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EFFECT OF SODIUM SULFATE ON HAEMATOLOGICAL PARAMETERS IN *MUS MUSCULUS* AND ITS AMELIORATION BY *AZADIRACHTA INDICA* EXTRACT

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ABSTRACT

Sulfur compounds are wildly distributed in nature in the form of Sulfates and Sulfites as prominent compound, inter the natural environment through the Sulfure cycle and significantly influencing the human health and hygiene. The present investigation was conducted to evaluate the effect of Sodium sulfate solution in Albino mice and its amelioration by leaf extract of *Azadirachta indica*. For this investigation 24 Albino mice (*Mus musculus*) were used. They were divided equally into three groups: A control group and two experimental groups. The control group received a normal diet along with distilled water. First experimental group (8 mice) was orally feed with 1% Sodium sulfate solution (0.5 ml/mice/day) for 28 days, while the second experimental group received both Sodium sulfate (1%) and freshly prepared (2%) aqueous extract of *A. Indica* (0.5 ml/mice/day) for the same period i.e. 28 days and was maintained on a normal diet with distilled water, similar to control group. Certain haematological parameters including RBC, WBC and Haemoglobin concentration in all the three groups (control and experimental) were estimated after the treatment of 28 days. The above mention parameters were found significantly decreased in case of mice of second group treated with Sodium sulfate solution only. Whereas in the third group of mice treated with both Sodium sulfate as well as the aqueous leaf extract of *A. indica* showed an increasing trend. The results clearly revealed that the aqueous leaf extract of *A. indica* could treated mice.

KEYWORD: Sodium sulfate, *Azadirachta indica*, Amelioration, Albino mice, Haematological parameters.

INTRODUCTION

Humans are continuously and inevitably exposed to environmental chemical pollutants, and in the context of modern times, avoiding such exposure appears to be challenging. Sulfur compounds are widely distributed in nature, with

sulfites and sulfates being prominent components. These compounds often enter the natural environment through the sulfur cycle, which is significantly influenced by human activities (Wu & Farquhar, 2013). As a result, sulfate concentrations in seawater and ocean water have risen to approximately 2700 mg/L (Risueño et al.,1968). Human activities continually contribute to the accumulation of sulfur compound residues, with major contributors including the steel industry, metallurgy, mining, pulp and paper production, acid gas processing industries, and the burning of fossil fuels (Doroftei & Antofie, 2013). Given the critical role of sulfates in maintaining human health, monitoring their levels in polluted soils and water is essential (Wang et al., 2013).

Due to the undesirable side effects associated with synthetic anticarcinogenic agents, there has been growing interest in recent years in exploring and utilizing antioxidant agents of natural origin, particularly plant extracts. *Azadirachta indica* (Neem), a large evergreen tree belonging to the Meliaceae family, is believed to have originated in Assam and Burma, South Asia **(**National Research Council, Board on Science & Technology for International Development, 1992). It is considered one of the most versatile medicinal plants, traditionally used as a remedy for various ailments **(**Bandyopadhyay et al., 2002). Neem has been suggested by traditional medicine practitioners to be effective in treating conditions such as leprosy, malaria, typhoid fever, respiratory disorders, constipation, cancer, and chronic syphilitic sores (Choudhary & Sharma, 2023).

Scientific research has also validated several pharmacological properties of neem leaf extract. For example, studies have demonstrated its hypolipidemic, antioxidant, and anti-diabetic effects in diabetic rat models (Halim, 2003; Gupta et al., 2004; Ekaidem, 2007). Additionally, neem has been shown to possess immunostimulatory (Sen P et al., 1992; Baral & Chattopadhyay, 2004), anti-inflammatory (Chattopadhyay et al., 1993; Kaur, 2004) and hepatoprotective properties (John et al., 2011) in various experimental settings. Various parts of the plant, particularly the leaves, have demonstrated significant cancer chemo preventive activity against different types of malignancies in several animal models. Additionally, neem leaf extract has been reported to influence both cell-mediated and humoral immune responses (Sen et al., 1992; Ray et al., 1996; Haque et al., 2006), also contain antifertility activity (Choudhary et al., 1990 & 1991; Choudhary & Sharma, 2023).

Research by Haque et al., 2006 revealed that neem leaf extract stimulates the haematological system, as evidenced by increases in the total counts of RBCs, WBCs, platelets, and haemoglobin levels. Furthermore, the observed immunostimulatory effects were closely associated with the restricted growth of murine carcinoma. Therefore, the present work was under taken to study the haematological effect of *A. indica* on Sodium sulfate induced *Mus musculus*.

MATERIALS AND METHODS

Animal

Swiss albino mice, aged 4–6 weeks and averaging 28-30 grams in body weight, were used as test animals. The mice were housed in groups and maintained under standard laboratory conditions with appropriate nutrition throughout the experimental period.

Preparation of Sodium sulfate solution

1% sodium sulfate solution was prepared for the experiment.

Preparation of *A. indica* leaves extract

Fresh *A. indica* leaves were collected and allowed to air dry for two weeks in a shaded, dark room. Once completely dried, the leaves were finely ground into a powder. The powder was then sifted using a thin cloth to remove any unground portions. The resulting fine powder was stored in a jar.

To prepare the aqueous extract, the powdered *A. indica* leaves were mixed with distilled water as needed. 2% solution of *A. Indica* was prepared for the experiment (Choudhary & Sharma, 2023; Sharma & Choudhary, 2024).

Study Design

A study was conducted to evaluate the effects of *A. indica* extract on Sodium sulfate induced mice. A total of 24 albino mice were divided into three groups: a control group (8 mice) and two experimental groups (8 mice each). The control group was fed a normal diet with distilled water.

The first experimental group received Sodium sulfate solution (0.5 ml/mice/day)for 28 days, while the second experimental group received both Sodium sulfate and freshly prepared aqueous *A. indica* extract (0.5 ml each separately at the same interaval of half an hour daily) for the same period i.e. 28 days and was maintained on a normal diet with distilled water, similar to the control group.

Haematological Assay

Collection of Blood Samples: Blood samples were obtained from the tail vein of *Mus musculus* following the completion of the dosage regimen.(Samiran et al.,2023).

Estimation of Red Blood Cell (RBC) Count: RBC counts were determined using the standard procedure with a Neubauer hemocytometer (Dacie & Lewis, 1975; Hrubee et al., 1996; Mishra et al., 1977; Masand & Singh, 2013; Sharma and Choudhary, 2024).

Estimation of White Blood Cell (WBC) Count: WBC counts were determined using the standard procedure with a Neubauer hemacytometer (Dacie & Lewis, 1975; Mgbenka et al., 2003; Masud and Singh, 2013).

Estimation of Haemoglobin (Hb): Haemoglobin levels were measured using Sahli's method (Dacie & Lewis, 1975; Sharma and Choudhary, 2024).

RESULT AND DISCUSSION

RBC COUNT

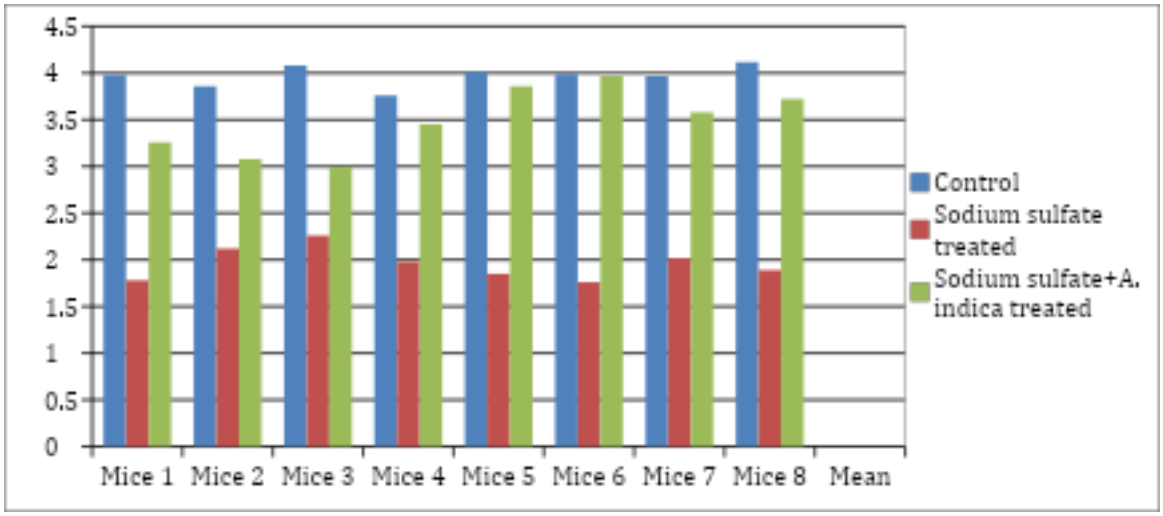
This study revealed a statistically significant difference in the total RBC count among control mice, Sodium sulfate-treated mice, and those in the amelioration group, supplemented with *A. indica* extract.

Sodium sulfate-treated mice exhibited a significantly lower mean RBC count (1.95 ± 0.06) compared to the control group (3.97 ± 0.04), with a t-value of 27.4292 and a p-value <0.0001 (Table 1). The decrease in RBC count may be attributed to increased ROS production and nonenzymatic glycosylation of haemoglobin and RBC membrane proteins, which compromise deformability, promote aggregation, and accelerate RBC aging (Asmah et al., 2015; Cho et al., 2008; Hamed, 2016). In the amelioration group, the mean RBC count significantly increased to 3.48 ± 0.12 (t-value = 3.6220, p-value = 0.0028), indicating that *A. indica* extract effectively mitigated the adverse effects of sodium sulfate

on RBC count. According to Biney et al. (2020), *A. indica* contains phenol, a key phytochemical that plays a crucial role in neutralizing free radicals in the body. This compound is attributed to the enhancement of antioxidant enzyme activity in experimental animals (Ezeigwe et al., 2020).

Table 1: Total No of RBC Count in Different Groups of Mice (In Million/mm3).

S. No.	Control	Sodium sulphate treated	Sodium sulfate+*A. indica* treated
Mice 1	3.98	1.78	3.26
Mice 2	3.86	2.12	3.08
Mice 3	4.08	2.26	2.99
Mice 4	3.76	1.98	3.45
Mice 5	4.01	1.85	3.86
Mice 6	3.99	1.76	3.97
Mice 7	3.97	2.02	3.58
Mice 8	4.12	1.89	3.72
Mean	3.97 ±0.04	1.95±0.06	3.48±0.12



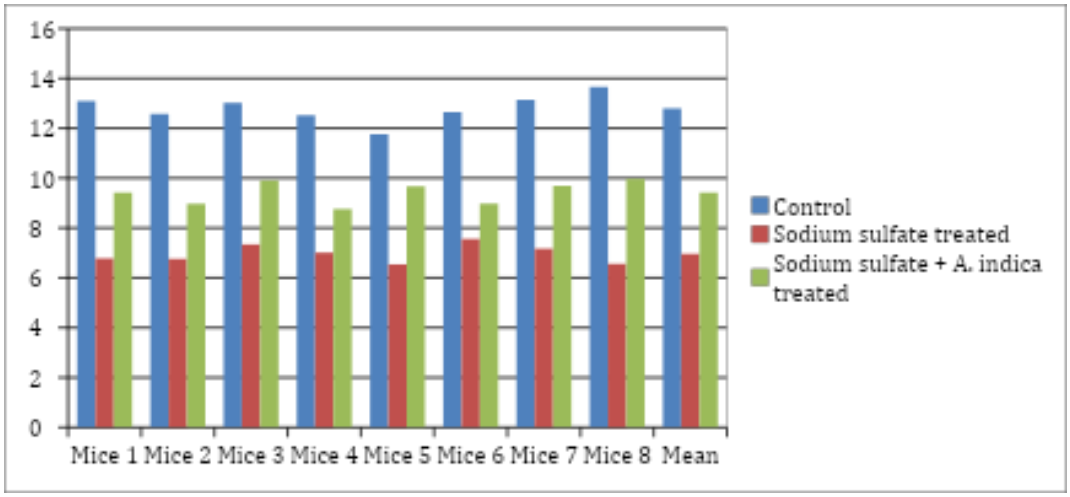
Graph 1: Total No of RBC Count in Different Groups of Mice (In Million/mm3).

WBC COUNT

In Table 1, it was observed that the WBC count significantly decreased in sodium sulfate-treated mice (6.96 ± 0.13) compared to the control group (12.8 ± 0.19), with a t-value of 24.4516 and a p-value of < 0.0001. However, the WBC count increased significantly in the group of mice treated with *A. indica* extract (9.42 ± 0.16), with a t-value of 13.0899 and a p-value of < 0.0001.

Table 2: Total No of WBC Count in Different Groups of Mice (1×10^3/mm^3).

S. No.	Control	Sodium sulfate treated	Sodium sulfate + *A. indica* treated
Mice 1	13.09	6.78	9.42
Mice 2	12.57	6.76	8.97
Mice 3	13.01	7.34	9.91
Mice 4	12.52	7.01	8.76
Mice 5	11.76	6.54	9.67
Mice 6	12.65	7.56	8.98
Mice 7	13.13	7.17	9.68
Mice 8	13.67	6.56	9.97
Mean ± S.E.	12.8 ± 0.19	6.96 ± 0.13	9.42 ± 0.16



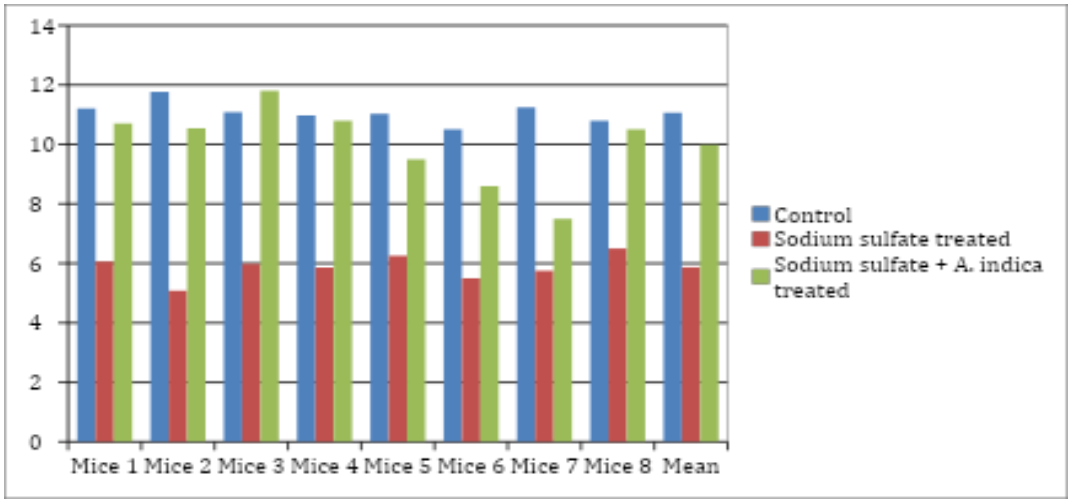
Graph 2: Total No of WBC Count in Different Groups of Mice ($1\times10^3/mm^3$).

HB%

Additionally, the current study revealed a significant reduction in haemoglobin (Hb) levels in sodium sulfate-treated mice (5.87 ± 0.15) compared to the control group (11.07 ± 0.12), with a t-value of 25.6634 and a p-value of < 0.0001. In contrast, Hb levels showed a significant improvement (9.99 ± 0.48) in mice treated with *A. indica* extract, with a t-value of 8.0310 and a p-value of < 0.0001.These findings align with Iyare & Obaji (2014), who reported that *A. indica* acts as a hematopoietic agent with the potential to improve anaemia during pregnancy.

Table 3: Total Amount of Hb Count in Different Groups of Mice (in gm%).

S. No.	Control	Sodium sulfate treated	Sodium sulfate + *A. indica* treated
Mice 1	11.21	6.06	10.7
Mice 2	11.76	5.08	10.55
Mice 3	11.08	5.98	11.8
Mice 4	10.98	5.86	10.8
Mice 5	11.02	6.25	9.5
Mice 6	10.5	5.5	8.6
Mice 7	11.25	5.75	7.5
Mice 8	10.8	6.5	10.5
Mean	11.07 ± 0.12	5.87 ± 0.15	9.99 ± 0.48



Graph 3: Total Amount of Hb Count in Different Groups of Mice (in gm%).

The RBC count, WBC count and Hb gm%, and showed improvement in Sodium sulfate-treated mice when administered with *A. indica*. The observed improvement in blood parameters can be attributed to its constituents, such as flavonoids and quercetin, which possess hematopoietic properties (Raja et al., 2011) or may be due to an alkaloid. Nimbidin present in *A. indica* may be the another region. Additionally, *A. indica* has been shown to boost macrophage activity, stimulate the lymphatic system, and enhance WBC production (Ray et al., 1996; Sen et al., 1992).

Table 4: Comparative Data of Control & Treated Group of Mice.

S. No.	Animal group	RBC COUNT (In Million/mm^3)	WBC COUNT ($1\times10^3/mm^3$)	HB% (In gram%)
1.	**Control group**	3.97±0.04	12.8±0.19	11.07±0.12
2.	**Treated with Sodium sulfate**	1.95±0.06 (p<0.0001)	0.96±0.13 (p<0.0001)	5.87±0.15 (p<0.0001)
3.	**Treated with Sodium sulfate +*A. indica***	3.48±0.12 (p<0.0028)	9.42±0.16 (p<0.0001)	9.99±0.48 (p<0.0001)

CONCLUSION

The present study clearly revealed that the standardized aqueous extract of *Azadirachta indica* leaves has the potential to mitigate the effects of Sodium sulfate by significantly enhancing RBC count, WBC count, and Hb gm% in mice. Nevertheless, further large-scale studies are required to validate the safety and efficacy of this extract. Additionally, investigations into the underlying mechanisms of action are essential to better understand its therapeutic potential. Such research could pave the way for its development as a viable natural remedy for haematological disorders.

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