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Efficacy of Jambu (Seed) as an Antidote in Kuchala Poisoning an Experimental Study

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Abstract: ***Kuchala (Strychnos nux-vomica) is spinal poison which is also classified as Upavisha in Ayurveda.Various poisons have been described in Ayurveda along with their antidotes. Mode of action of these antidotes is not mentioned in texts. In Ayurvedic literature According to Basavrajeeyam under the heading of Vish-Prativishani, Jambu (seed) have been described to be possessing antidote action which may act by some way to counter toxicity of Kuchala. It is necessary to verify the efficacy of these antidotes on scientific parameters so that it can be useful in emergencies.***
Objectives: To establish the action and mechanism of Jambu (seed) against toxic effects of Kuchala on albino wistar rats.
Methods: albino wistar rats were selected as an animal model and antidote potential of Jambu (seed) against Kuchala is evaluated by measuring convulsions and histopathological reports of brain and spinal cord.
Results: Jambu (seed) administration before Kuchala poisoning reduced the toxic effects of Kuchala such as convulsion and histopathological changes in brain and spinal cord.
Conclusion: From this study, we can conclude that Jambu (seed) can resist the toxic effects of Kuchala up to some extent.
Keywords: Kuchala, Jambu (seed), Agad, Toxicity, Antidote.

I. INTRODUCTION

One of the ancient disciplines of life, *Ayurveda,* is used to achieve total wellness. It is not only a relic of a bygone era of medicine. It is a science that is founded on the observation of living organisms and their real responses to their surroundings.[1]
The goal of *Ayurveda* can be achieved by maintaining metabolic homeostasis & psycho-somatic condition in the human and to restore it to normalcy when it is disrupted.[2]
Agadtantra is the branch of *Ayurveda* which deals with the toxicity of various snakes, spiders, insects, rats etc. animals and its treatments. The word '*Gada'* means poison and the antidote used is called as '*Agada*'.[3] According to modern science, Toxicology is the branch which deals with the study of poison regarding their sources properties, mode of action, symptoms which they produce, lethal dose, fatal period, treatment of their detection estimation & autopsy findings.[4,5] *Kuchala (Strychnos Nux-vomica*) is spinal poison which is also classified as *Upavisha* in *Ayurveda.*[6,7]
Kuchala, a medium-sized deciduous tree, 15-30 meters in height and up to 70 cm in diameter with fairly straight and cylindrical lobe having dark gray or yellowish gray bark and minute tubercles. Fruits are globular and contain disk-shaped seeds. The seeds are round, disk shaped, concave on one side and convex on the other side. Seeds are ash gray in color and covered with silky fibers. The seeds are about 2.5 cm in diameter and 5 mm in thickness. The pericarp of seed is tough.[8]
If seeds are swallowed uncrushed, the hard pericarp resist digestion and seeds are passed in feces without any poisonous symptoms. With crushed seeds, symptoms begin to appear within 15 to 30 minutes. Bitter taste in mouth. Sense of uneasiness, restlessness, fear and anxiety. Increase difficulty in breathing and swallowing. Muscle twitching and spasm of muscle followed by convulsions. The convulsions last for 30 seconds to 2 minute and are precipitated by slightest stimuli such as sudden noise, a current of air or gentle touching of patient. The convulsions are first clonic but eventually becomes tonic. In between the convulsions, the muscles are completely relaxed and it is an important diagnostic feature.
Various poisons have been described in *Ayurveda* 6-8 along with their antidotes. These antidotes are readily available in nature. Mode of action of these antidotes is not mentioned in texts. In *Ayurvedic* literature according to *Basavrajeeyam, Jambu* (seed) mentioned as an *Prativisha* (Antidote) in *Kuchala* Poison.
Main chemical constituents of *Jambu* (seed) are Jambosine, gallic acid, ellagic acid, corilagin.
It is necessary to verify the efficacy of these antidotes on scientific parameters. so that it can be useful in emergencies. Hence present study "Efficacy of *Jambu* (Seed) as an Antidote in *Kuchala* Poisoning an Experimental Study" in mice was carried out to establish the action and mechanism of *Jambu* (seed) *churna* against toxic effects of *Kuchala* on albino wistar rats.

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II. AIMS AND OBJECTIVES

In vivo study of *Jambu* (seed) as an antidote of *Kuchala* poisoning in wistar rats.

1) To study the toxic effects of *Jambu* (seed) according to OECD Gudieline 423.

2) Experimental study to the efficacy of *Jambu* (seed) as an antidote of *Kuchala.*

III. MATERIAL AND METHODS

A. Sample Collection & Plant Identification

Collection of test sample: A test sample of *Jambu* Seed was collected by the scholar himself from nearby surroundings of Dr S.R. Rajasthan Ayurved University. The test sample was authenticated from BSI (Botanical Survey of India Jodhpur), having Reference no: BSI/AZRC/1.12012/TECH./2021-22 (Pl. Id.) / 13. Collected Jambu (seed) were dried in shade. The samples were used in powder form.

B. Animals

6 albino wistar rats for study the toxic effects of *Jambu* (seed) and 18 rats for Experimental study to the efficacy of *Jambu* (seed) as an antidote of *Kuchala.* were selected for the animal experiment and animals were maintained as per animal ethical committee regulations approved by the Committee for the Purpose of the Control with and Supervision of Experiments on Animals (CPCSEA).

C. Preparation and Administration of Jambu seed churna (Powder)

For preparing *churna* of *Jambu* (seed), the collected *Jambu* (seed) was properly washed then dried, the *Jambu (*seed*)* was then grind in a grinder and then sieved through a fine cloth. Then, the 20 gm *Jambu* (seed) *churna* of was given by mixing it with 1% CMC (carboxy methyl cellulose) as it does not alter any therapeutic value of a drug to be given with the help of oral feeding needle to the rats.

IV. DOSE CALCULATION & JUSTIFICATION

A. Acute oral toxicity study of Jambu (seed) according to OECD Guideline 423.

Group 1: 3 rats and dose will be 300 mg/kg test sample.

Table 1: Group 1 rats marking, weight (in gm), dose (in mg & ml).

S.N.	Marking	Weight (in gm)	Dose (in mg)	Dose (in ml)
1.	H	181	54.3	0.2715
2.	B	128	38.4	0.192
3.	T	137	41.1	0.2055

Group 1: 3 rats and dose will be 2000 mg/kg test sample.

Table 2: Group 2 --rats marking, weight (in gm), dose (in mg & ml).

S.N.	Marking	Weight (in gm)	Dose (in mg)	Dose (in ml)
1.	HB	118	236	1.18
2.	BT	110	220	1.1
3.	HT	158	316	1.58

- *Observations*

Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days. All observations are systematically recorded with individual records being maintained for each animal. Additional observations will be necessary if the animals continue to display signs of toxicity. Observations include changes in skin and fur, eyes, mucous membranes, salivation, lethargy, sleep, coma, convulsions, tremors, diarrhoea, morbidity, mortality. Blood sample was withdrawn with help of capillary from orbital sinus site and all Haematology and Biochemical Analysis was done in automatic haematology and biochemistry analyser.

B. Strychnos nux-vomica induce convulsion in wistar albino rats.

Eighteen healthy albino wistar rats had been selected in this animal model and divided in three groups each group contain six rats. All rats orally received 45 mg/kg of suspension of *Strychnos nux-vomica* for 10 days for induce convulsion.

Group-1: Six Convulsion induced albino wistar rats

Dose form and time - distilled water (control group) (5 ml/kg/P.O.) for 30 days.

Table 3: Group 1 rats marking, weight (in gm), dose (in ml).

S.N.	Marking	Weight (in gm)	Dose (in ml)
1.	H	88	0.44
2.	B	96	0.48
3.	T	106	0.53
4.	HB	175	0.87
5.	BT	134	0.67
6.	HT	113	0.56

Group-2: Six Convulsion induce albino wistar rats

Dose form and time - *Jambu* (seed) (test drug) 200 mg/kg/P.O. for 30 days.

Table 4: Group 2 rats marking, weight (in gm), dose (in mg & ml).

S.N.	Marking	Weight (in gm)	Dose (in mg)	Dose (in ml)
1.	H	105	21	0.105
2.	B	113	22.6	113
3.	T	96	19.2	0.096
4.	HB	96	19.2	0.096
5.	BT	151	30.2	0.151
6.	HT	93	18.6	0.093

Group-3: Six Convulsion induce albino wistar rats

Dose form and time - Standard drug Valporic acid 45 mg/kg.

Table 5: Group 3 rats marking, weight (in gm), dose (in mg & ml).

S.N.	Marking	Weight (in gm)	Dose (in mg)	Dose (in ml)
1.	H	100	4.5	0.02
2.	B	99	4.45	0.02
3.	T	112	5.04	0.02
4.	HB	115	5.17	0.02
5.	BT	99	4.45	0.02
6.	HT	110	4.95	0.02

- *Parameters*

Convulsions and histopathological changes in brain and spinal cord.

V. RESULTS AND DISCUSSION

A. *Acute oral toxicity study of Jambu (seed) according to OECD Guideline423.*

There are no changes in skin and fur, eyes, mucous membranes, salivation, lethargy, sleep, coma, convulsions, tremors, diarrhoea, morbidity, mortality.

Table 6: Hematology and Biochemical Analysis of group 1 & group 2.

S. No.	Parameters	Group 1 Mean Value	Group 2 Mean Value
1.	Haemoglobin (grams per decilitre)	13.58	13.98
2.	WBC (x 10^3/$mm^{3)}$	8.15	8.35
3.	RBC (x 10^6/ $mm^{3)}$	7.25	6.81
4.	Neutrophils (x10^3/ mm^3)	2.85	2.69
5.	Lymphocytes (x 10^3/ mm^3)	6.49	6.82
6.	Eosinophils (x 10^3/ mm^3)	0.05	0.05
7.	Monocytes (x 10^3/ mm^3)	0.02	0.02
8.	Basophiles (x 10^3/ $mm^{3)}$	0	0
9.	SGOT (IU/L)	79.96	81.69
10.	SGPT (IU/L)	67.65	65.96
11.	Serum Creatinine	0.51	0.49
12.	Serum Urea	48.95	45.95

B. *Strychnos nux-vomica induce Convulsion in wistar Albino Rats*

Table 7: Observation Recorded various phases of convulsion of group 1 to group 3.

Marking	Flexion (Mean ± SEM)	Extensor (Mean ± SEM)	Clonus (Mean ± SEM)	Stupor (Mean ± SEM)
Group 1	3.67±0.494	8.50±0.764	3.17±0.601	96.00±4.243
Group 2	2.67±0.615	2.67±0.558	0.00±0.000	35.50±3.519
Group 3	2.33±0.422	0±0.00	0±0.00	0±0.00

Fig. 1: Graphical chart representing observations of group I to group 3 on flexion (convulsions) in nux-vomica poisoning.

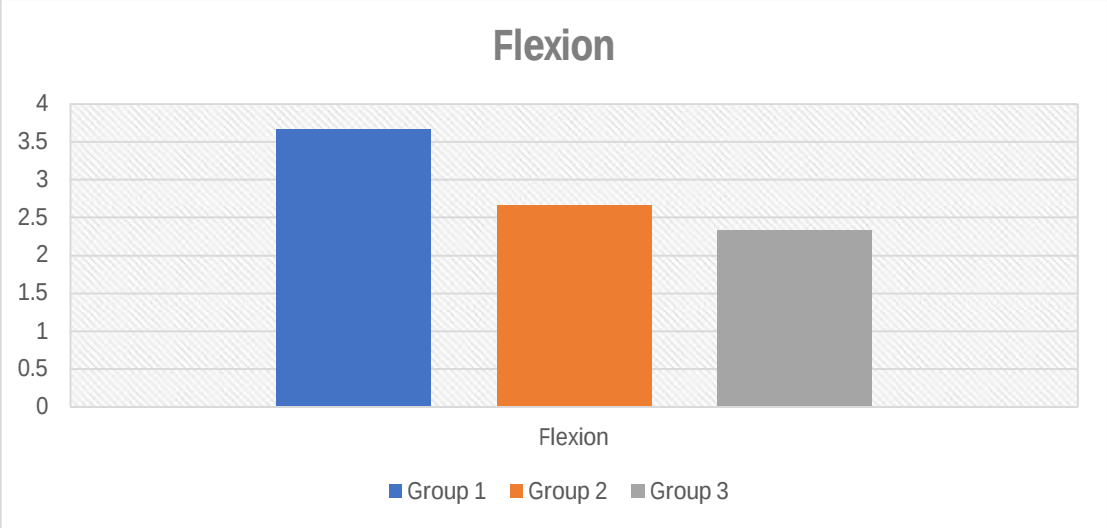


Fig. 2: Graphical chart representing observations of group I to group 3 on Extensor (convulsions) nux-vomica poisoning.

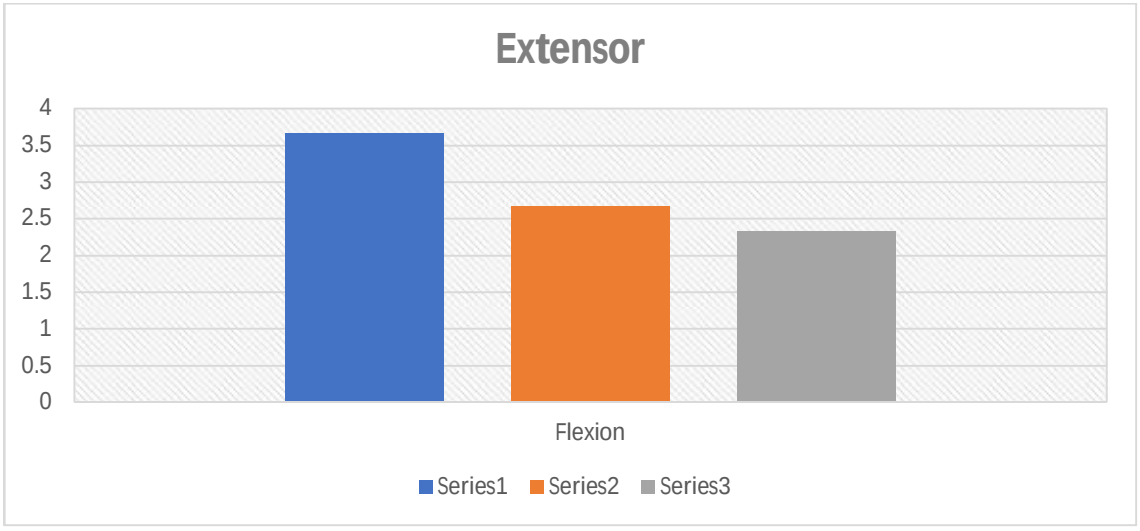


Fig. 3: Graphical chart representing observations of group I to group 3 on Clonus (convulsions) nux-vomica poisoning.

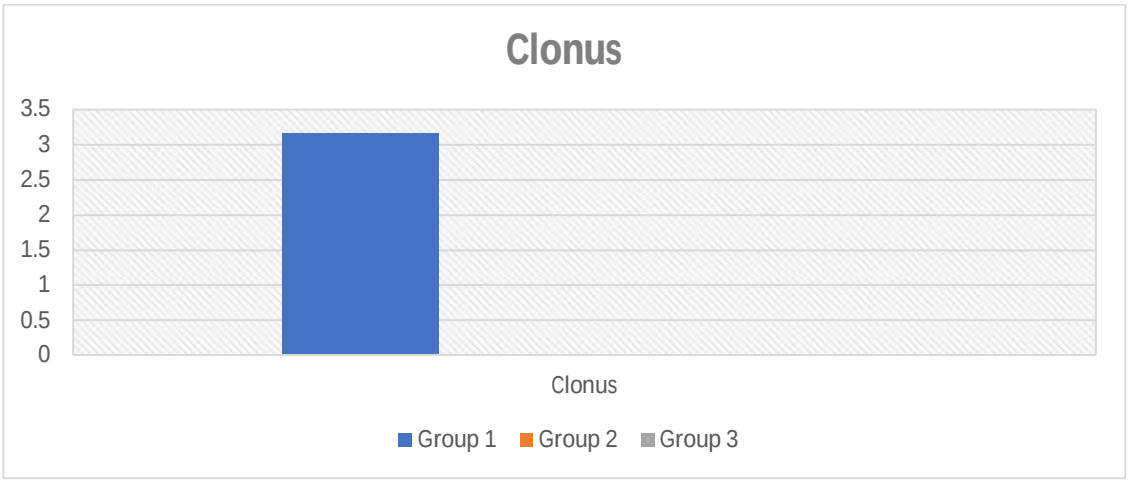
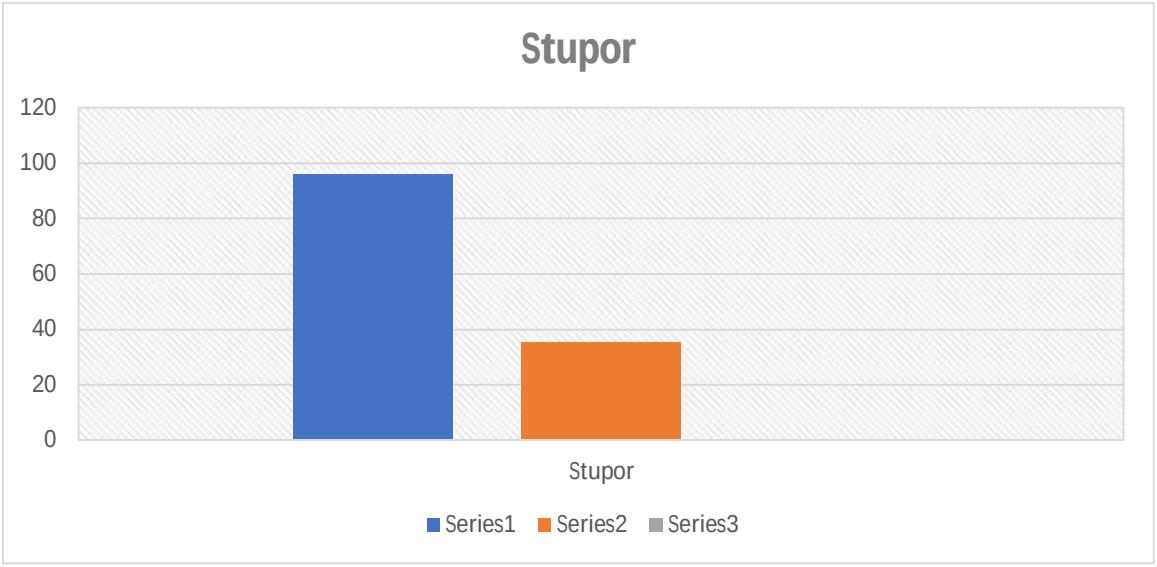


Fig. 8: Graphical chart representing observations of group I to group 3 on Stupor (convulsion nux-vomica poisoning.



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Table 8: Statistical analysis of Flexion (Convulsion) of Group 1 to 3

Flexion					
Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	1.000	-0.7814 to 2.781	No	ns	0.3156
Group 1 vs. Group 3	1.333	-0.4481 to 3.115	No	ns	0.1525

In this study it was observed that when the Dunnett's multiple comparison test was applied between Group 1 vs Group 2, the adjusted 'p" value was 0.3156 and the result was not significant.

Table 9: Statistical analysis of Extensor (Convulsions) of Group 1 to 3

Extensor					
Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	5.833	3.950 to 7.717	Yes	****	<0.0001
Group 1 vs. Group 3	8.500	6.616 to 10.38	Yes	****	<0.0001

In this study it was observed that when the Dunnett's multiple comparison test was applied between Group 1 vs Group 2, the adjusted 'p" value was <0.0001 and the result was significant.

Table 10: Statistical analysis of Clonus (Convulsions) of Group 1 to 3

Clonus					
Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	3.167	1.970 to 4.364	Yes	****	<0.0001
Group 1 vs. Group 3	3.167	1.970 to 4.364	Yes	****	<0.0001

In this study it was observed that when the Dunnett's multiple comparison test was applied between Group 1 vs Group 2, the adjusted 'p" value was <0.0001 and the result was significant.

Table 11: Statistical analysis of Stupor (Convulsions) of Group 1 to 3

Stupor					
Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	60.50	49.52 to 71.48	Yes	****	<0.0001
Group 1 vs. Group 3	96.00	85.02 to 107.0	Yes	****	<0.0001

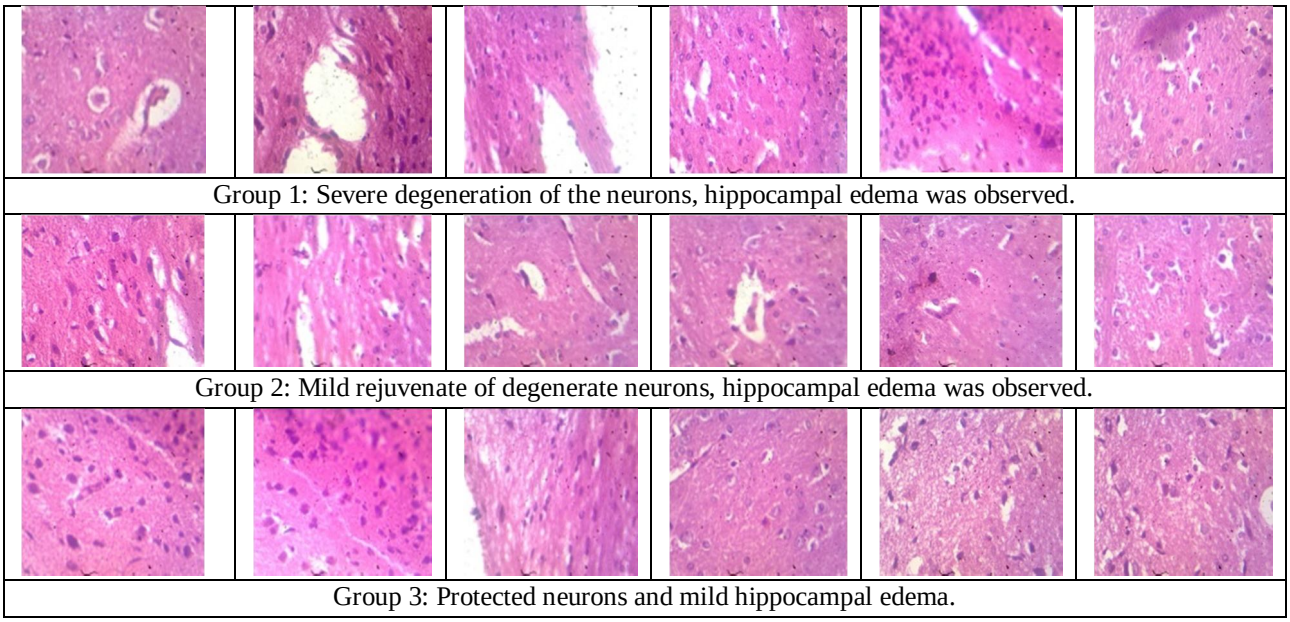
In this study it was observed that when the Dunnett's multiple comparison test was applied between Group 1 vs Group 2, the adjusted 'p" value was <0.0001 the.
The overall result of experimental study on the efficacy of *Jambu* (seed*)* As an antidote in *Kuchala* poisoning is found 64.7%.

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VI. HISTOPATHOLOGICAL CHANGES IN BRAIN AND SPINAL CORD

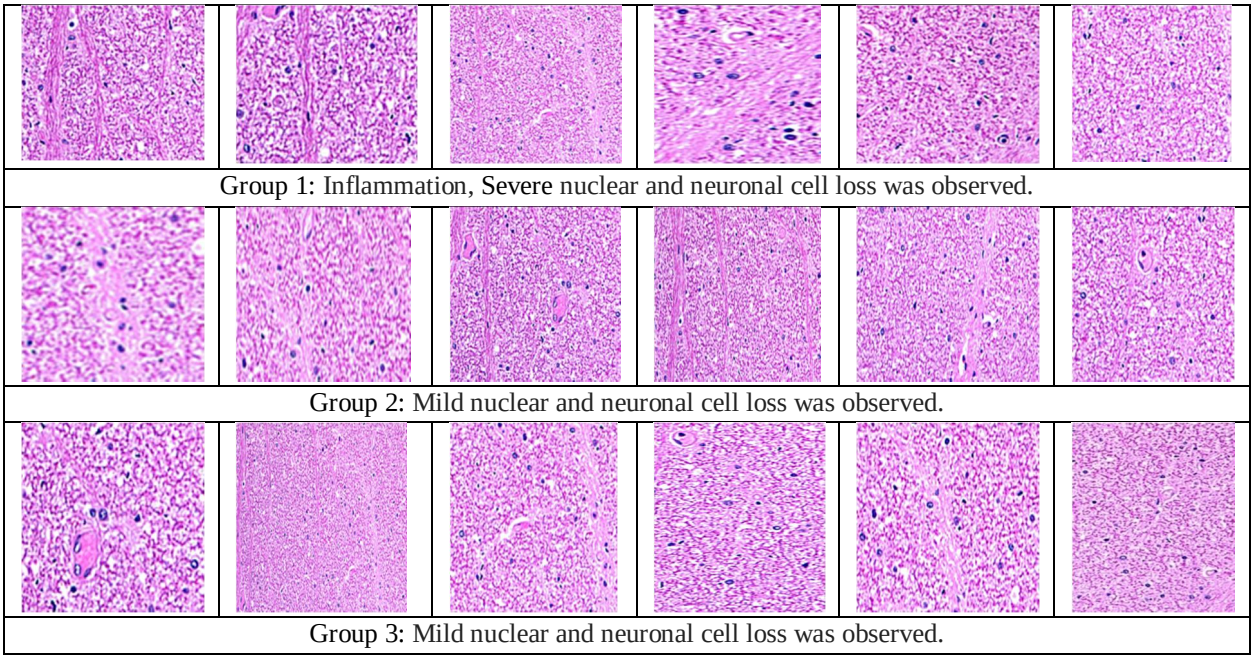
A. *Histopathology of Brain*

Fig. 9: Images of brain after histopathology of group 1 to group 3



B. *Histopathology of Spinal Cord*

Fig. 10: Images of Spinal Cord after histopathology of group 1 to group 3.



In present study "Efficacy of Jambu (Seed) as an Antidote in Kuchala Poisoning an Experimental Study" histopathological report of brain showed mild rejuvenate of degenerate neurons, hippocampal oedema was observed and histopathological report of Spinal cord showed mild nuclear and neuronal cell loss was observed.

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VII. CONCLUSION

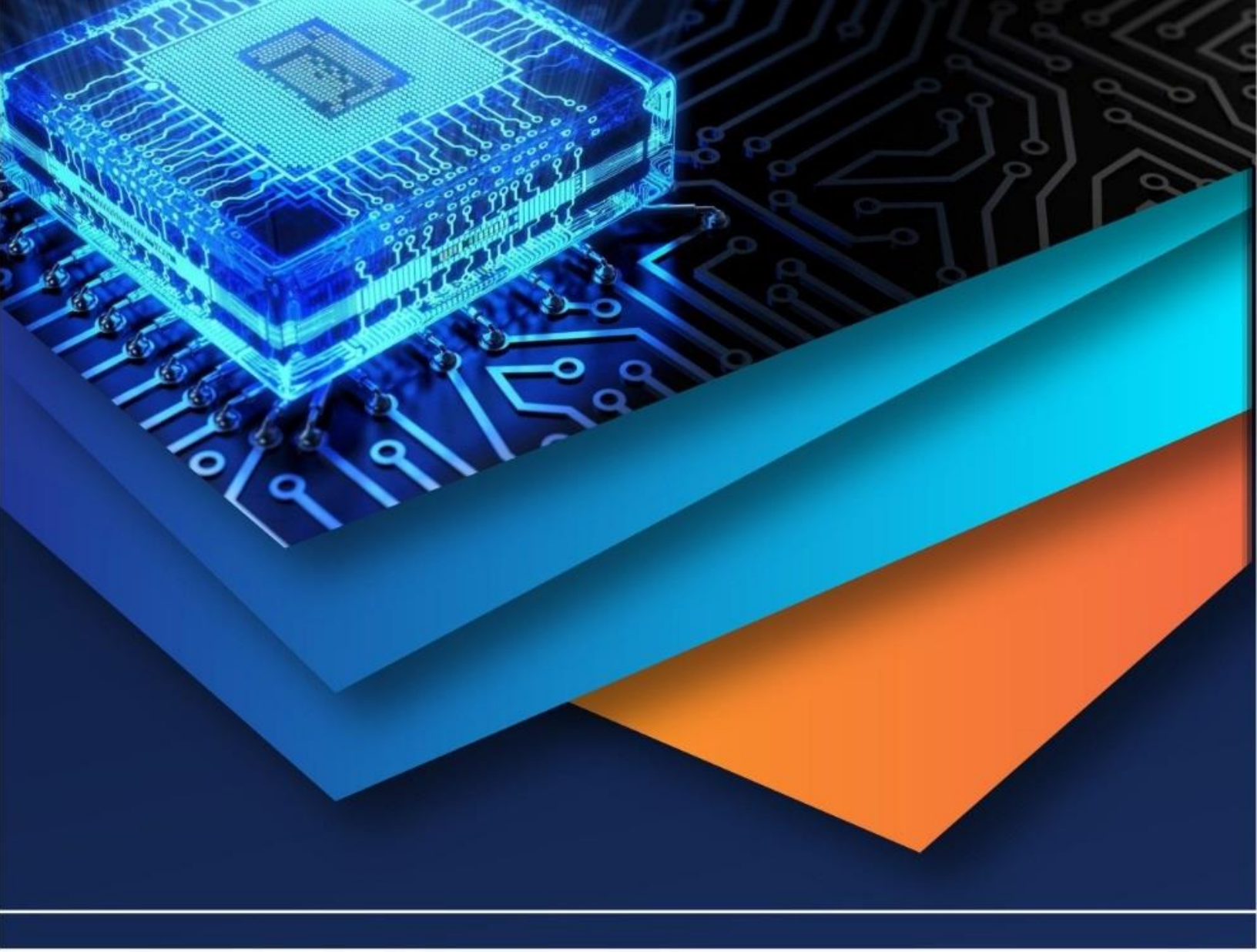
Based upon the results observed during present study "Efficacy of *Jambu* (seed) as an Antidote in *Kuchala* Poisoning an Experimental Study" following conclusions may be drawn: -

1) In Acute Oral Toxicity Study, *Jambu* (seed*) churna* test dose was found to be safe at 2000 mg/kg.
2) Our drug shows 64.7% efficacy against convulsion in *nux-vomica* induced wistar rats.
3) In histopathological report shows the test drug is mild regenerating for severely degenerate neurons in the brain.
4) The histopathological report shows the test drug reduces inflammation and mild regenerates nuclear and neuronal cells of the spinal cord.

Therefore, our test drug is biologically active and showing efficacy against *Kuchala* (nux-vomica) Poisoning.

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