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Acute Oral Safety and Comparative Anti-Aggressive Effects of Methanolic Leaf Extracts of *Piper methysticum G. Forst* and *Buchanania lanzan* Spreng. in Mouse Models

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Abstract

Background: Aggression is a multidimensional behaviour influenced by stress, arousal, social isolation and central inhibitory control. This study evaluated the acute oral safety of methanolic leaf extracts of *Piper methysticum* G. Forst. and *Buchanania lanzan* Spreng., selected experimental doses and compared their anti-aggressive effects in food shock-induced and isolation-induced aggression models. Methods: In an OECD Test Guideline 425 limit study, female Swiss albino mice received a single oral dose of 2000 mg/kg of either extract and were observed for 14 days. For each aggression model, male Swiss albino mice were allocated to vehicle control, diazepam 2 mg/kg, *Piper methysticum* 100, 200 or 400 mg/kg, or *Buchanania lanzan* 100, 200 or 400 mg/kg groups (n=6). Treatments were administered orally. Food shock consisted of 0.5 mA stimulation for 0.5 s at 10-s intervals over a 5-min observation period. In the isolation model, mice were housed individually for 7 days and challenged with a similar-weight male intruder for 5 min. Results: Neither extract produced mortality or major toxicity at 2000 mg/kg; body weight remained stable, indicating an approximate oral LD50 greater than 2000 mg/kg. Doses of 100, 200 and 400 mg/kg were therefore selected. Both extracts reduced aggressive behaviour dose-dependently. In the food shock model, *Piper methysticum* and *Buchanania lanzan* at 400 mg/kg reduced fights by 64.60% and 56.64%, respectively. In isolation-induced aggression, the corresponding reductions in attack frequency were 64.18% and 55.22%. Both high-dose effects were highly significant versus control ($p<0.001$), although diazepam remained more active. Conclusion: The extracts showed preliminary acute tolerability and anti-aggressive-like effects in stress- and isolation-related models, with *Piper methysticum* consistently more active. The findings are preclinical and require repeated-dose toxicity, locomotor controls and mechanistic validation before therapeutic translation.

Keywords: Piper methysticum, Buchanania lanzan, acute oral toxicity, aggression, food shock, social isolation, diazepam

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1. Introduction

Aggressive behaviour is not a single emotional state but an observable response produced by interactions among threat perception, arousal, stress, reward, impulse control and social context. In animal pharmacology, aggression is quantified through outcomes such as attack frequency, fighting episodes, biting attempts, latency to the first attack, chasing and total duration of aggressive behaviour. This operational approach is necessary because a general impression that an animal is 'calmer' cannot distinguish selective behavioural regulation from weakness, sedation or motor impairment.

The neurobiology of aggression involves coordinated activity across the prefrontal cortex, amygdala, hypothalamus, septal regions, striatum and brainstem, together with GABAergic, serotonergic, dopaminergic and glutamatergic signalling. Reduced inhibitory control or excessive excitatory drive can lower the threshold for attack-like behaviour. GABA generally limits neuronal excitability, serotonin contributes to impulse control and emotional regulation, and dopamine influences motivation, reward and behavioural activation (Fritz et al., 2023). Because aggression has multiple triggers, evidence from more than one experimental model is more informative than evidence from a single test.

Food shock-induced aggression models an aversive, stress-provoked response. Mild foot shock can increase arousal, defensive reactivity, vocalization, jumping and fighting between paired animals. A test agent may reduce these responses by lowering anxiety, fear or stress reactivity, but it may also reduce them by producing analgesia or motor suppression. Isolation-induced aggression examines a different dimension. Individual housing increases territorial sensitivity and emotional reactivity and may alter stress hormones, serotonergic regulation, dopamine signalling and GABAergic inhibition. The introduction of another male into the isolated animal's cage then evokes attacks, fights, biting and chasing. Activity in both models suggests a broader influence on stress-related and social aggression.

Piper methysticum, or kava, was selected because its kavalactones have established central nervous system relevance. The major kavalactones include kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin and desmethoxyyangonin. These compounds may influence GABA-A receptors, voltage-gated ion channels, monoamine systems and other neurobehavioural targets (Chua et al., 2016; Volgin et al., 2020). Kava's anxiolytic and calming profile makes it a plausible candidate for reducing aggressive responses driven by hyperarousal or impaired inhibition. At the same time, concerns about hepatotoxicity, drug interactions and variability among products require safety assessment before dose selection (Soares et al., 2022).

Buchanania lanzan , commonly known as chironji, has a different pharmacological profile. Its leaves and other parts have been associated with phenolic compounds, flavonoids, tannins, glycosides and terpenoids and with antioxidant, anti-inflammatory, adaptogenic and protective effects (Elias et al., 2021). Direct anti-aggressive evidence is limited, but stress, oxidative disturbance and inflammation can influence behavioural regulation. A chemically diverse extract could therefore reduce aggressive responding indirectly by improving stress adaptation or neuronal protection. The comparison is useful because it places a CNS-oriented plant beside a broader stress-protective phytomedicine under identical experimental conditions.

Safety and efficacy must be interpreted together. A substance that reduces fighting because animals are severely sedated, ataxic or ill is not a useful anti-aggressive candidate. Acute toxicity testing provides an initial estimate of tolerability and a rational ceiling from which lower pharmacological doses can be selected. OECD Test Guideline 425 provides a recognized framework for single-dose oral toxicity assessment, observation of clinical signs and estimation

of whether the LD50 is above a limit dose (OECD, 2022). Acute tolerability does not establish chronic safety, but it is an ethical and scientific prerequisite for behavioural screening.

The present paper deliberately restricts the wider thesis to acute oral toxicity, dose selection and two aggression paradigms: food shock-induced aggression and isolation-induced aggression. Resident-intruder, water competition, open-field and receptor-binding findings are not presented as primary outcomes here. The objectives were to assess visible acute toxicity at 2000 mg/kg, select low, medium and high doses below the limit dose, and determine whether the two extracts reduced aggressive responses dose-dependently in models representing aversive stress and social isolation. It was hypothesized that both extracts would show anti-aggressive-like activity, with a stronger response from *Piper methysticum* because of its documented central inhibitory relevance.

The use of a standard comparator was important for both experimental interpretation and internal validity. Diazepam was not included to suggest that benzodiazepines are universally appropriate treatments for aggression; rather, its established facilitation of GABA-A receptor signalling provided a pharmacological reference for a model expected to respond to enhanced inhibition. A successful standard response shows that the apparatus, timing and behavioural scoring were capable of detecting a centrally mediated reduction under the conditions of the experiment.

Model selection also determines the meaning of a positive result. Food shock combines aversive stimulation with social proximity, whereas isolation produces a history-dependent change in territorial and social responsiveness. Agreement between the two models reduces the likelihood that the extract acted only by changing pain perception during shock. Conversely, the experiments do not represent all forms of aggression. Predatory, maternal, dominance-reward and resource-competition behaviours may involve overlapping but non-identical circuits, so the findings should remain tied to the tested paradigms.

Dose-response interpretation should consider both statistical significance and biological magnitude. At 100 mg/kg, both extracts produced relatively modest inhibition, while 200 and 400 mg/kg progressively shifted several endpoints. The parallel increase in latency and decrease in frequency and duration is more informative than a p value alone because it shows a coherent behavioural pattern. Nevertheless, formal trend analysis, confidence intervals and direct statistical comparison between the two plants would provide a stronger estimate of relative potency in future work.

Animal welfare was particularly relevant in the shock and confrontation procedures. Shock intensity and duration were kept low and the observation window was limited to 5 min. Animals required continuous monitoring, and trials should be stopped when injury risk exceeds the approved humane endpoint. Refinement can include video-based scoring, barriers that prevent severe biting, optimized intruder matching and use of the minimum shock sufficient to elicit a reliable response. Such measures improve both ethics and data quality by reducing uncontrolled distress.

2. Materials and Methods

2.1 Ethical framework and experimental animals

The work was conducted after approval from the Institutional Animal Ethics Committee under CPCSEA Registration No. 1397/Po/Re/S/10/CPCSEA. Reporting principles were aligned with ARRIVE 2.0, including specification of species, sex, body weight, housing, treatment, outcomes and statistical analysis (Percie du Sert et al., 2020). Swiss albino mice were obtained

from a recognized animal facility. Animals showing injury, infection, weakness, abnormal behaviour or poor activity were excluded.

2.2 Housing

Mice were acclimatized for 7 days. Standard polypropylene cages of approximately 30 x 20 x 20 cm were used. The room was maintained at 25 +/- 2 degrees C and 50-60% relative humidity with a 12-h light/12-h dark cycle. Standard pellet feed and clean water were available ad libitum except where fasting was required for toxicity testing. Bedding was changed on alternate days. Behavioural experiments were conducted in a quiet room between 09:00 and 14:00 h, and animals were allowed at least 30 min to adapt to the testing room.

2.3 Test preparations and administration

Crude methanolic leaf extracts of *Piper methysticum* and *Buchanania lanzan* were freshly suspended in 1% carboxymethyl cellulose (CMC). All doses were given orally by gavage at 10 mL/kg, calculated from body weight on the day of treatment. The vehicle control received 1% CMC, while diazepam at 2 mg/kg served as the standard comparator. Extract-treated animals were tested 60 min after dosing; diazepam-treated animals were tested after 30 min.

2.4 Acute oral toxicity

Healthy adult female Swiss albino mice weighing 20-30 g were used because female rodents are commonly employed in acute oral toxicity guidance. Animals were acclimatized for 7 days and fasted for 4 h before dosing, with water available. Food was withheld for 1 h after administration. Two extract groups were used, with five mice per group. Each animal received a single oral limit dose of 2000 mg/kg of *Piper methysticum* or *Buchanania lanzan* extract in 1% CMC.

Toxicity observations. Animals were observed individually during the first 30 min, periodically during the first 4 h and once daily for 14 days. Recorded outcomes included mortality, general activity, abnormal posture or behaviour, sedation, sleep, tremors, convulsions, salivation, diarrhoea, piloerection, food intake, water intake and visible distress. Body weight was measured on days 0, 7 and 14. In the absence of mortality and severe toxicity at the limit dose, the approximate oral LD50 was considered greater than 2000 mg/kg, and substantially lower doses were chosen for behavioural screening.

2.5 Dose selection and behavioural grouping

Three dose levels were selected: 100 mg/kg (low), 200 mg/kg (medium) and 400 mg/kg (high). For each behavioural model, 48 healthy adult male Swiss albino mice, 6-8 weeks old and weighing 20-30 g, were randomly divided into eight groups (n=6): vehicle control; diazepam 2 mg/kg; *Piper methysticum* 100, 200 and 400 mg/kg; and *Buchanania lanzan* 100, 200 and 400 mg/kg. Separate sets of animals were used for the two models to avoid carry-over effects from shock, isolation, repeated fighting or behavioural fatigue.

2.6 Food shock-induced aggression

A pair of mice from the same treatment group was placed in an aggression chamber fitted with a grid floor. A controlled stimulator delivered a mild foot shock of 0.5 mA for 0.5 s at intervals of 10 s. Behaviour was observed for 5 min. The recorded variables were number of fights, attack frequency, biting attempts, latency to the first aggressive response and total duration of aggression; jumping and vocalization were also monitored during the procedure. The chamber was cleaned with 70% ethanol and allowed to dry between trials to reduce odour cues.

2.7 Isolation-induced aggression

Mice assigned to this model were housed individually for 7 days before testing, with food and water ad libitum and the same environmental conditions as other animals. After oral treatment, one non-isolated male mouse of similar body weight was introduced into the cage of the isolated mouse. Aggressive behaviour was observed for 5 min. Outcomes were latency to the first attack, number of attacks, number of fights, biting attempts, chasing behaviour and total duration of aggression. The intruder was removed immediately after the observation period, or earlier if welfare concerns required termination.

2.8 Outcome interpretation and percentage inhibition

A reduction in fights, attacks, biting, chasing and duration, together with an increase in attack latency, was interpreted as anti-aggressive-like activity. Percentage inhibition was calculated from the primary frequency endpoint relative to control: [(control mean - treated mean) / control mean] x 100. Because the models use different primary behaviours, number of fights was used for food shock-induced aggression and number of attacks for isolation-induced aggression.

2.9 Statistical analysis

Results were expressed as mean +/- standard error of the mean (SEM), with six animals per group. Group means were analysed by one-way analysis of variance followed by Dunnett's post-hoc test against the vehicle control. Significance thresholds were $p<0.05$, $p<0.01$ and $p<0.001$. Acute body-weight changes were treated descriptively and tested for significant reduction; $p>0.05$ was considered non-significant.

3. Results

3.1 Acute toxicity and dose selection

No mortality occurred in either extract group following 2000 mg/kg orally (0/5 for each extract). General activity remained normal, and no severe sedation, tremors, convulsions, excessive salivation, diarrhoea, piloerection or behavioural abnormality was recorded. Food and water intake remained normal. *Piper methysticum* group body weight increased from 24.20 +/- 0.66 g on day 0 to 24.80 +/- 0.58 g on day 7 and 25.30 +/- 0.53 g on day 14. *Buchanania lanzan* group body weight increased from 23.90 +/- 0.62 g to 24.50 +/- 0.55 g and 25.00 +/- 0.57 g, respectively. Neither group showed a significant body-weight reduction ($p>0.05$). The findings supported an approximate oral LD50 greater than 2000 mg/kg and justified selection of 100, 200 and 400 mg/kg for behavioural experiments.

Table 1: Acute toxicity studies

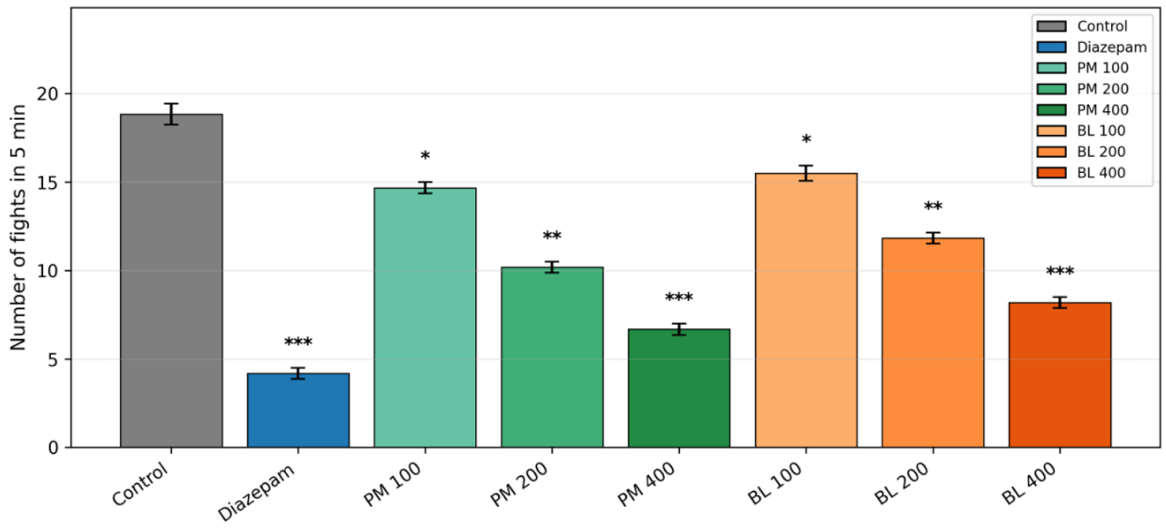
Extract	Dose	Mortality	Major toxic signs	Day 0 weight	Day 14 weight
Piper methysticum	2000 mg/kg	0/5	Absent	24.20 +/- 0.66 g	25.30 +/- 0.53 g
Buchanania lanzan	2000 mg/kg	0/5	Absent	23.90 +/- 0.62 g	25.00 +/- 0.57 g

3.2 Food shock-induced aggression

Vehicle-treated mice displayed frequent fighting, attacks and biting, a short attack latency and prolonged aggression. Diazepam markedly reduced all aggressive parameters and increased latency, confirming model sensitivity. Both extracts produced dose-related effects. *Piper methysticum* reduced fights from 18.83 +/- 0.60 in control animals to 14.67 +/- 0.33, 10.17 +/- 0.31 and 6.67 +/- 0.33 at 100, 200 and 400 mg/kg, representing 22.12%, 46.02% and 64.60% inhibition. *Buchanania lanzan* reduced fights to 15.50 +/- 0.43, 11.83 +/- 0.31 and 8.17 +/- 0.31, corresponding to 17.69%, 37.17% and 56.64% inhibition.

Table 2: Food shock-induced aggression

Group	Fights	Attack frequency	Biting attempts	Latency (s)	Duration (s)	% inhibition
Control	18.83 +/- 0.60	21.50 +/- 0.76	14.67 +/- 0.61	18.17 +/- 1.08	142.33 +/- 3.56	-
Diazepam 2 mg/kg	4.17 +/- 0.31***	5.50 +/- 0.43***	3.33 +/- 0.33***	83.17 +/- 2.48***	38.67 +/- 2.32***	77.87
Piper 100 mg/kg	14.67 +/- 0.33*	16.33 +/- 0.49*	11.17 +/- 0.31*	31.33 +/- 1.45*	109.50 +/- 2.28*	22.12
Piper 200 mg/kg	10.17 +/- 0.31**	12.33 +/- 0.49**	8.00 +/- 0.37**	49.50 +/- 1.87**	78.83 +/- 2.18**	46.02
Piper 400 mg/kg	6.67 +/- 0.33***	8.17 +/- 0.31***	5.50 +/- 0.34***	67.83 +/- 2.18***	52.50 +/- 1.89***	64.60
B. lanzan 100 mg/kg	15.50 +/- 0.43*	17.67 +/- 0.49*	12.17 +/- 0.40*	28.67 +/- 1.33*	118.17 +/- 2.54*	17.69
B. lanzan 200 mg/kg	11.83 +/- 0.31**	14.00 +/- 0.37**	9.17 +/- 0.31**	42.17 +/- 1.56**	89.67 +/- 2.03**	37.17
B. lanzan 400 mg/kg	8.17 +/- 0.31***	10.17 +/- 0.31***	6.67 +/- 0.33***	58.50 +/- 1.71***	65.33 +/- 1.76***	56.64



PM = Piper methysticum; BL = Buchanania lanzan. Values are expressed as mean ± SEM. Behavioural experiments: n = 6 animals per group. Receptor-binding assays were performed in triplicate.

Figure 1: Food-Shock-Induced Number of Fights

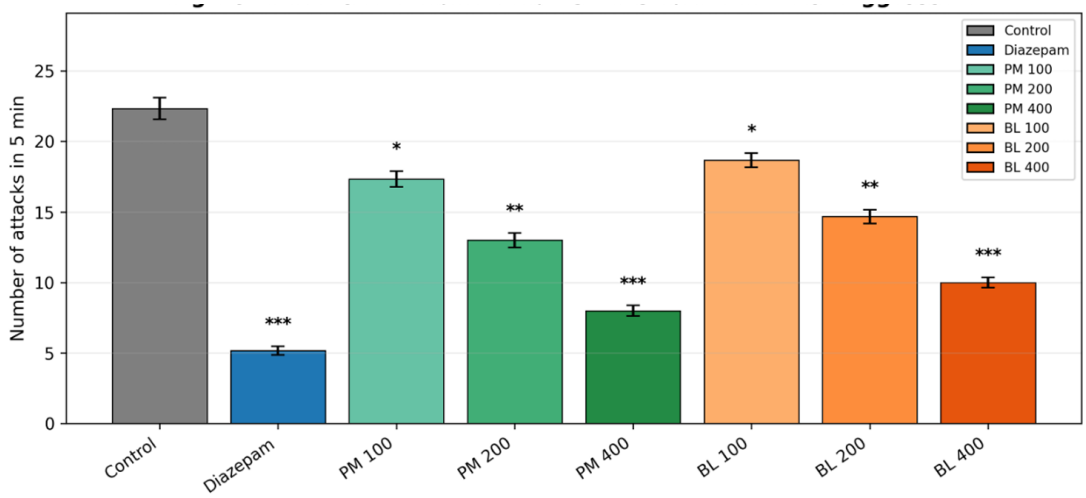
3.3 Isolation-induced aggression

Individually housed control mice showed short attack latency, frequent attacks and fights, biting, chasing and prolonged aggressive behaviour. Diazepam again produced a strong inhibitory effect.

Table 3: Isolation-induced aggression

Group	Latency (s)	Attacks	Fights	Biting	Chasing	Duration (s)	% inhibition
Control	20.50 +/- 1.12	22.33 +/- 0.76	17.83 +/- 0.60	13.83 +/- 0.48	16.50 +/- 0.67	151.67 +/- 3.35	-
Diazepam 2 mg/kg	91.33 +/- 3.18***	5.17 +/- 0.31***	4.00 +/- 0.26***	2.83 +/- 0.31***	4.33 +/- 0.33***	36.50 +/- 1.82***	76.85
Piper 100 mg/kg	34.67 +/- 1.63*	17.33 +/- 0.56*	13.83 +/- 0.48*	10.67 +/- 0.42*	12.83 +/- 0.48*	118.33 +/- 2.74*	22.40
Piper 200 mg/kg	53.50 +/- 1.82**	13.00 +/- 0.52**	10.17 +/- 0.31**	7.83 +/- 0.31**	9.67 +/- 0.42**	84.67 +/- 2.46**	41.78
Piper 400 mg/kg	73.83 +/- 2.29***	8.00 +/- 0.37***	6.33 +/- 0.33***	5.00 +/- 0.26***	6.33 +/- 0.33***	57.17 +/- 1.83***	64.18
B. lanzan 100 mg/kg	30.83 +/- 1.35*	18.67 +/- 0.49*	15.00 +/- 0.52*	11.50 +/- 0.43*	14.00 +/- 0.52*	127.50 +/- 2.60*	16.39
B. lanzan 200 mg/kg	47.00 +/- 1.55**	14.67 +/- 0.49**	11.67 +/- 0.42**	8.83 +/- 0.31**	10.83 +/- 0.40**	96.33 +/- 2.22**	34.31
B. lanzan 400 mg/kg	63.67 +/- 1.96***	10.00 +/- 0.37***	8.00 +/- 0.37***	6.17 +/- 0.31***	7.67 +/- 0.33***	69.83 +/- 1.92***	55.22

Values are mean +/- SEM; n=6 per group. One-way ANOVA followed by Dunnett's test versus control: *p<0.05, **p<0.01, ***p<0.001.



PM = Piper methysticum; BL = Buchanania lanzan. Values are expressed as mean ± SEM. Behavioural experiments: n = 6 animals per group. Receptor-binding assays were performed in triplicate.

Figure 2: Isolation-induced aggression

Piper methysticum reduced attacks from 22.33 +/- 0.76 to 17.33 +/- 0.56, 13.00 +/- 0.52 and 8.00 +/- 0.37 at 100, 200 and 400 mg/kg, corresponding to 22.40%, 41.78% and 64.18% inhibition. *Buchanania lanzan* reduced attacks to 18.67 +/- 0.49, 14.67 +/- 0.49 and 10.00 +/- 0.37, corresponding to 16.39%, 34.31% and 55.22% inhibition. All measured aggressive behaviours followed the same dose-related direction, and *Piper methysticum* was consistently more active at matched doses.

4. Discussion

The acute toxicity findings provided the dose-selection foundation for the behavioural work. A single oral dose of 2000 mg/kg caused no mortality or major clinical toxicity in either group over 14 days. Normal food and water intake, preserved activity and small increases in mean body weight argue against an acute systemic illness severe enough to confound the subsequent dose range. On the basis of the limit test, the approximate oral LD50 was above 2000 mg/kg, allowing 100, 200 and 400 mg/kg to be used as low, intermediate and high screening doses.

This result should not be interpreted as proof that either extract is completely safe. Acute oral toxicity evaluates the consequences of one exposure over a short period. It does not characterize cumulative hepatotoxicity, reproductive effects, genotoxicity, organ pathology or interactions with sedatives and other medicines. This distinction is particularly important for *Piper methysticum* because safety reports have varied with plant part, chemotype, extraction solvent, dose, duration and product quality (Soares et al., 2022). The absence of acute signs supports short-term experimental use at lower doses but does not resolve the wider kava safety debate.

The food shock model produced the expected pattern of aversive aggression in control mice: frequent fights and attacks, repeated biting, rapid onset and prolonged aggressive behaviour. Diazepam strongly suppressed these responses, supporting the sensitivity of the model to centrally acting inhibitory treatment. Both plant extracts significantly reduced the behavioural response, and the graded changes from 100 to 400 mg/kg indicate a dose-response relationship rather than a single isolated difference.

Piper methysticum showed the stronger food shock effect. At 400 mg/kg it reduced fights by 64.60%, compared with 56.64% for *Buchanania lanzan* and 77.87% for diazepam. The high dose also increased attack latency from 18.17 to 67.83 s and reduced aggression duration from 142.33 to 52.50 s. These parallel changes strengthen the inference because the effect was not confined to one count. Kava's central calming properties offer a plausible explanation. Kavain can potentiate GABA-A receptor responses, and kavalactones have been associated with several ion-channel and monoamine-related effects (Chua et al., 2016; Volgin et al., 2020). Enhanced inhibitory control could reduce the hyperarousal, defensive reactivity and impulsive attacks triggered by repeated shock.

Buchanania lanzan also produced a clear, significant and dose-dependent food shock response, although it was less pronounced. The extract may act through a broader stress-protective mechanism rather than a strongly direct GABAergic mechanism. Flavonoids and phenolic compounds can support antioxidant and anti-inflammatory defence and may influence neuronal signalling. A previous study reported adaptogenic activity of methanolic *Buchanania lanzan* leaves, which is compatible with reduced behavioural reactivity under aversive stress (Mehta et al., 2011). Such an interpretation remains provisional because oxidative markers, inflammatory mediators and neurotransmitters were not measured in the restricted study reported here.

The isolation model addressed a different trigger. Seven days of individual housing produced rapid attacks, fighting, biting, chasing and long aggression duration when another male was introduced. Social isolation can alter neuroendocrine regulation, emotional reactivity and territorial behaviour. An effect in this model cannot be explained solely by reduction of shock perception because no electric stimulus is involved. The activity of both extracts across shock and isolation conditions therefore suggests a behavioural influence that extends beyond one experimental trigger.

At 400 mg/kg, *Piper methysticum* reduced attack frequency by 64.18%, increased latency from 20.50 to 73.83 s and reduced aggression duration from 151.67 to 57.17 s. *Buchanania lanzan* reduced attacks by 55.22%, increased latency to 63.67 s and reduced duration to 69.83 s. The high-dose effects were highly significant, while the low and intermediate doses produced smaller but statistically meaningful responses. Diazepam remained more active, with 76.85% inhibition, indicating that neither plant extract matched the standard under these conditions.

The consistent advantage of *Piper methysticum* at equivalent doses supports the original mechanistic expectation. Social isolation may increase anxiety-like arousal and reduce inhibitory behavioural control. Human neuroimaging research has explored kava-associated changes in central GABA, while preclinical literature supports kavalactone interaction with inhibitory systems (Savage et al., 2023). These findings do not prove that the observed mouse effect was mediated by GABA-A receptors, but they make inhibitory neuromodulation a reasonable hypothesis for future antagonist or receptor-assay studies.

For *Buchanania lanzan* , moderate activity in both models is pharmacologically interesting because the plant is not usually classified as a classical CNS sedative. Its chemical profile, reported in the broader thesis, includes flavonoids, phenols, tannins, glycosides and terpenoids. These constituents may act together on oxidative stress, inflammation, stress adaptation or serotonergic regulation. A multi-target effect could reduce emotional reactivity without the strong direct inhibitory profile expected from kava. Confirmation would require measurement of brain monoamines, oxidative stress indices, inflammatory cytokines and receptor-sensitive reversal of the behavioural response.

One major interpretive issue is nonspecific sedation. A less mobile animal may fight less even when aggressive motivation is unchanged. The full thesis included locomotor assessment, but the present paper was intentionally restricted to acute toxicity and two aggression models. Therefore, this article cannot independently exclude all sedative contribution from the data presented here. The preserved general activity in the acute limit study is reassuring but is not a substitute for a locomotor test at the pharmacological doses. Future model-specific work should include open-field activity, rotarod performance or automated movement tracking alongside aggression endpoints.

Another limitation concerns the crude nature of the extracts. The administered preparations contained multiple chemical classes, and their exact active constituents and concentrations were not established in this paper. Batch-to-batch variation could therefore alter efficacy. *Piper methysticum* should be standardized for major kavalactones and potentially safety-relevant flavokavains. *Buchanania lanzan* requires chromatographic markers linked to its phenolic and flavonoid composition. Without such standardization, the results describe the tested batches rather than all preparations of either species.

The experimental design also used small groups of six animals, a common size for exploratory behavioural pharmacology but insufficient for precise effect-size estimation across biological subgroups. Only one sex was used for each phase: females for acute toxicity and males for

aggression. The results may not generalize to female aggression or to repeated dosing. Behavioural scoring may be vulnerable to observer bias unless allocation is concealed and videos are analysed by blinded raters. Future studies should report randomization and blinding in detail, preregister primary outcomes and use sample-size calculations.

Despite these limitations, the convergence of several endpoints is a strength. In both models, treatment increased latency while reducing attack or fight counts, biting and duration. The pattern was dose-dependent and reproducible across two distinct behavioural triggers. The standard drug produced the expected direction and magnitude, supporting assay validity. These features justify describing the extracts as showing anti-aggressive-like activity in mice, while avoiding claims that they treat human aggression.

Translation to clinical use would require a much longer development pathway. Repeated-dose toxicity, clinical chemistry, haematology and histopathology should precede chronic behavioural studies. Mechanistic experiments should test whether receptor antagonists reverse the effects and should quantify GABA, serotonin, dopamine, stress hormones and oxidative markers. Only after chemical standardization, pharmacokinetic characterization and robust animal replication would controlled human studies be ethically defensible. Human aggression is shaped by psychological, psychiatric, cultural and social factors that are not reproduced by these rodent paradigms.

Overall, the findings support two conclusions at the preclinical level. First, the selected doses were substantially below a tolerated single oral limit dose. Second, both extracts reduced stress-provoked and isolation-related aggression in a graded manner, with *Piper methysticum* consistently producing the stronger response. The study therefore identifies *Piper methysticum* as the more promising direct neurobehavioural candidate and *Buchanania lanzan* as a plausible multi-target comparator deserving further mechanistic investigation.

5. Conclusion

Methanolic leaf extracts of *Piper methysticum* and *Buchanania lanzan* produced no mortality or major visible toxicity after a single oral dose of 2000 mg/kg, supporting an approximate oral LD50 above the limit dose and selection of 100, 200 and 400 mg/kg for screening. Both extracts significantly and dose-dependently reduced aggressive behaviour in food shock-induced and isolation-induced models. At 400 mg/kg, *Piper methysticum* produced 64.60% inhibition of food shock fighting and 64.18% inhibition of isolation-induced attacks, compared with 56.64% and 55.22% for *Buchanania lanzan* . Diazepam remained more effective. The results support preliminary anti-aggressive-like activity, not clinical efficacy. Repeated-dose safety studies, locomotor controls, chemical standardization and receptor- or biomarker-based mechanistic experiments are required before therapeutic interpretation.

6. Declarations

Ethics approval: Institutional Animal Ethics Committee approval was reported under CPCSEA Registration No. 1397/Po/Re/S/10/CPCSEA.

Conflict of interest: The author declares no conflict of interest.

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Author contribution: Poonam Yadav contributed to conceptualization, methodology, investigation, data collection, formal analysis, and preparation of the original manuscript.

Sushila Kaura provided supervision, validation, project administration, and critical review and editing. Both authors reviewed and approved the final manuscript.

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