EFFECT OF METHANOLIC EXTRACT OF ALSTONIA SCHOLARIS LEAVES AGAINST AGGRESSION IN MICE, AND ITS ACUTE TOXICITY PROFILE

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ABSTRACT
Predatory and affective aggression garners support as a classification system from clinical, social, biopsychological and forensic databases. Different parts of Alstonia scholaris (L.) R.Br. (Family- Apocynacea) have been traditionally used as an ethno medicine in Ayurvedic system for a number of disorders including laxative, antipyretic, fever, leprosy, skin diseases, and asthma. This study was aimed to evaluate antiaggressive/antidepressant activities of Alstonia scholaris (AS) leaves extract and its acute toxicity profile including haematological/histopathological aspects. Mice were randomly divided into four groups (Gr.) consisting of 6 animals each. Gr. I animals treated with saline(10ml/kg body weight) served as control, Gr. II and III treated with leases extract of AS (250 and 500mg/kg body weight, p.o. respectively) and Gr. IV mice administered Standard drug- Diazepam (2mg/kg, i.p.) for three consecutive days. The different test models such as Isolation-induced aggression, Resident-intruder aggression, Open field test and Water competition test were carried out. In addition, acute toxicity studies which included haematological and histopathological aspects were studied in 14 days treatment of AS extract. The results demonstrate significant antiaggressive activity of AS extract in experimental mice. There were no signs of toxic
evident. The body and organ weights, histopathology and hematological parameters were found to be normal in treated animals. HPTLC analysis indicated the presence of alkaloids, carbohydrates, phenolic compounds, steroids and saponins as the active chemical constituents.

**KEYWORDS:** Antiaggressive, Isolation-Induced Aggression, Resident-Intruder Aggression, Open Field Test, Acute toxicity profile.

**INTRODUCTION**

One of the most important and intensely studied social behaviors exhibited by animals is aggression.[1-3] Aggression is a notoriously nebulous concept that has been defined and categorized in a multitude of ways. Aggression has traditionally been defined as overt behavior with the intention of inflicting physical damage upon another individual or “goal entity”. [4] The behavioral biology of mouse aggression offers insights to understanding the neurobiological and molecular mechanisms mediating behavior in social conflict, the critical developmental and adult determinants, and the functional significance of conflict in males and females.[5] In an effort to trace the behavioral phenotypes of aggressive behavior to chromosomal candidate mechanisms, classic genetic analyses proceeded via strain comparisons and selective breeding to quantitative trait loci (i.e. ‘top-down’ genetics).[6,7] More recently, the ‘bottom-up’ genetic approach has attracted an audience in scientific and popular journals finding that single gene mutations can engender unusual aggressive behavior.[8,9] Violence and aggression are the most serious problems facing humanity.[10-13]

The most general definition of aggressive behavior relies on the intent to harm.[10] A commonly employed classification scheme was described by Moyer[14], who divided aggression into specific subtypes based on differences in social conditions in which the behavior was observed. These subtypes of aggression include: predatory aggression, inter-male aggression, fear-induced aggression, irritable aggression, maternal aggression, territorial aggression, and instrumental aggression.[15]

*Alstonia scholaris* (L.) R. Brown (AS), a 20 to 40 m high tree in family Apocynaceae, is widely distributed in the tropical regions of Africa and Asia.[16] As a folk medicinal pant, AS has been historically used in "Dai" ethno pharmacy to treat chronic respiratory diseases.[17] AS is a popular Philippine medicinal plant, where its root bark is known for its antimalarial properties.[18] The plant is traditionally being used in debility,[19] arthritis,[20] impotence,[21] wounds and earache,[22] asthma,[23,24] leucorrhoea,[25] dog bite,[26] fever,[27] cancer, tumour,
jaundice, hepatitis, malaria, skin diseases\textsuperscript{28}, diarrhea\textsuperscript{29}, leprosy, mental disorders, cardiopathy, helminthiasis, pruritus, agalactia\textsuperscript{30}, hypertension\textsuperscript{31}, dental or gum problem\textsuperscript{32}, abdominal pain after delivery\textsuperscript{33,34} and swelling\textsuperscript{33}. It is also used as aphrodisiac\textsuperscript{21}, antidote to poison\textsuperscript{28}, abortifacient\textsuperscript{35}, astringent, thermogenic, cardiotonic\textsuperscript{30}, stomachic and expectorant\textsuperscript{32}. Reports are available on its ethnoveterinary use such as fever in cattle.\textsuperscript{36, 37} Ayurvedic use is found in phosphaturia and as a blood purifier.\textsuperscript{38} The leaves of AS were reported to contain several alkaloids such as scholaricine, 19, 20 dihydrocondylocarpine, Vallesamine, alstonamine, rhasizamine, 19 epischolaricine, N\textsuperscript{b} methylscholaricine, methylburnamine, vallesamine N\textsuperscript{b} oxide, nareline ethyl ether, 5-epi-nareline ethyl ether and scholarine-N\textsuperscript{4}-oxide, picrinine, angustilobine Bacid, losbanine (6,7-seco-6-nor-angustilobine B), tubotaiwine, itoxides, lagunamine, manilamine, 19, 20 E-alstoscholarine and 19, 20 Z-alstoscholarine, alschomine isoalschomine, netulin.\textsuperscript{39} As herbal medicines are widely available and often used by the general public, more clinical research is needed to establish their safety and efficacy. Based on the literature survey available on \textit{Alstonia scholaris}, it is found to be an interesting plant with the presence of CNS active principles, which have not been studied in details and its Anti-aggressive activity not reported till date.

Therefore, present study was conducted to determine anti-aggressive activity of AS in mice. Further, study also reveals preliminary phytochemical analysis and acute toxicity profile of the AS extract.

**MATERIAL AND METHODS**

**Drugs and chemicals**

Diazepam (Sun Pharma Pvt. Ltd., Mumbai, India as a gift sample) was diluted in 1\% CMC and administered orally. Volume of administration was 1ml / 100 g/body weight. All the drugs were administered in the morning session i.e. 8 AM- 9 AM on each day. Other chemicals used were dragandroff’s reagent, Conc. HCl, H\textsubscript{2}SO\textsubscript{4}, Fehling’s solution, Acetic Anhydride, Chloroform, and Methanol were purchased locally.

**Plant Collection and Authentication of Plant Material**

Fresh leaves of AS were collected from local area of Kanpur (India) in the month of November 2011. The Sample was identified & authenticated taxonomically at National Botanical Research Institute (NBRI), Lucknow. A voucher specimen (NBRI/CIF/259/2011) of the collected sample was deposited in the institutional herbarium for future reference. The Sample was finely powdered in a blender, weighed and stored in a dry polythene bags.
Preparation of extract
The leaves were dried in shade at room temperature. The dried leaves were powdered by using grinder to coarse powder and packed in an air tight container. The leaves powdered (500g) was successively extracted with Methanol: Water (7:3,v/v)\textsuperscript{[40]} by Soxhlet apparatus at 60°C. The solvent was removed from the extract under reduced pressure. The collected extract was stored in sterile container in the refrigerator till further analysis.

Animal and housing
Swiss albino mice (20 ± 2) g of either sex was obtained from the Institutional Animal house. Animals were randomly housed in groups of six in polypropylene cages at an ambient temperature of 25 ± 1°C and 45-55% relative humidity, with a 12 h light/dark cycle (lights on at 7 am). The animals had free access to standard pellet diet from Hindusthan Liver Ltd., Bangalore, India, and water \textit{ad libitum}. Experiments were conducted between 8:00 and 14:00 according to the norms of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Prior permission was obtained from the Institutional Animal Ethics Committee (IAEC) to carry out the experiments.\textsuperscript{[41]}

Phytochemical analysis
The various tests were conducted such as Wagner’s test, Ninhydrin test, Fehling’s test, ferric chloride test, Salkowaski’s test, Kellar Kiliani test, Spot test, Chloroform test, Foam test to identify the constituents present in the AS leaves extract.\textsuperscript{[42]}

High performance thin layer chromatography (HPTLC)
Activation of precoated Silica Gel 60 TLC Plates by placing in oven at 110'-120°C for 30 min prior to sample spotting was done. The Sample was applied on these activated Plates through capillary. Plates were developed with the solvent system of Benzene: Ethyl acetate (86:14 v/v).\textsuperscript{[43]} Rf values of the separate constituent of fractions were detected. Then samples were loaded on the thin layer Chromatographic plates and processed for HPTLC. Results observed were analyzed in HPTLC monitor by using CAMAG software. Precoated aluminum silica gel 60 F\textsubscript{254} plates were used as stationary phase and Toluene: Ethyl acetate: Formic acid (7:3:0.1) as mobile phase for development of chromatogram. Samples (10 μl) were applied using Linomat 5 Applicator (CAMAG).
Experimental design
The animals were randomly divided into four groups (Gr.) containing six mice each. The different groups of mice received vehicle saline (Control, Gr. I), AS (250 mg/kg, p.o., Gr. II), AS (500 mg/kg, p.o., Gr. III) and Standard drug, Diazepam (1mg/kg, i.p., Gr. IV). This group pattern was used to assess the neuropharmacological activity.

Pharmacological screening of anti-aggressive activity

Isolation-Induced Aggression (IIA)
Male mice of Swiss albino strain weighing 20-30 g were used. Mice were kept isolated in small cages for a period of 6 weeks. Prior to the administration of the test drug, the aggressive behavior of the isolated mouse was assessed against a male mouse (similar in weight to that of isolated mouse, and accustomed to live in a group) into the cage of an isolated mouse for 5 min. immediately, the isolated mouse started to attack the "intruder". The aggressive behavior of the isolated mouse was characterized by hitting the tail on the bottom of the cage, screaming and biting. Isolated mice without exhibiting aggressive behavior were excluded from the test. One day after the initial trial, isolated animals were distributed into four groups (5 in each) and treated as Group I (control), Gr. II (250 mg/kg extract of AS), Gr. III (500 mg/kg AS) orally and Gr. IV(2 mg/kg, of diazepam, i.p.) for 3 days. One hour after the last dose, aggressive behavior of isolated mouse against a male mouse was evaluated again for 5 min.\textsuperscript{[44,45]} Aggressive behavior related parameters assessed during this test were latency to first attack, screaming, pursuit frequency, tail rattling, aggressive posture and total number of fighting bouts.

Resident-Intruder Aggression (RIA)
Resident male mice (40 ± 2 g) were tested in their home cages for aggression against a smaller (20 ± 2 g) male intruder. Before the start of the experiments, each resident male mouse was kept in pair with one female mouse in a polypropylene cage for 15 days, and they were randomly divided into four groups (5 pair in each). Drug treatment was started 16th day onward, and only male mice of each pair were treated as Group I (control), Gr. II (250 mg/kg extract of AS), Gr. III (500 mg/kg AS) and Gr. IV (2 mg/kg, of diazepam) orally for 3 days. Resident female was removed from the cage 30 min prior to the start of the test. One hour after the last treatment, a male intruder (~200 g) was placed in the territorial cage of the resident male, and behavior of the resident male was observed for the next 15 min. During
this period, the time until the first attack (in seconds), number of attacks, and duration of each attack (in seconds) were recorded by a blind observer.\[46\]

**Water Competition Test (WCT)**

Two male Swiss albino mice of equal body weight (200 ± 20 g) were paired and housed in one cage for 6 days. After 6 days, the animals were deprived of water for 23 h, and then a water bottle was introduced with a shielded spout so that only one animal of a pair can drink at a time. Duration and frequency of spout possession and water consumption of dominant mice were recorded for 5 min (day 1 of experiment) and the aggressive animal of the pairs was marked for identification (test 1). Animals were then allowed another 55 min for water consumption and again deprived of water for next 23 h. The same procedure was repeated on next day (test 2). At the end of this, aggressive mice was treated as Group I (control), Gr.II (250 mg/kg extract of AS), Gr. III (500 mg/kg AS) and Gr. IV (2 mg/kg, of diazepam) orally for 3 days. Frequency and time in seconds of spout possession of same mice (dominant) were again recorded for 5 min as mentioned above on the third day. Treatment effects were assessed by comparing the values before drug treatment with the values obtained after the drug treatments. Duration of water consumption in this test is considered to be a more specific parameter for evaluating effects of agents on aggressiveness of more dominant mice.\[44,47\]

**Open Field Test (OPT)**

Male mice were randomly divided into 4 groups containing 5 animals each and treated as Group I (control), Gr. II (250 mg/kg extract of AS), Gr. III (500 mg/kg AS) and Gr. IV (2 mg/kg, of diazepam) orally for 3 days. The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The numbers of squares visited by the animals were counted for 3 min at 0, 30, 60, 90 and 120 min after oral administration of the test drugs.\[48\]

**Acute Toxicity Studies**

Acute toxicity studies were carried out in healthy male mice as per OECD guidelines (OECD Guidelines, 2001). Mice were randomly divided into 2 groups, Gr. I was given vehicle (saline water, 10ml/kg body weight, p.o.) served as control. While Gr. II animals were administered orally with the methanolic extract of AS (2000 mg/kg body weight dissolved in water with 1% CMC) for 14 days. Food/water intake and gross behavioral changes were recorded daily for 1-2 hr after the drug administration daily for toxic effects (if any). Autopsy of animals
from control and treated groups was done on day 15. Blood samples were collected from tail region before autopsy in pre-coated ethylene diamine tetra acetic acid (EDTA) vials for haematology. The vital body organs, viz. brain, liver, heart, spleen and kidney organs were dissected out, freed from connective tissues/blood clots, weighed and fixed in Bouin’s solution (24 h) for histology purpose.

**Hematological studies**

Blood samples from control and treated animals were processed in MS-9 Haematology Analyzer (Germany) to study haematological parameters, viz. packed cell volume (PCV), Haemoglobin (Hb), white blood cell (WBC) counts, platelets counts, haematocrit (HCT), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), polymorphs, neutrophil, lymphocytes and monocytes.\(^{[49]}\)

**Histopathology**

Bouin’s fixed tissues from brain, liver, heart, spleen and kidneys were dehydrated in graded ethanol series, cleared in xylene, infiltrated and embedded in moulton paraffin wax (at 58\(^{0}\)C). Tissues blocks were cut at 5 µm and stained with routine haematoxylin and eosin staining, and the slides were examined under Olympus Trinocular Microscope (Olympus BX 51, Tokiyo, Japan) and photomicrographed at X200 Magnification.\(^{[50]}\)

**Statistical analysis**

The values were represented as mean ± S.E.M for six mice. Analysis of variance (ANOVA) test was followed by individual comparison by Newman–Keuls test using Prism Pad Software for the determination of level of significance. Statistical significance between same treatment groups was analysed by Student's \(t\)-test and \(p\) values less than 0.05 were considered statistically significant.

**RESULTS**

**Preliminary phytochemical Analysis**

The Phytochemical analysis confirmed the presence of alkaloids, carbohydrates, Phenolic compounds, steroids and saponins in the extract of AS (Table 1).
Separation of active compounds by HPTLC

Compounds from methanolic extract of AS were detected using the solvent system Toluene: Ethyl acetate: Formic acid (7:3:0.1) and analyzed in HPTLC. The plant extract showed seven compounds having an Rf value of 0.11, 0.19, 0.35, 0.42, 0.72, 0.76, 0.82 and λ max at 254 nm. The compound 1 was the major compound with 60.03% area (Table 2, Fig.1).

Table 1. Phytochemical Screening Tests for methanolic extract of Alstonia scholaris leaves.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Identification Test</th>
<th>AS leaves extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorff Test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mayer’s test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Wagner’s test</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Levigre’s test</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam Test</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Sodium hydroxide test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Lead acetate test</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>FeCl3 test</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Molisch’s test</td>
<td>+</td>
</tr>
</tbody>
</table>

† present, - Absent.

Table 2. Rf values and Area% by HPTLC screening of AS extract.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Start Position</th>
<th>End Position</th>
<th>Area%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.04 Rf</td>
<td>0.11 Rf</td>
<td>60.03%</td>
</tr>
<tr>
<td>2.</td>
<td>0.11 Rf</td>
<td>0.14 Rf</td>
<td>5.17%</td>
</tr>
<tr>
<td>3.</td>
<td>0.16 Rf</td>
<td>0.18 Rf</td>
<td>5.02%</td>
</tr>
<tr>
<td>4.</td>
<td>0.19 Rf</td>
<td>0.23 Rf</td>
<td>11.69%</td>
</tr>
<tr>
<td>5.</td>
<td>0.31 Rf</td>
<td>0.36 Rf</td>
<td>2.68%</td>
</tr>
<tr>
<td>6.</td>
<td>0.54 Rf</td>
<td>0.61 Rf</td>
<td>15.41%</td>
</tr>
</tbody>
</table>

Figure 1: HPTLC analysis of methanolic Alstonia scholaris extract.
Effect of *Alstonia scholaris* on Isolation-Induced Aggression (IIA)

The extract extended latency period to first attack and the number of fighting episodes. Number of aggressive postures, number of screaming’s and tail rattle frequency were also significantly reduced by the extract. Qualitatively, these effects of extract were identical to that of the diazepam (Fig. 2).

![Figure 2: Effect of methanolic extract of AS on Isolation-induced aggression test (n = 6, *p* < 0.001, **p** < 0.05, ***p** < 0.01, #p > 0.05-Non significant, Treated vs. Control).](image)

**Effect of AS on Resident-intruder aggression (RIA)**

The extract treatment prolonged the latency period of first attack and reduced the total duration and mean number of fights. Mean numbers of lateral threats and aggressive grooming were also lowered in the extract treated group as compared to control group. The observed effects of diazepam in this model were qualitatively similar to those of extract (Figure 3).

![Figure 3: Effect of methanolic extract of AS on Resident- intruder aggression test (n = 6, *p* < 0.001, **p** < 0.05, ***p** < 0.01, #p > 0.05-Non significant, Treated vs. Control).](image)
Effect of AS on Water competition test (WCT)
In control group rats, duration of water consumption of the animals did not change significantly over the four different test days, indicating that a stable relationship of water consumption and aggressive behavior had been established in each pair. The extract treatment significantly reduced the duration of water intake by the dominant rats. Similar relationship was obtained during frequency of water spout possession (Figure 4).

Figure 4: Effect of methanolic extract of AS on water competition test (n = 6, *p< 0.001, **p< 0.05, ***p< 0.01, #p> 0.05-Non significant, treated vs. control).

Effect of AS on Open field test (OPT)
There was a significant increase in the number of squares crossed in the centre with all the treatment groups compared to control. There was also a significant decrease in the total number of squares crossed in all the tested groups when compared to control, which indicates the decrease in motor activity (Figure 5).

Figure 5: Effect of methanolic extract of AS on open field test (n = 6, *p< 0.001, **p< 0.05, ***p< 0.01, #p> 0.05-Non significant, treated vs. control).
Acute Toxicity Study

The test animals after 14 days did not exhibit any visible change and survived beyond recommended duration of observation. Hence, AS extract was safe up to 2000 mg kg\(^{-1}\) were selected for further experimentation.

Hematological Indices

There were no significant changes in RBC, WBC, HGB, HCT, MCV, MCH and MCHC of rats treated with AS extract at the dose of 2000 mg/kg body weight when compared with those of controls (Table 3).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Control</th>
<th>AS-Treated Group (2000mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>WBC (m/mm(^3))</td>
<td>6.94 ± 1.09</td>
<td>6.07 ± 0.15</td>
</tr>
<tr>
<td>2.</td>
<td>RBC (m/mm(^3))</td>
<td>8.99 ± 0.04</td>
<td>8.58 ± 0.29</td>
</tr>
<tr>
<td>3.</td>
<td>Platelets (%)</td>
<td>513 ± 1.71</td>
<td>509 ± 2.21</td>
</tr>
<tr>
<td>4.</td>
<td>HGB (g/dl)</td>
<td>13.7 ± 0.18</td>
<td>13.1 ± 0.20</td>
</tr>
<tr>
<td>5.</td>
<td>MCV (fl.)</td>
<td>48.3 ± 0.23</td>
<td>47.9 ± 0.22</td>
</tr>
<tr>
<td>6.</td>
<td>HCT (%)</td>
<td>42.2 ± 0.18</td>
<td>41.9 ± 0.21</td>
</tr>
<tr>
<td>7.</td>
<td>MCHC (g/dl)</td>
<td>31.2 ± 0.23</td>
<td>31.4 ± 0.21</td>
</tr>
<tr>
<td>8.</td>
<td>Polymorphs</td>
<td>31.1 ± 0.30</td>
<td>30.7 ± 0.20</td>
</tr>
<tr>
<td>9.</td>
<td>Lymphocytes</td>
<td>58.2 ± 0.13</td>
<td>59.8 ± 0.07</td>
</tr>
<tr>
<td>10.</td>
<td>Monocytes (%)</td>
<td>3.6 ± 0.17</td>
<td>3.9 ± 0.15</td>
</tr>
<tr>
<td>11.</td>
<td>Eosinophils</td>
<td>9.1 ± 0.11</td>
<td>8.9 ± 0.08</td>
</tr>
<tr>
<td>12.</td>
<td>Basophils</td>
<td>1.0 ± 0.21</td>
<td>1.10 ± 0.06</td>
</tr>
</tbody>
</table>

Histopathology

No marked histopathological changes were observed in treated mice as compared to controls after 14 days treatment of AS extract at 2000 mg/kg dose level (Figure 6).
Figure 6: Histology of liver, brain, kidney, heart and spleen from control group of mice showing normal histoarchitecture. Treatment of methanolic extract of AS (2000 mg/kg) for 14 days did not show any deleterious effects but show normal histoarchitecture of liver, brain, kidney, heart and spleen comparable to controls. Haematoxylin-Eosin stained; Magnification: X400 for all figures.

DISCUSSION

One of the difficult medical and social problems associated with neuropsychiatric disturbances is aggression. Analysis of aggression in different animal species could provide a firm understanding of human violence and the therapeutic measures to be taken to combat it.[51] Exposure of an animal to a threatening situation elicits a behavioral repertoire referred to as aggression. The behavioral profile may be manifested in the form of offensive and defensive aggression.[52] Offensive behavior is characterized by initiative of the aggressor and devastation to the opponent.[53] On the other hand defensive behavior lacks initiative and the animal does not impose intentional damage.[54] The term aggression is widely employed to indicate various patterns of psychological or sociological behavior resulting from pathological, biochemical or physiological alteration of central nervous system constituents. There are many psychiatric disorders such as schizophrenia and Alzheimer's disease which show close association with aggression.[55]

The qualitative phytochemical studies carried out so far reveal the presence of alkaloids, coumarins, flavonoids, reducing sugars, simple phenolics, steroids, saponins and tannins. Of the various phytochemicals estimated, lipid and saponin were found in larger amounts than others. The above different chemical compounds detected in AS which could make the plant useful in treating different ailments and having potential for providing useful drug for human use.[56] Various antidepressant drugs have been reported to be effective in the treatment of aggression.[57,58] Alkaloids has been identified as one of the main components of Alstonia scholaris extract responsible for its antidepressant effects.[59-63] Therefore, observed
antiaggressive activity of alstonine and scholaricine adds a new potential use in the wide spectrum of AS for the treatment of neurological disorders. The reported HPTLC method was found to be rapid, simple and accurate for quantitative estimation of phytochemicals in methanolic leaves extract of AS. HPTLC analysis of the sample revealed a wide-variability in the methanolic extract. The scanning at λ max 254 nm, showed the presence of seven spots having the percentage from 3.70 to 56.76. The major spot at Rf.0.11 had the concentration of 56.76%.

With accurate determination of hematologic parameters, about 80% of hematologic diagnoses can be made and information collected to evaluate the stage of a particular disease or to diagnose some diseases that may not be directly related to the hematopoietic system. Information generated from white blood cell count, without differential count may only be partial and in some cases misleading. Thus, granulocytes are estimated in differential count to establish the nature of infection. The results of the present study suggested that total leukocyte and differential counts were normal in both test models, therefore being indicative of the non-allergic nature of the extracts. It was also an indication that the extract did not compromise the immune status of the animals.

In view of the fact that the therapeutic dose of the extract is by far lower than the amounts used in toxicity tests, it may be appropriate to suggest that the extract may not affect erythropoiesis, hemoglobin synthesis or other factors related to RBC metabolism when used at the therapeutic dose.

The organ pathology scores of the mice after oral administration of the methanolic extract of AS leaves indicated that the liver, kidney, brain, spleen and heart of the mice had normal architectures and comparable with in controls.

The Open Field Test provides simultaneous measures of locomotion, exploration and anxiety. The number of line crosses and the frequency of rearing are usually used as measures of locomotor activity, but are also measures of exploration and anxiety. A high frequency of these behaviors indicates increased locomotion and exploration and/or a lower level of anxiety. The number of central square entries and the duration of time spent in the central square are measures of exploratory behavior and anxiety. A high frequency/duration of these behaviors indicates high exploratory behavior and low anxiety levels. In the present study,
there was an increase in the number of squares crossed in the centre on treatment with methanolic extract which indicate anti-aggressive activity.

In water-competition test, water-finding task provides a measure of memory related mainly to the spatial construction of the test apparatus and to the specific objects in it. In this task, there is no need to use any form of motivation to train the animals. Animals are deprived of water only before the test trial to promote recall of the location of the water tube in the alcove of the apparatus in which they have been exposed in the training trial. The end of the water tube is set farther above the floor in the test trial than in training to decrease the probability of it being found by chance. The finding latency of the trained mice is markedly shorter than that of naive mice, indicating finding latency provides a measure of latent learning (attention) in mice. In the present study, extract treatment significantly reduced the duration of water consumption and frequency of water spout possession.

In Isolated-induced aggression test, the serotoninergic system is implicated in aggressive states and it has been hypothesized that decreasing serotoninergic activity may encourage aggressive behavior. Since; both anti-anxiety and anti-aggressive effects are seen with 5HT1A antagonists, it is assumed that extract may also interact with the 5-HT1A receptors. The most frequently used methodological approach in the laboratory to induce mice to fight is to isolate males for some time, ranging from 24 h to 8 weeks and subsequently, to confront the isolate with a group housed stranger in an unfamiliar test arena or in the isolate’s home cage (isolation-induced aggression). Depending on strain, a considerable proportion of isolated male mice do engage in non-agonistic social interactions or respond defensively toward a stimulus mouse rather than deliver attack bites (isolation-induced timidity). Functionally, aggressive behavior by isolated laboratory male mice has been interpreted as pathological and abnormal, representing a cardinal symptom of the ‘isolation syndrome’. By contrast, in the wild, dominant male mice that exclude other males from their territory are behaviorally isolated and their aggression may be an adaptive behavior that increases their relative fitness.

In Resident-intruder test, fighting is known to occur frequently in male mouse groups. In this study, the possible impact of individual aggressiveness on fighting in groups and on the social status of animals was studied. Male mice were pre-tested in a resident-intruder test and rated as initially aggressive or non-aggressive according to their attack behavior against an intruder. Thereafter they were randomly allocated to new social groups, with four mice per
cage. Fighting in groups was increased when several initially aggressive animals were included in the group. Within the groups, animals were rated as dominants and subordinates according to their behavior toward a strange intruder introduced into their home-cage test and the occurrence of wounds.\textsuperscript{[76]} In the present study, extract (250 and 500 mg/kg) produced a significant reduction in total fighting time in comparison with vehicle, suggesting that extract has an anti-aggressive effect.

The effect of diazepam on the values of aggressive behavior shows the tranquilizer activity of this drug. This is because of its capacity as an anxyolytic agent that has activity to the level of the GABAA receptors.\textsuperscript{[77]} Aggression is associated with low cerebrospinal fluid concentrations of the serotonin (5-HT) metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in humans and nonhuman primates, and reduced 5-HT level or turnover in the brain of laboratory animals.\textsuperscript{[78]} Brain levels of gamma-aminobutyric acid (GABA) and glutamic acid decarboxylase in the striatum and the olfactory bulbs are low in mice and rats that exhibited aggressive behavior.\textsuperscript{[79,80]} Pharmacological strategies of increasing 5-HT levels, such as the use of 5-HT precursors and 5-HT reuptake inhibitors are able to reduce aggressive behavior in rodents.\textsuperscript{[81-84]} Likewise, increased GABAergic transmission is therapeutically beneficial in aggression (\textit{e.g.} benzodiazepines). Methanolic extract of AS act as a neurotransmitter reuptake inhibitor, affecting the synaptosomal uptake of serotonin, dopamine, noradrenalin, glutamate and GABA with similar efficiencies. Therefore, increased serotonergic transmission due to reuptake inhibition may be responsible for observed antiaggressive activity of AS. The findings of this study are consonant and elucidate that methanolic extract of AS may be potentially responsible for the observed antiaggressive activity.

**CONCLUSION**

In conclusion, the methanol extract of AS shows potential antiaggressive activity in rats and found to be efficacious in producing serenity and masking the constellation of behavioral changes encountered during aggressive bouts making it a promising naturally derived product.

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