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Effect of Sodium Sulfate on Certain Biochemical Parameters in *Mus Musculus* and its Amelioration by *Azadirachta Indica* Extract

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ABSTRACT

Sulphate occurs naturally in the environment as a component of the sulphur cycle, involving both atmospheric and terrestrial processes. Sulphur compounds are widely distributed in the natural environment, with sulphate and sulphite being the predominant forms. The present investigation was carried out to examine the effects of sodium sulfate solution in albino mice and to evaluate the protective role of *Azadirachta indica* leaf extract. A total of 24 albino mice (*Mus musculus*) were used and equally divided into three groups: one control group and two experimental groups. The control group was maintained on a standard diet with distilled water. The first experimental group (n = 8) was orally administered 1% sodium sulfate solution (0.5 ml per mouse per day) for 28 days. The second experimental group (n = 8) received both 1% sodium sulfate solution and a freshly prepared 2% aqueous extract of *Azadirachta indica* (0.5 ml per mouse per day) for the same duration of 28 days, while being maintained on a normal diet with distilled water similar to the control group. After 28 days of treatment, biochemical parameters such as blood glucose, serum cholesterol, and plasma protein were measured in all three groups (control and experimental). A significant reduction in these parameters was observed in the second group of mice treated only with sodium sulfate. However, mice in the third group, which received both sodium sulfate and the aqueous leaf extract of *Azadirachta indica*, showed a noticeable improvement. These results suggest that the aqueous leaf extract of *A. indica* has a beneficial effect in mitigating the adverse changes induced in mice.

KEYWORDS: *Sodium sulfate, Azadirachta indica, Amelioration, Albino mice, Biochemical parameters.*

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INTRODUCTION

Sulphate occurs naturally in the environment as a component of the sulphur cycle, involving both atmospheric and terrestrial processes. Sulphur-containing compounds are widely utilized in agricultural and industrial activities (Prasad, 2014). They are important in the production of fertilizers, fungicides, algicides used for controlling algae, and insecticides. Moreover, sulphur compounds are extensively applied in industries such as glass manufacturing, paper and wood pulp processing, soap and detergent production, pharmaceuticals, hide and skin processing, and water treatment systems (Kutney, 2023).

A significant portion of atmospheric sulphur is generated from anthropogenic activities. It is estimated that nearly one-third of the sulphur released into the atmosphere originates from human sources, and about 90% of atmospheric sulphur dioxide (SO_2) emissions are attributed to activities such as fossil fuel combustion, petroleum refining, and various industrial processes (Gonzalez and Bergovist, 1986).. In the modern era, humans are continually exposed to a wide range of environmental chemical pollutants, making it difficult to completely avoid such exposure. These compounds enter and circulate within the environment through the sulphur cycle, which is strongly influenced by anthropogenic activities (Zhou et al., 2025; Choudhary et al., 2025).

The chemicals play a very important role in controlling various crop pests in agriculture sectors. Various chemicals also serve as medicine to cure different types of human diseases and skin problems but excessive use of these chemicals in the form of pesticides and biopesticides have been proved harmful to the ecosystem and resulted in the loss of biodiversity including human health (Lemly, 2004). Sodium sulfate is widely used as a model chemical in environmental toxicology because of its high water solubility and frequent occurrence in industrial waste. Elevated sulfate levels in soil and water can alter plant and microbial communities, and in animal models it is often used to evaluate the protective effects of pharmacological or plant-based agents (Hill et al., 2025).

There is limited information available regarding the inhalation and oral toxicity of sulphate, particularly concerning chronic and sub-chronic exposure, as well as its carcinogenic, developmental, and reproductive effects in humans and animals (Knighton., 1982). Sulphate penetrates mammalian cellular membranes slowly and is rapidly excreted through the kidneys (Mitchell and Waring 2016; Markovich, 2000)). No reference dose has been established for sulphate. The sulphate ion is poorly absorbed from the human intestine, which limits its systemic toxicity in humans (Choudhary, 1996; World Health Organisation, 2003).

Nimbidin, an important bioactive compound isolated from neem seeds, has been reported to reduce fasting blood glucose levels (Biswas et al., 2002). In addition, it is widely used as a natural pesticide and serves as a raw material in the cosmetics industry and in the production of various commercial products (Baby et al., 2022)

Neem is a reservoir of bioactive constituents, with over 140 bioactive compounds isolated from various parts of the tree. Key phytochemicals include limonoids such as azadirachtin, nimbin, salannin and nimbidin; flavonoids like quercetin and kaempferol; tannins, phenolic acids, triterpenoids, polysaccharides, saponins and steroids (Nagini and Priyadarsini, 2013; Varshini and Wadhwani, 2026). In addition to their bioactive phytochemicals, neem leaves are rich in vitamins (A, C, E and B-complex), amino acids and minerals (calcium, phosphorus, iron, potassium), which further support their nutraceutical and therapeutic effects (Rao et al., 2024).

Biochemical analysis of neem extracts has revealed a high presence of proline, which is currently being investigated for its therapeutic potential in the treatment of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, as well as in type 2 diabetes mellitus and polycythemia (Atoki et al., 2023).

The extract was shown to normalize hematological parameters altered by toxic exposure. This includes improving RBC count, hemoglobin levels, WBC count and platelet numbers, which may be disturbed during sodium sulfate-induced oxidative injury (Pillai et al., 2014). Research by Haque et al., 2006 reported that neem leaf extract stimulates the haematological system, as indicated by increased counts of RBCs, WBCs, platelets, and haemoglobin levels. These immunostimulatory effects were also associated with the restricted growth of murine carcinoma.

Therefore, the present study was undertaken to investigate the biochemical effects of *Azadirachta indica* on sodium sulfate–induced *Mus musculus*.

MATERIALS AND METHODS :

Animals

Healthy adult mice weighing between 25–30 g were selected for the study. The animals were housed in cages at the Department of Zoology, Tilka Manjhi Bhagalpur University, Bhagalpur animal facility. They were maintained under standard laboratory conditions and provided with a standard diet and water throughout the experimental period (Choudhary et al., 2025).

Preparation of Sodium sulfate solution

A 1% solution of sodium sulfate was prepared and used for the experiment.

Preparation of the *A. indica* leaves extract

Fresh and mature leaves of *Azadirachta indica* were collected from trees located within the campus of the Department of Zoology, Tilka Manjhi Bhagalpur University, Bhagalpur. The leaves were thoroughly washed with water to remove dust and debris and then air-dried at room temperature. After complete drying, the leaves were ground into a fine powder. The powdered material was then sieved through a thin cloth to remove any coarse particles or unwanted materials. The resulting fine powder was collected and stored in a clean, airtight jar for further experimental use (Choudhary and Sharma, 2023).

For the preparation of the aqueous extract, powdered *A. indica* leaves were mixed with distilled water in the required proportion. A 2% aqueous solution of *A. indica* was prepared for the experiment (Sharma and Choudhary, 2024).

Study Design:

A study was carried out to assess the effects of *A. indica* extract on sodium sulfate–induced toxicity in mice. A total of 24 albino mice were randomly divided into three groups: one control group consisting of 8 mice and two experimental groups, each containing 8 mice. The control group was maintained on a standard diet and supplied with distilled water throughout the experimental period.

The first experimental group was administered sodium sulfate solution (0.5 ml per mouse per day) for a period of 28 days. The second experimental group received both sodium sulfate solution and freshly prepared aqueous *A. indica* extract (0.5 ml each), which were administered separately at an interval of 30 minutes daily for the same duration of 28 days. This group was also maintained on a normal diet and provided with distilled water, similar to the control group.

Biochemical Assay

Collection of Blood Samples

At the end of the dosing period, blood samples were obtained from the tail vein of *Mus musculus* (Wang et al., 2026).

Blood Glucose

The estimation of blood glucose was carried out by the O-Toulidine method (Plumer, 1971; Gitay and Bano, 2013)).

Total serum Cholesterol

Blood samples were collected from the treated mice, and the total serum cholesterol was determined using Zak's method (Zak, 1953).

Total plasma Protein

Estimation of plasma protein was carried out using Lowry's method (Lowry 1951).

RESULT AND DISCUSSION:

BLOOD GLUCOSE:

After the dosage period was completed for all groups, several tests were carried out and the results were recorded. The results of this study showed a statistically significant variation in the total RBC count among the control group, sodium sulfate–treated mice, and the amelioration group receiving *A. indica* extract.

The average blood glucose level in the control group was 89.12 ± 5.40 mg/dL. In contrast, the sodium sulfate-treated group showed a marked increase in blood glucose level, reaching 187.62 ± 10.82 mg/dL, indicating that Sodium sulfate group induces hyperglycaemia, possibly through pancreatic stress or insulin resistance. Ammonium sulfate readily dissociates into NH_4^+ and SO_4^{2-} ions within biological systems, and elevated levels of ammonium are known to lead to hyperammonemia and its associated toxic effects (Acar et al., 2018). These adverse effects have been linked to heightened oxidative stress, increased permeability of cellular membranes, and alterations in chromatin organization and DNA structure (Wiren and Merrick, 2004). However, in the amelioration group treated with *A. indica*, the mean blood glucose level decreased to 115.75 ± 4.90 mg/dL and t-value 3.6472. The administration of *A. indica* extract significantly reduced ($p < 0.05$) the elevated blood glucose levels in sodium sulfate-treated mice. Similarly, the SS+AI group (SS with *A. indica*) also demonstrated a significant improvement, with glucose levels lowered to 115.75 ± 4.90 and a t-value of 3.6472 ($p<0.05$).

The group receiving *A. indica* (SS + AI) showed considerable recovery, with blood glucose levels recorded at 115.75 ± 4.9, which was close to that of the control group. Statistical comparison with the control group yielded a t-value of 3.6472 ($p < 0.05$), indicating that the difference was not statistically significant.

Table 1:Blood Glucose(mg/dl) of control and different treated groups of mice.

Glucose (mg/dl)			
S. No.	**C**	**SS**	**SS+AI**
Mice 1	80	145	127
Mice 2	75	155	110
Mice 3	95	188	97
Mice 4	96	176	103
Mice 5	87	194	116
Mice 6	105	239	139
Mice 7	110	187	109
Mice 8	65	217	125
Mean	89.12 ± 5.40	187.62 ±10.82	115.75 ±4.90

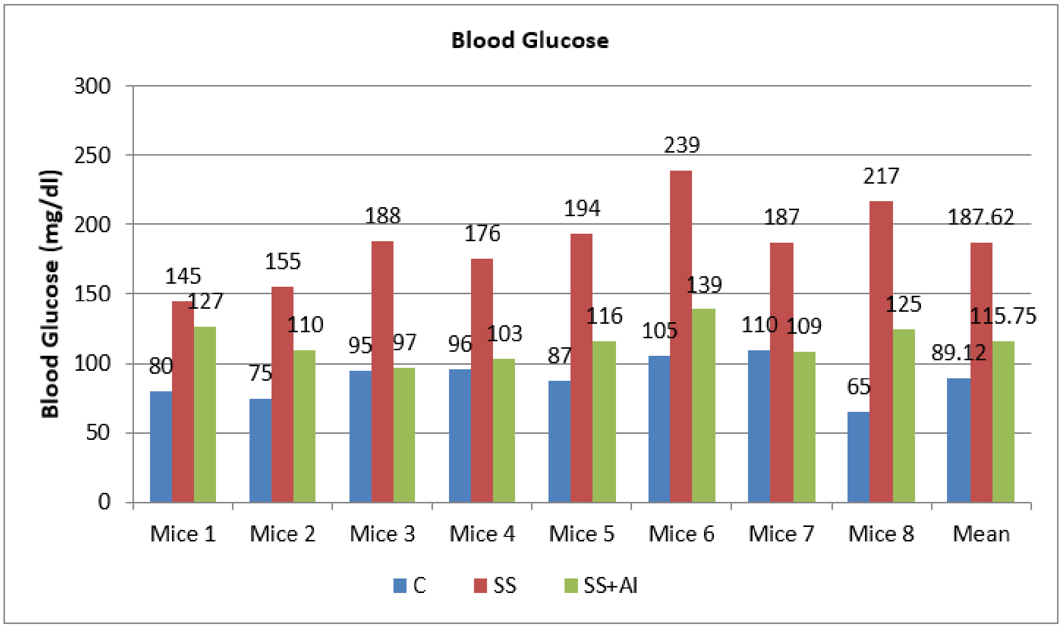

Fig.1: Blood Glucose (mg/dl)of control and different treated groups of mice.

SERUM CHOLESTEROL:

Table 2 presents the serum cholesterol levels in the control group, sodium sulfate (SS) treated group, and the group treated with *A. indica*. The effects of sodium sulfate (SS) and the protective role of *A. indica* (AI) on serum cholesterol levels in mice were evaluated.

The control group exhibited a mean cholesterol level of 162 ± 11.12, which was considered the physiological baseline. Mice administered sodium sulfate (SS) alone showed a marked increase in serum cholesterol, with a significantly higher mean level of 238.75 ± 5.23, indicating that SS induces substantial dyslipidaemia. Statistical comparison between the SS and control groups revealed a t-value of 6.2445 ($p < 0.05$) (Table 2, Fig. 2), confirming that SS has a strong hypercholesterolemic effect, possibly due to disturbances in hepatic lipid metabolism or oxidative stress-induced damage. Sodium sulfate, like other inorganic salts, may promote oxidative stress and inflammation, thereby disrupting liver function, which is crucial for cholesterol synthesis and metabolism(Khan et al., 2019).

It leads to the destruction of microbial cells. The potent bactericidal property of blood serum is mainly due to the presence of lysozyme, which causes lysis of microorganisms.(Lesyk et al ., 2022).

Mice treated with *A. indica* (SS + AI) showed a reduced serum cholesterol level of 186 ± 6.08. Statistical comparison between the SS and SS + AI groups indicated a t-value of 1.8934 ($p < 0.05$), suggesting that *A. indica* produced a partial reduction in cholesterol levels. The metabolism of ammonium sulfate mainly occurs in the liver and kidneys, making hepatocytes and renal cells particularly vulnerable to its harmful effects (Acar et al., 2018).

However, the decrease was not sufficiently large to indicate a strong lipid-lowering effect. These findings suggest that *A. indica* may exert mild hypocholesterolemic activity, which might become more pronounced with higher doses or prolonged treatment.

Table.2.: Serum cholesterol(mg/dl)of control and different treated groups of mice.

SERUM CHOLESTEROL (mg/dl)			
S. No.	C	SS	SS+AI
Mice 1	136	235	154
Mice 2	129	217	199
Mice 3	157	228	176
Mice 4	188	243	191
Mice 5	183	251	178
Mice 6	199	226	211
Mice 7	187	261	194
Mice 8	117	249	185
Mean	162 ±11.12	238.75 ± 5.23	186 ±6.08

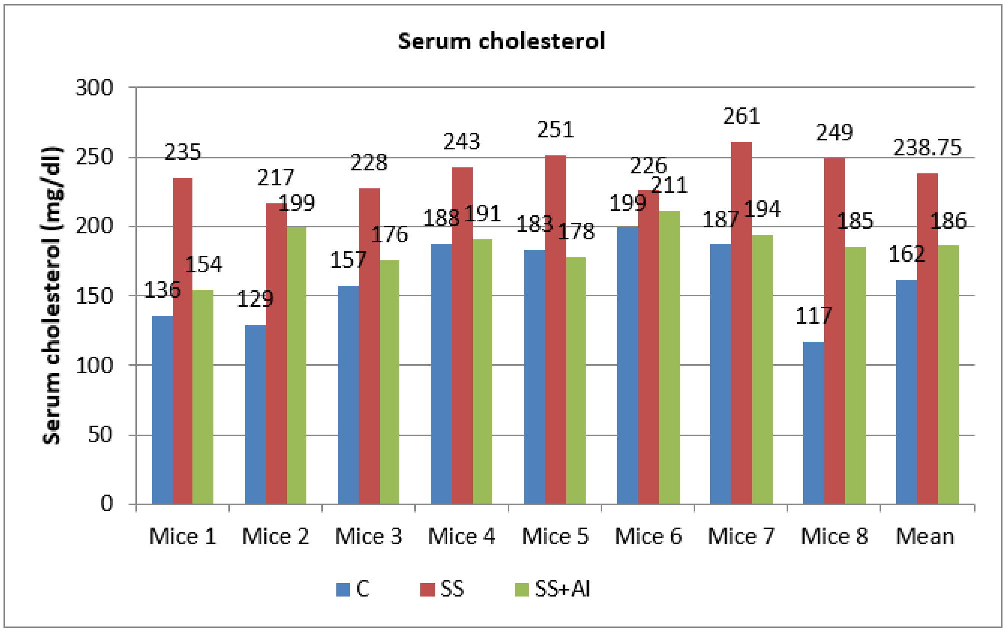

Fig.2: Serum cholesterol (mg/dl) of control and different treated groups of mice.

PLASMA PROTEIN :

The analysis of plasma protein levels revealed marked alterations following sodium sulfate administration and notable improvement after treatment with *A. indica* extract. In the control group, the mean plasma protein concentration was 2.03 ± 0.03, representing normal physiological levels. Exposure to sodium sulfate alone caused a substantial decline in plasma protein concentration to 1.31 ± 0.04, indicating severe hepatic and metabolic dysfunction. A highly significant reduction was observed relative to the control group ($t = 12.5303$; $p < 0.05$), confirming the deleterious effect of sodium sulfate on plasma protein synthesis or stability (Yang et al.,2025).

Research indicates that *Azadirachta indica* increases the activity of antioxidant enzymes in the liver and decreases the production of reactive oxygen species (ROS), thereby helping to prevent oxidative damage (Gautam et al., 2020; Subapriya and Nagini, 2005)..

In contrast, the group treated with *A. indica* extract (SS + AI) exhibited a mean plasma protein concentration of 1.82 ± 0.07, showing a significant improvement compared with the SS group ($t = 2.6098$; $p < 0.05$). This result reflects the hepatoprotective and antioxidant potential of *A. indica* (Table 3, Fig. 3).

Overall, the results suggest that *A. indica* extract provides protective effects against sodium sulfate-induced biochemical alterations, possibly due to its bioactive constituents that enhance hepatic protein synthesis, maintain cellular membrane integrity, and reduce oxidative stress (Li et al., 2015).

Table3.: Plasma Protein (mg/dl)of control and different treated groups of mice.

PLASMA PROTEIN (mg/dl)			
S. No.	C	SS	SS+AI
Mice 1	1.99	1.32	1.86
Mice 2	1.86	1.07	1.93
Mice 3	2.12	1.46	1.54
Mice 4	2.09	1.38	1.61
Mice 5	2.11	1.19	1.98
Mice 6	1.97	1.33	1.72
Mice 7	2.04	1.29	1.81
Mice 8	2.13	1.47	2.18
Mean	2.03 ±0.03	1.31 ±0.04	1.82 ±0.07

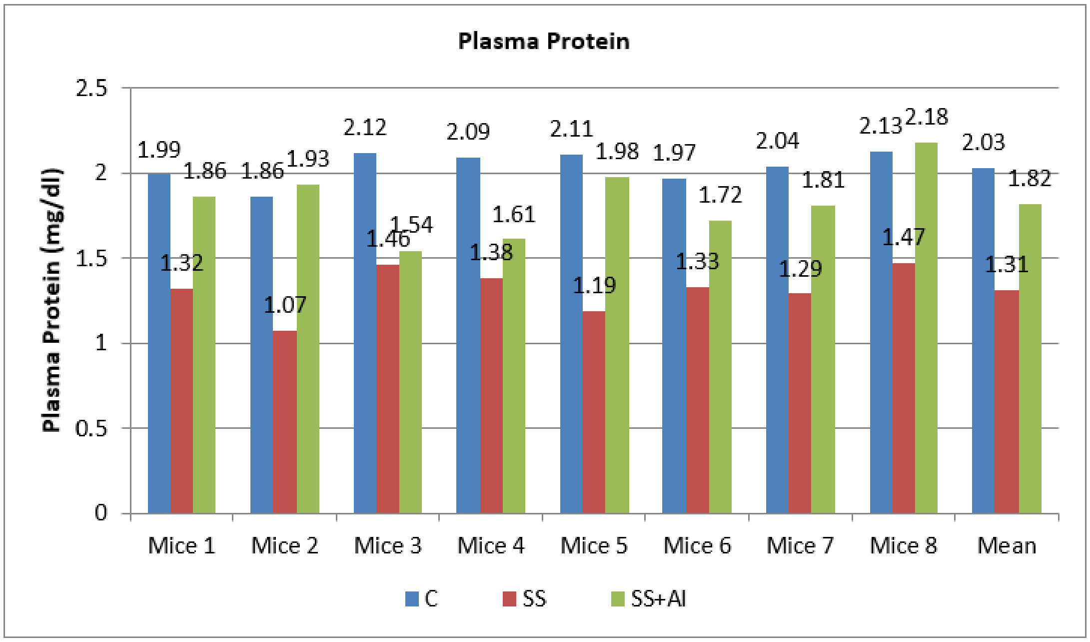

Fig.3.: Plasma Protein (mg/dl)of control and different treated groups of mice.

CONCLUSION :

The present study indicates that the standardized aqueous extract of *A. indica* leaves can effectively reduce the harmful effects of sodium sulfate in mice. The treatment significantly improved important biochemical parameters such as blood glucose count, erum cholesterol, and plasma protein levels. These results suggest that *A. indica* may play a protective role in maintaining normal biochemical parameters. However, further large-scale studies are needed to confirm its safety and effectiveness and also to explore the mechanisms responsible for its biological activity to explore the mechanisms responsible for its biological activity. Such investigations may help in developing *A. indica* as a potential natural herbal drug for the treatment of biochemical disorders.

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