OPEN ACCESS

Saudi Journal of Medical and Pharmaceutical Sciences
Abbreviated Key Title: Saudi J Med Pharm Sci
ISSN 2413-4929 (Print) | ISSN 2413-4910 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article **Zoology**

Assessment of Acute Oral Toxicity, Shrimp Lethality Assay, and Histopathological Effects of Crude Extract of *Chiropsoides buitendijki* in Female Sprague Dawley Rats

Dolnar, J. L[1]*, Zodape, G. V[1]

[1]Department of Zoology, S. S. & L.S. Patkar College of Arts & Science & V.P. Varde College of Commerce & Economics S.V. Road Goregaon (west), Mumbai – 400 104, Maharashtra, India

DOI: https://doi.org/10.36348/sjmps.2025.v11i02.006 | **Received:** 15.01.2025 | **Accepted:** 20.02.2025 | **Published:** 22.02.2025

***Corresponding author:** Dolnar, J. L
Department of Zoology, S. S. & L.S. Patkar College of Arts & Science & V.P. Varde College of Commerce & Economics S.V. Road Goregaon (west), Mumbai – 400 104, Maharashtra, India

Abstract

This study evaluated the acute oral toxicity of a crude extract of *Chiropsoides buitendijki* in female Sprague-Dawley rats and its toxic effects in brine shrimp (*Artemia salina*). Rats were treated with the extract for 14 days, and no clinical signs of intoxication, lethality, or significant changes in body weight, food intake, or behavior were observed. The LD50 value for the rats was determined to be greater than 2000 mg/kg body weight. In brine shrimp lethality assays, the LC50 was calculated to be 20.89 µg/mL. Histological analysis of organs such as the liver, kidney, lungs, brain, spleen, ovary, and uterus revealed no pathological changes at both 300 mg/kg and 2000 mg/kg doses, with tissues maintaining normal structure. These results suggest that while the crude extract demonstrates potent toxicity in brine shrimp, it is non-toxic at high doses in rats and does not cause harm to major organs. The extract shows promise for potential pharmaceutical use, warranting further investigation into its bioactive constituents.

Keywords: Toxicity, lethality, histological, pharmaceutical.

INTRODUCTION

Bioactive compounds are versatile substances with significant potential across various industries due to their health-promoting properties and functional benefits (Banwo *et al.*, 2021). Their applications continue to expand as research explores new sources and potential uses. Currently, 80% of bioactive chemicals are employed in pharmaceuticals, while about 30% are used in the food industry and cosmetics industries (de Jesus Raposo *et al.*, 2013).

Cnidarians exhibit remarkable diversity in their physical forms, categorized into five main groups: Anthozoa, which encompasses true corals, sea anemones, and sea pens; Cubozoa, known for box jellies that possess powerful toxins; Hydrozoa, the most varied group that includes siphonophores, hydroids, fire corals, and numerous medusae; Scyphozoa, referred to as true jellyfish; and Staurozoa, which consists of stalked jellyfishes (Rocha *et al*., 2011). Positioned at the upper levels of the marine food chain, cnidarians can 12 effectively compete with fish for food resources. Consequently, extensive removal of top-predatory fish species through commercial fishing practices could potentially release food sources for jellyfish (Mills, 2001). Additionally, research suggests that salinity plays a significant role in influencing jellyfish behavior and distribution (Catalano *et al*., 1985). Additionally, it was observed that the anticipated decrease in ocean pH levels due to rising CO2 concentrations could result in a long-term increase in jellyfish frequency (Attrill, 2007).

Jellyfish are classified as free-swimming creatures within the phylum Cnidaria. This phylum derives its name from the unique cells known as cnidocytes that are found in cnidarians. Among the many species of edible jellyfish are *Lobonema smithii, Rhopilema esculentum, Nemopilemanomurai, and Lobonemoidesgracilis* (Nishimoto *et al*., 2008). The latter, primarily located in Southeast Asia, yields an annual harvest exceeding 750,000 tons, with demand expanding beyond Asian markets. Jellyfish are

recognized for their potential as a nutritious food source (Sugahara *et al*., 2006) and are known for their nutritional and medicinal value in traditional Chinese medicine. Furthermore, jellyfish species provide cost-effective raw materials for pharmaceuticals, nutraceuticals, and cosmetics, thanks to their high collagen and protein levels (Omori & Nakano, 2001; Li Q.-M *et al*., 2017), along with their antioxidant characteristics and recognition as novel foods in Europe. Earlier research has demonstrated that polysaccharides derived from *N. nomurai* and *R. esculentum* possess anti-inflammatory, antioxidant, and immunomodulatory effects. Additionally, proteins and other compounds extracted from jellyfish show notable antioxidant and various other biological activities.While there are numerous studies focused on cnidocytes for 13 various applications, literature regarding their toxic potential remains limited (Assaw *et al*., 2010).

These creatures are rich in various nutrients such as proteins, amino acids, carbohydrates, vitamins, and minerals, which render them significant both economically and nutritionally (Cao *et al*., 2021). Dried jellyfish is esteemed as a delicacy in numerous Asian nationsRhizostomes are preferred due to their larger size and firmer bodies compared to other scyphozoan orders. When prepared, these rhizostomes yield a product that boasts a desirable, nearly crunchy texture. Furthermore, jellyfish are believed to offer medicinal benefits and are traditionally utilized for treating conditions like arthritis, hypertension, and back pain. Rhizostomes are preferred due to their larger size and firmer bodies compared to other scyphozoan orders. When prepared, these rhizostomes yield a product that boasts a desirable, nearly crunchy texture.

MATERIALS AND METHODS

a) Collection of Samples:

The box jellyfish-*Chiropsoides buitendijki* (Horst, R 1907) were collected during low tides from Edwan village, of West Coast of Mumbai. Animals were taken alive to the laboratory in sea water and then washed the animal two times under sea water and then rinse in distilled water. The collected samples were stored in ice cubes until they were transferred to the deep freezer at - 8º C at the Department of Zoology, S.S. & L.S. Patkar College of Arts & Science, and V. P. Varde College of Commerce & Economics, Goregaon west, Mumbai.

Photograph: Google Map showing the Collection site-Edwan village, of West Coast of Mumbai, Maharashtra

b) Identification of box jellyfish:

Preliminary identification was done by studying the shape and no. of tentacles and by referring the relevant literature and final confirmation of identification was done by Dr. Ramkumar, scientist, at the Central Marine Fisheries Research Institute (CMFRI), Mumbai.

c) Preparation crude Extract of box jellyfish:

Crude extract of *Chiropsoides buitendijki* (Horst, R 1907) was obtained following the 80 % methanol and 1% acetic acid by applying method of Braekman *et al.*, 1992 with some modifications. 10 grams of box jellyfish samples was grinded with blender and then 10 ml mixture of equal volume of 80 % methanol and 1% acetic acid, was added and kept standing for 24 hrs in a water bath at 45º C. The aliquot mixture obtained was filtered through Whatman filter paper No.1. The homogenate centrifuged at 5000 rpm for 15 minutes in cold centrifuge at -8º C (Remi centrifuge serial No. VCDX- 5983). The supernatant was collected

in a conical flask and the aliquot was concentrated in at low pressure using rotary vacuum evaporator at 45º C. The resultant compound was subjected to Millipore filter system and finally dried in vacuum desiccators and stored at -20º C in a refrigerator till further use.

d) Ethical Approval:

Ethical approval is sought from Maharashtra State Biodiversity Board, Nagpur, Maharashtra for collection of box jellyfish samples for research purpose (No.: MSBB/Desk-5/ /Research/ 841/2022-23) and (No.: MSBB/Desk-5/ /Research/ 397/2023-24). The voucher specimen of ***Chiropsoides buitendijki*** was submitted to the repository at the Zoological Survey of India, Western Regional Office, Pune (ZSI-WRC Misc/19), India.

1. ACUTE ORAL TOXICITY

i) Procurement of animals

Twelve female Sprague-Dawley rats (150-200 g) were used for acute oral toxicity testing, sourced from the National Toxicology Centre, Pune. The study was approved by the APT Foundation Ethical Committee (CPCSEA RP. No APTRF/RP-02/2223). Rats were acclimatized for one week in controlled conditions (50-60% humidity, 22 ± 3°C, 12-hour light/dark cycle) and housed in polypropylene cages with commercial food, water, and paddy husk bedding.

Table No. 1: Showing the food consumption, dosing period of female Sprague- Dawley rats administered with crude extract of box jellyfish-*Chiropsoides buitendijki*

1. Species	Rats
2. Strain	Sprague Dawley Rats
3. Source	APT Testing and Research Pvt Ltd, Pune
4. Age and Sex	Female, 06-08 weeks
5. Body weight range	150 to 200 gm
6. Identification	By unique identification number marked by writing on cage tag and by corresponding colour body markings.
7. No. of animals	Three animals were tested in per group.
8. Acclimatization	The rats were housed in their cages for five days prior to start of dosing in the experimental room after veterinary examination.
9. Environmental Conditions	Room temperature was maintained between 22$\pm$3^{0}C, relative humidity 50-60 % and illumination cycle was set to 12 hours light and 12 hours dark.
10. Accommodation	Three rats per cage housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of clean paddy husk.
11. Diet	Pelleted feed supplied by Supplier.
12. Water	Potable water passed through 'Aquaguard' water filter was provided ad libitum in plastic bottles with stainless steel sipper tubes.

ii) Administration of test article

Four groups of female Sprague-Dawley rats (three per group) were given single oral doses of 300 mg/kg (Groups 1 & 2) and 2000 mg/kg (Groups 3 & 4) of the test material via intubation. Observations were made within 30 minutes, periodically in the first 24 hours, and daily for 14 days. Weights were recorded before dosing and weekly thereafter. Weight changes were calculated and documented.

2. SHRIMP LETHALITY ASSAY

The test, based on (Goh *et al*., 2022), involved hatching *Artemia shrimp* cysts in seawater and exposing nauplii to concentrations ranging from 10 to 100 µg/mL. A negative control (seawater) and positive control (0.1 mg/mL potassium dichromate) were used. Mortality was assessed after 24 hours by counting immobile nauplii under a microscope.

3. HISTOPATHOLOGICAL EXAMINATION

After 14 days, the female Sprague-Dawley rats were euthanized, and tissues from the liver, kidney, lung, brain, spleen, ovary, and uterus were collected. These were fixed in 10% formalin, dehydrated through graded ethanol (50-100%), cleared in xylene, and embedded in paraffin. Five-micron sections were prepared, stained with hematoxylin and eosin (H&E), and examined under a Motic B1 Series biological digital microscope for histological changes such as necrosis, fatty degeneration, and other tissue alterations.

RESULTS AND DISCUSSION

Table No. 2: Showing the body weight (gm) of female Sprague- Dawley rats administered with crude extract of box jellyfish-*Chiropsoides buitendijki*

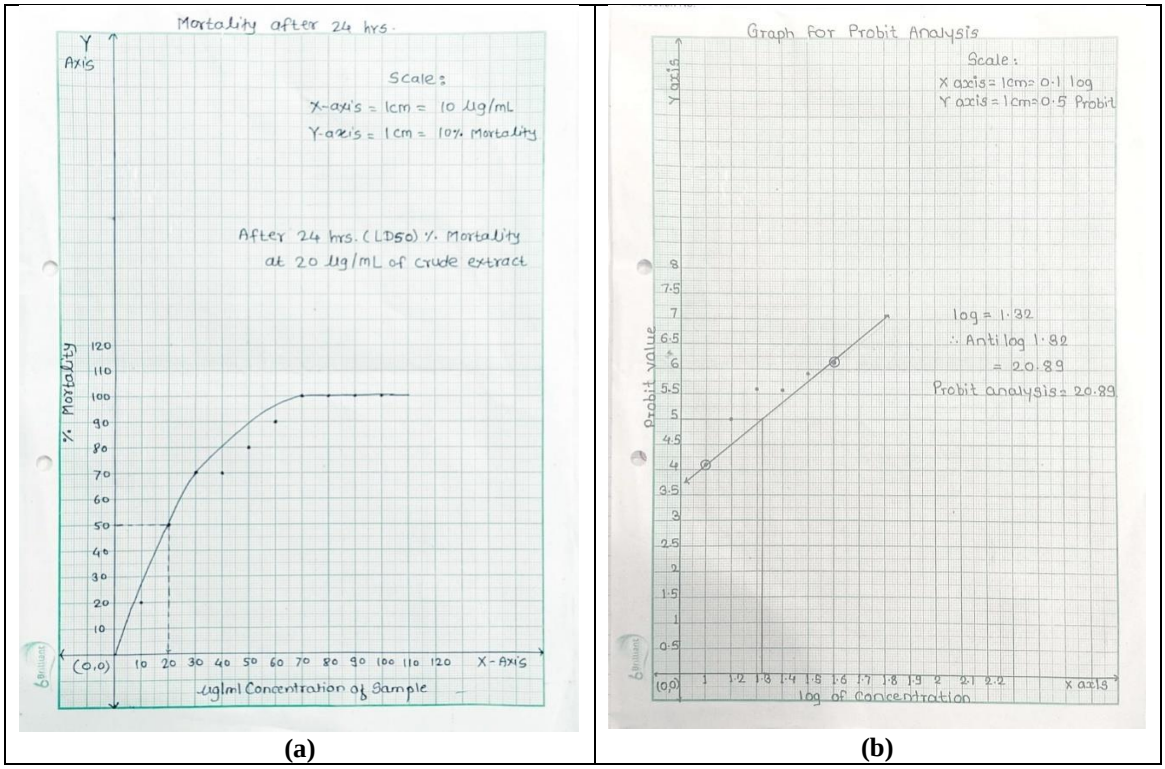
Group		Body Weight (0 Day)	Body Weight (7 Day)	Body Weight (14 Day)
Step 1	Mean	167.8	202.3	217.0
300mg/kg	(±)SD	12.9	16.6	13.8
Step 2	Mean	194.7	215.3	226.7
300mg/kg	(±)SD	43.8	36.5	36.1
Step 3	**Mean**	203.8	221.0	227.7
2000mg/kg	(±)SD	29.3	18.1	10.0
Step 4	Mean	199.0	224.3	232.3
2000mg/kg	(±)SD	17.1	26.4	32.2

(The experiment was conducted in duplicates)

Table No. 3: Showing the effect of crude extract of box jellyfish-*Chiropsoides buitendijki* on *Artemia* shrimp

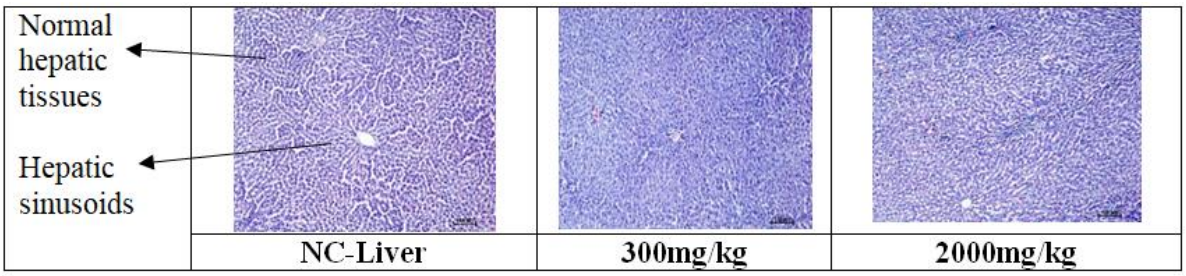
Sr. No	Conc. of Extract	Total no. of Shrimp	Dilution 10ml (%)	Log of conc.	No. of dead Shrimp	% Mortality	Probit value
1	100 µg/ml	10	10	1.000	2	20%	4.16
2	200 µg/ml	10	20	1.3010	5	50%	5.00
3	300 µg/ml	10	30	1.4771	7	70%	5.52
4	400 µg/ml	10	40	1.6028	7	70%	5.52
5	500 µg/ml	10	50	1.6910	8	80%	5.84
6	600 µg/ml	10	60	1.7782	9	90%	6.28
7	700 µg/ml	10	70	1.8451	10	100%	00
8	800 µg/ml	10	80	1.9031	10	100%	00
9	900 µg/ml	10	90	1.9542	10	100%	00
10	1000 µg/ml	10	100	2.000	10	100%	00

(Each analysis was achieved by triplicates)

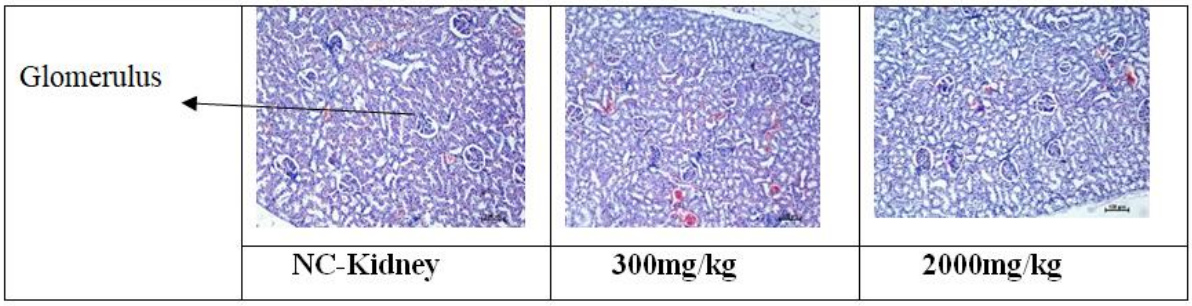


(a) **(b)**

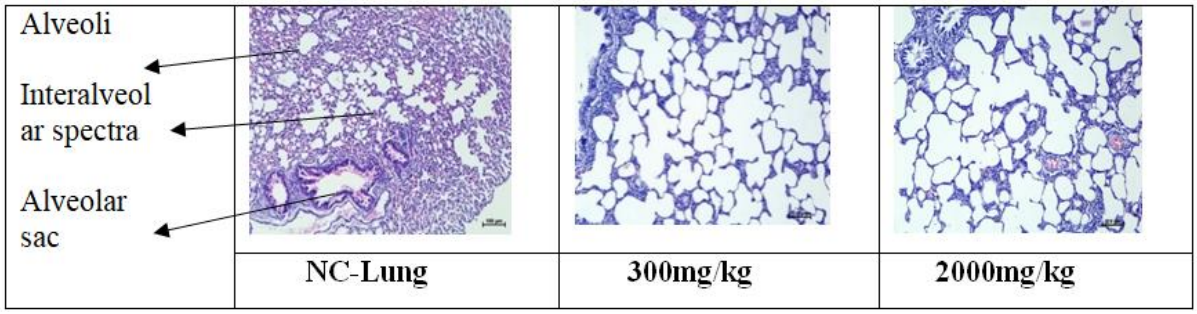
Graph No. (a, b): Showing effect of concentration crude extract of box jellyfish-*Chiropsoides buitendijki*and Probit value by shrimp lethality assay



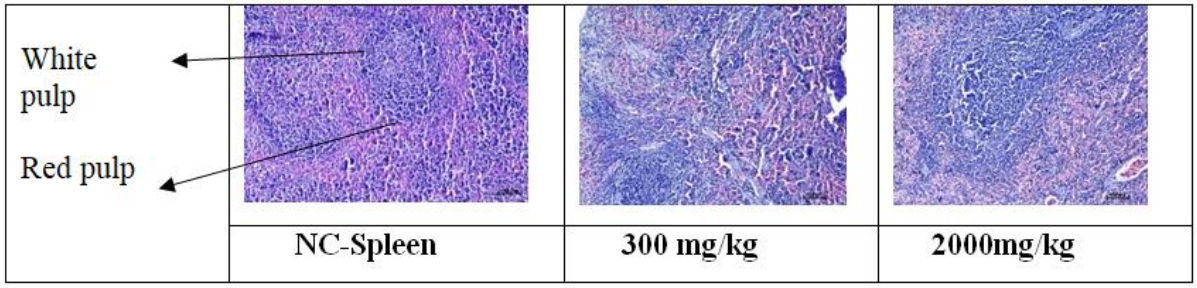
Photograph No. (1.1): Showing histopathological consideration of Liver tissues of female Sprague dawley rats



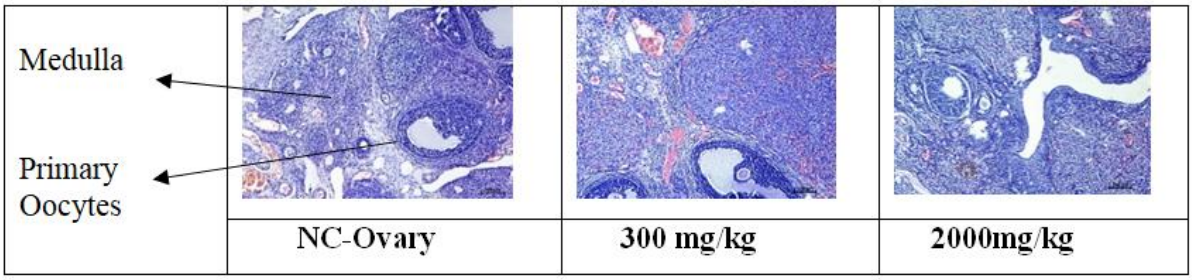
Photograph No. (1.2): Showing histopathological consideration of kidney tissues of female Sprague dawley rats



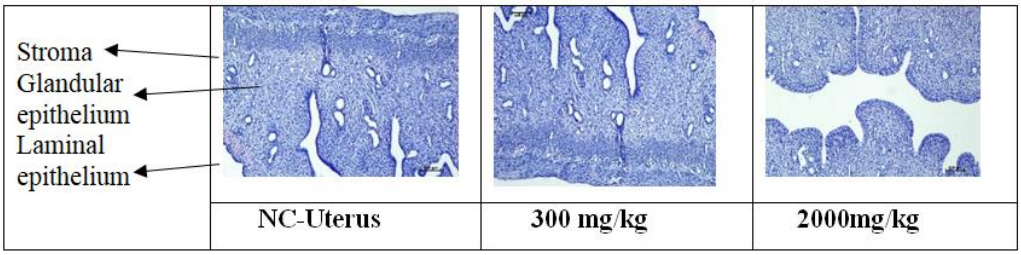
Photograph No. (1.3): Showing histopathological consideration of Lungs tissues of female *Sprague dawley* rats



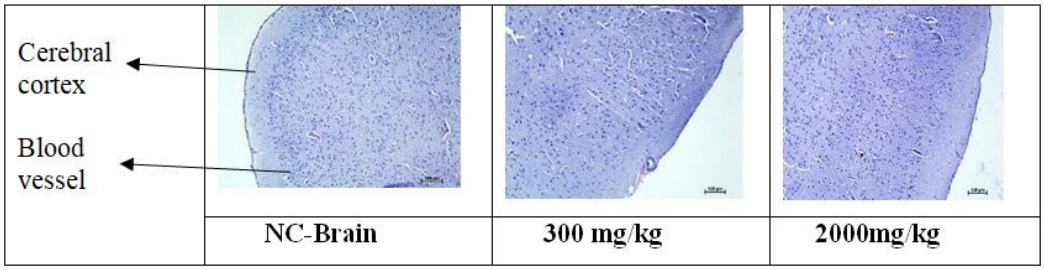
Photograph No. (1.4): Showing histopathological consideration of Spleen tissues of female *Sprague dawley* rats



Photograph No. (1.5): Showing histopathological consideration of Ovary tissues of female *Sprague dawley* rats



Photograph No. (1.6): Showing histopathological consideration of Uterus tissues of female *Sprague dawley* rats



Photograph No. (1.7): Showing histopathological consideration of Brain tissues of female *Sprague dawley* rats

This study investigated the acute oral toxicity of *Chiropsoides buitendijki* crude extract in female Sprague-Dawley rats. Organ weight changes, a key indicator of toxicity, were assessed, as they often reflect organ damage or stress (Piao *et al*., 2013; Sellers *et al*., 2007). Unlike other marine toxins, such as those from *Cyanea capillata* and *Anthopleura asiatica*which exhibit severe toxicity (Liang *et al*., 2011; Ramkumar *et al*., 2012). In the present study,Table No. (1) and (2), the crude extract of *Chiropsoides buitendijki* did not cause significant alterations in organ weight or histology. There were no signs of intoxication or lethality, suggesting a lack of acute toxicity at doses up to 2000 mg/kg. Comparatively, other jellyfish species have shown potent toxicities (Takenori *et al*., 2013), but the extract from *Chiropsoides buitendijki* appears to be relatively safe at the tested dose, warranting further research into its bioactive components.

The brine shrimp lethality assay is a cost-effective and reliable alternative to traditional bioassays for studying toxicity, with applications in evaluating marine toxins (Ruebhart *et al*., 2008). Numerous studies have shown that marine organisms, including corals and jellyfish, exhibit significant toxicity in Artemia salina, with LC50 values ranging from 3.2 to 125.9 mg/mL (Goh *et al*., 2022; Lee *et al*., 1981). Toxins from species like *Callyspongia* and *Palythoacaribaeorum* also demonstrated lethal effects on brine shrimp (McCaffrey & Enden, 1985; Liany *et al*., 2012). Box jellyfish toxins, such as those from *Carybdearastoni* and *Chiropsalmus quadrigatus*, are known for their lethal effects on crustaceans (Nagai *et al*., 2000; 2002). These findings suggest that marine toxins, including those from box jellyfish, contain potent bioactive compounds that may have pharmacological applications, particularly in anticancer and pain-relief therapies (Towle *et al*., 2001; Rinehart, 2000).In this study, the acute oral toxicity of the crude extract of *Chiropsoides buitendijki* was evaluated in female Sprague-Dawley rats, revealing an LD50 value greater than 2000 mg/kg. From Table No. (3) and Graph (a, b), toxicity testing using brine shrimp (*Artemia salina*) showed an LC50 of 20.89 µg/mL, indicating the presence of potent toxins in the extract. These findings suggest that the extract contains bioactive compounds with potential pharmaceutical applications. The toxicity level was found to be influenced by solvent type and environmental factors, which affect the production of toxic metabolites in the jellyfish.

Ingestion of box jellyfish toxins, known for their neurotoxic effects, causes human poisoning, often from contaminated seafood. Despite extensive research on box jellyfish toxins, data on their acute oral toxicity in humans is limited, necessitating animal model studies. Abal *et al*., (2017) demonstrated that box jellyfish toxins were detected in mice blood within hours, while Hong *et al*., (2017) reported rapid absorption in rats, with distribution to organs like the stomach, kidneys, and intestines. Histopathological studies on *Chiropsoides buitendijki* are rare. For instance, Suvik and Mohd (2017) found no significant liver or kidney damage in rats given *Lobonema smithi* extract, suggesting safety. In contrast, Ramkumar *et al*., (2012) observed nephrotoxicity and hepatotoxicity from *Anthopleura asiatica* venom, highlighting the need for further histological research on *Chiropsoides buitendijki* toxins. In our study, we examined the histological changes in various organs of female Sprague Dawley rats after administering crude extract of *Chiropsoides buitendijki* at doses of 300 mg/kg and 2000 mg/kg. The organs studied included the liver, kidneys, lungs, spleen, ovary, uterus, and brain. Histopathological examination revealed no significant alterations in the tissues at either dose level. Fig No. (1.1) showing the effect of crude extract on liver, normal hepatic parenchyma with normal histomorphology of hepatocytes with intact cellular features. No metabolic or pathological cellular lesions in

the liver tissues observed. Fig No. (1.2) showing the effect of crude extract on kidney, normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma observed. Fig No. (1.3) showing the effect of crude extract on lungs, displayed healthy bronchi and alveolar tissue. Fig. No. (1.4) showing the effect of crude extract on spleen, normal histomorphological features of red pulp and white pulp. Adequate lymphoid cellular population in the lymphoid tissue observed. Fig No. (1.5) showing the effect of crude extracton ovary, normal histomorphological features of ovarian tissue with presence of developing follicles and corpus luteum observed. Fig No. (1.6) showing the effect of crude extract on uterusnormal histomorphology of myometrial glands in uterine tissues were observed. maintained normal histoarchitecture, with no evidence of inflammation or pathological changes observed. Fig No. (1.7) showing the effect of crude extract on brain,normal histomorphology of neuronal tissue and supporting tissue matrix in the brain cerebrum and cerebellum observed. Based on these findings, it can be concluded that the crude extract of *Chiropsoides buitendijki* at doses of 300 mg/kg and 2000 mg/kg does not cause adverse effects on the histology of the studied organs, suggesting that these doses may be well-tolerated in rats. Further studies are needed to determine the potential effects of higher doses beyond 2000 mg/kg.

SUMMARY

In our study, we assessed the acute oral toxicity of *Chiropsoides buitendijki* crude extract in female Sprague Dawley rats. The rats were treated for 14 days with no clinical signs of intoxication, behavioral changes, or significant alterations in body weight. This indicates that the LD50 for acute oral toxicity in rats is greater than 2000 mg/kg. Additionally, toxicity was evaluated using the brine shrimp lethality assay, where the LC50 was calculated at 20.89 µg/ml. Histological examination of organs (liver, kidney, lungs, brain, spleen, ovary, and uterus) showed no adverse effects at both 300 mg/kg and 2000 mg/kg doses. All tissues exhibited normal histoarchitecture, confirming that the crude extract did not cause histopathological damage at these dose levels.

CONCLUSION

According to the results above, we discovered that the LD50 value in the female Sprague dawley rats is greater than 2000 mg/kg. However, the brine shrimp-*Artemia salina*, lethality assays showed LC50 calculated at 20.89 µg/mL. In histological examination of liver, kidney, lungs, brain, spleen, ovary and uterus of the female Sprague dawley rats showed normal histo-architecture thus, it confirms that the screened crude extract *Chiropsoides buitendijki* showed LD50 is greater than 2000 mg/kg, in Sprague dawley rats.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Ramkumar, scientist, at the Central Marine Fisheries Research Institute (CMFRI), Mumbai for final identification and confirmation of species. Authors are also thankful to the Director, Maharashtra State Biodiversity Board, Nagpur, Maharashtra for giving permission for collection of species. Dr. Kishori Apte, National Toxicological Centre, APT Testing & Research Pvt. Ltd. Pune, Maharashtra for supervising and assisting during the toxicological studies.

Conflict of Interest: Authors have no conflict of interest.

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