in drinking water + *Tamarindus indica* @ 200 mg/kg b.w. daily	53.53^{Ca}±0.67	63.15^{Bab}±2.18	71.70^{Aa}±1.63
IV	Sodium fluoride @ 100 ppm in drinking water + *Terminalia arjuna* @ 50 mg/kg b.w. daily	52.91^{Ba}±0.46	62.97^{ABab}±2.70	73.18^{Aa}±5.26
V	Sodium fluoride @ 100 ppm in drinking water + *Tamarindus indica* @ 100 mg/kg + *Terminalia arjuna* @ 25 mg/kg b.w. daily	52.51^{Ca}±0.61	61.25^{Bab}±1.63	70.13^{Aa}±3.41
VI	Sodium fluoride @ 100 ppm in drinking water + Ascorbic acid @ 100 mg/kg b.w. daily	53.63^{Ca}±0.73	64.88^{Ba}±1.00	73.83^{Aa}±2.08

Means bearing different superscripts between rows (lower case) and columns (upper case) differ significantly ($p<0.05$).

Table 6: Effect of *T. indica, T. arjuna* and Ascorbic acid administration on Monocyte percentage (%) in NaF intoxicated rats

Group	Treatment	Day 0	Day 29	Day 56
I	R.O. water (less than 0.5 ppm fluoride)	2.21±0.08	2.01±0.01	1.43±0.38
II	Sodium fluoride @ 100 ppm in drinking water	1.60±0.21	1.90±0.19	1.86±0.20
III	Sodium fluoride @ 100 ppm in drinking water + *Tamarindus indica* @ 200 mg/kg b.w. daily	1.56±0.17	2.03±0.18	2.13±0.18
IV	Sodium fluoride @ 100ppm in drinking water + *Terminalia arjuna* @ 50 mg/kg b.w. daily	1.75±0.23	1.83±0.25	1.80±0.35
V	Sodium fluoride @ 100 ppm in drinking water + *Tamarindus indica* @ 100 mg/kg + *Terminalia arjuna* @ 25 mg/kg b.w. daily	1.64±0.17	2.06±0.24	1.50±0.20
VI	Sodium fluoride @ 100 ppm in drinking water + Ascorbic acid @ 100 mg/kg b.w. daily	1.83±0.25	1.81±0.21	1.76±0.13

the size of the medullary canal due to osteosclerosis may also contribute to these alterations (Santhakumari and Subramanian, 2007). Another possible reason could be the harmful effects on essential organs, especially the spleen, liver, kidneys, and bone, which are closely linked to hematopoiesis (Sharma *et al*., 2010; Rao and Vidyunmala, 2010). *In vitro* studies demonstrate that T cell development requires ascorbic acid, while ascorbic acid also enhances T cell proliferation and may influence T cell function. Ascorbic acid also enhances the proliferation of NK cells, a group of cytotoxic innate lymphocytes. Evidence indicates ascorbic acid positively influences lymphocyte development and function (Van Gorkom *et al*., 2018).

The mean monocyte percentage (mean ± SE) for the control and various treatment groups are presented in Table 6. The monocyte percentage values showed no significant difference between the control group and various treatment groups. These results are in good accordance with that obtained by Arpita and Bidyut, (2012) who gave fluoride to rats at 5, 10, 15, and 20 ppm for 60 days and did not observe any significant change in monocyte percentage. F (20 ppm in their drinking water) exposure to rats for 28 days failed to cause changes in monocyte percentage (Khan, *et al.*, 2013).

The mean neutrophil percentage (mean ± SE) for the control and various treatment groups are presented in Table 7. In comparison to the control group (I), the percentage of neutrophils did not vary significantly in rats exposed to fluoride (group II). Similarly, a study conducted by Khan *et al.* (2013) showed non-significant alterations in neutrophil percentage, in the F group (20 ppm for 28 days), when compared with the control group (I).

CONCLUSION

Table 7: Effect of *T. indica, T. arjuna* and Ascorbic acid administration on Neutrophil percentage (%) in NaF intoxicated rats

Group	Treatment	Day 0	Day 29	Day 56
I	R.O. water (less than 0.5 ppm fluoride)	30.95^{Aa}±1.37	31.00^{Aab}±1.72	19.53^{Ba}±3.24
II	Sodium fluoride @ 100 ppm in drinking water	32.66^{Aa}±0.85	35.83^{Aa}±1.53	26.53^{Aa}±4.73
III	Sodium fluoride @ 100 ppm in drinking water + *Tamarindus indica* @ 200 mg/kg b.w. daily	30.45^{Aa}±0.35	29.96^{Aab}±2.07	22.35^{Ba}±1.45
IV	Sodium fluoride @ 100 ppm in drinking water + *Terminalia arjuna* @ 50 mg/kg b.w. daily	33.09^{Aa}±0.64	30.60^{Aab}±0.87	24.41^{Ba}±1.28
V	Sodium fluoride @ 100 ppm in drinking water + *Tamarindus indica* @ 100 mg/kg + *Terminalia arjuna* @ 25 mg/kg b.w. daily	31.44^{Aa}±0.23	28.51^{Ab}±0.24	22.70^{Aa}±5.02
VI	Sodium fluoride @ 100 ppm in drinking water + Ascorbic acid @ 100 mg/kg b.w. daily	33.68^{Aa}±0.94	30.89^{Aab}±1.22	21.11^{Ba}±1.59

Means bearing different superscripts between rows (lower case) and columns (upper case) differ significantly ($p<0.05$).

The fluoride-treated group II showed a significant reduction in lymphocyte percentage compared to the control group. In contrast, the group receiving ascorbic acid exhibited a notable increase in lymphocyte percentage among the fluoride-exposed rats (group II). Additionally, treatments with *T. indica* and *T. arjuna*, both separately and in combination, resulted in measurable increases in lymphocyte percentages compared to the fluoride-treated group II.

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