Open Access Research Journal of
Science and Technology
Journals home page: https://oarjst.com/
ISSN: 2782-9960 (Online)

OARJ OPEN ACCESS RESEARCH JOURNALS

(RESEARCH ARTICLE)

Herbal formulation: Constac plus granules for severe constipation; an acute oral toxicity study

Ashwin Dhanrajji Porwal [1,*], Gajanan Bhagwan Bhagwat [2], Pravin Prakash Kamble [2], Mangesh Mohan Rode [2] and Sudhir Dinkar Borate [3]

[1] *Healing Hands Clinic, Ground Floor, Millennium Star Extension, Dhole Patil Road, Pune, 411001, Maharashtra, India.*
[2] *R and D Center, Healing Hands and Herbs Pvt. Ltd., Tilak Road, Pune, 411002, Maharashtra, India.*
[3] *Indian Drugs Research Association and Laboratory, Shivaji Nagar, Pune, 411005, Maharashtra, India.*

Open Access Research Journal of Science and Technology, 2023, 08(02), 048–058

Publication history: Received on 26 June 2023; revised on 04 August 2023; accepted on 07 August 2023

Article DOI: https://doi.org/10.53022/oarjst.2023.8.2.0046

Abstract

The main aim of this study was to evaluate the acute oral toxicity of Constac® Plus granules. Twelve rats were randomized into four groups of three at random. Groups 2 and 3 received doses of 2000 mg/kg body weight of Constac Plus granules, while group 4 received doses of 5000 mg/kg body weight of Constac Plus granules. Group 1 served as the control and merely received distilled water. For 14 days, the rats were closely monitored for any indications of toxicity. At the end of the study, all rats underwent necropsy and gross pathology was noted. The results demonstrated that during the 14-day observation period, none of the rats in the control or treatment groups showed any clinical symptoms of toxicity or mortality. It was determined that the LD_{50} value was more than 5000 mg/kg body weight. The acute oral toxicity study of Constac Plus granules in wistar rats concluded that there was no toxicity at dosages up to 5000 mg/kg body weight. These results proved that the usage of Constac Plus granules administered orally is safe.

Keywords: Constac® Plus; Severe Constipation; Acute Oral Toxicity Study; Herbal Formulation; LD_{50} value

1. Introduction

Ayurveda relies mainly on plants since they have a variety of therapeutic properties that can be used to treat and prevent a variety of diseases. As a result, Ayurvedic medicine has worked to establish a strong base by using plants as a source of its medicines [1]. Most Ayurvedic medicines are based on the idea of a single plant source or a mixture of several plant sources (polyherbal). In comparison, polyherbal medicines have higher efficacy than single herb medicines [2]. Currently, 70% of the world's population depends on herbal medicines. Due to lower dosage levels, convenience and ease of administration, herbal formulations have gained widespread recognition in comparison to crude plant materials and extracts that are the primary source of therapeutic agents to treat human diseases [3]. Plants have a variety of harmful phytochemical components despite having therapeutically useful secondary metabolites. These plant-based therapeutic medicines may have long-term harmful/toxic effects on individuals, primarily toxicity to the liver and kidneys [4]. Therefore, toxicity studies are equally crucial to demonstrating the potentially toxic effects of traditional herbal medicines in detail and ensuring their safety within a scientific framework for long-term therapeutic usage. Additionally, many people take these medicines for self-medication. The need to clarify the advantages and hazards of herbal medications for health should be increased because there is limited data available about the safety of the most widely used herbal remedies [5].

Acute toxicological studies look into the harmful outcomes of a single, high-dose exposure to a toxin that lasts no more than 24 hours. The individual may suffer severe biological consequences as a result (harm or death). The outcomes of acute toxicity are crucial for designing chronic toxicological investigations as well as for examining accidental chemical

* Corresponding author: Ashwin Dhanrajji Porwal

poisoning [6]. The development of tolerance is usually revealed by acute exposure. An acute encounter typically reveals the emergence of tolerance. The dose used in acute toxicity testing results in 50% of experimental animals dying is known as the LD_{50} value, and it is the starting point for the toxicological classification of drugs [7].

The formulation in the current study is mainly used for severe constipation. The earlier clinical study on Constac® Plus confirmed that the formulation is safe and effective for treating severe chronic constipation and its associated symptoms [8,9]. Constac Plus granules is an Ayurvedic polyherbal formulation consisting of Haritaki (*Terminalia chebula* Retz.), Baheda (*Terminalia bellirica* (Gaertn.) Roxb.), Amalaki (*Phyllanthus emblica* L.), Balharitaki (*Terminalia chebula* Retz.), Sanay (*Cassia angustifolia* Vahl), Yashtimadhu (*Glycyrrhiza glabra* L.), Yavani (*Trachyspermum ammi* Sprague), Shatapushpa (*Foeniculum vulgare* Mill.), Ela (*Elettaria cardamomum* (L.) Maton), Nishottar (*Operculina turpethum (L.)* Silva Manso), Erand tail (*Ricinus communis* L.) and Narikel lavan (Processed salt with coconut). *Terminalia chebula* Retz. increases the stools and has the property of evacuating the bowels completely as per the short-term clinical study carried out on patients with simple constipation [10, 11]. Fruits of *Terminalia bellirica* (Gaertn.) Roxb. are used as laxatives [12, 13]. Also, the study conducted on mice revealed that *Phyllanthus emblica* L. has a positive effect on gut disorders like indigestion and constipation [14]. As a laxative, *Cassia angustifolia* Vahl is widely known for its ability to relieve constipation [15]. Yashtimadhu (*Glycyrrhiza glabra* L.) in the present formulation has proven laxative activity [16, 17]. Yavani (*Trachyspermum ammi* Sprague) and Shatapushpa (*Foeniculum vulgare* Mill.) are used traditionally for flatulence [18, 19]. The seeds of (*Elettaria cardamomum* (L.) Maton) possess Antioxidant and laxative activity [20]. Furthermore, Nishottar (*Operculina turpethum (L.)* Silva Manso), Erand tail (*Ricinus communis* L.) and Narikel lavan have proven to have laxative activity [21-23].

In accordance with OECD Guidelines No. 423 [24], the objective of this study was to find out the acute oral toxicity profile of Constac Plus granules in wistar female rats. The results of this study may offer a scientific perspective on the safety of Constac Plus granules.

2. Material and methods

2.1. Polyherbal formulation

The Polyherbal formulation Constac® Plus granules (Batch No.: HCO2203) (https://pilospray.com/product/constac-plus-granules-100g-pack-of-2/) were obtained from Healing Hands & Herbs Pvt. Ltd. Pune, India., (www.myhealinghands.in) and it was manufactured under the GMP certified manufacturing unit at Eisen Pharmaceutical Co. (Pvt.) Ltd. Each 100 gm granules contain Haritaki (*Terminalia chebula* Retz.), Baheda (*Terminalia bellirica* (Gaertn.) Roxb.), Amalaki (*Phyllanthus emblica* L.), Balharitaki (*Terminalia chebula* Retz.), Sanay (*Cassia angustifolia* Vahl), Yashtimadhu (*Glycyrrhiza glabra* L.), Yavani (*Trachyspermum ammi* Sprague), Shatapushpa (*Foeniculum vulgare* Mill.), Ela (*Elettaria cardamomum* (L.) Maton), Nishottar (*Operculina turpethum* (L.) Silva Manso), Erand tail (*Ricinus communis* L.) and Narikel lavan (Processed salt with coconut). The 2000 mg and 5000 mg test suspensions were prepared in distilled water to yield 200 mg/ml and 500 mg/ml solutions, respectively. The oral route of administration was chosen because it was the intended clinical route of administration and was based on the human clinical dose (3.0-5.0 grams) [8], complying with OECD/WHO guidelines and the indication of Constac Plus granules. The rat received only fresh suspension through oral gavage. A volume of 10 ml/kg of the suspension was administered.

2.2. Experimental Animals

LACSMI Biofarms Pvt. Ltd. in Pune, Maharashtra, India, provided the wistar rats. They were kept in stainless steel cages and maintained in a room with a 12-hour light/dark cycle, 30–70% humidity, 19–25^{0}C temperature and adequate ventilation. All animals had complete access to food and water. Throughout the study period, individuals were recognized by tail marking, and information from the cage cards was used to identify the group of animals housed in each cage. The experiments were carried out in accordance with IDRAL's standard operating procedures and the requirements established by the Committee for Control and Supervision of Experiments on Animals (CCSEA), as published in The Gazette of India on December 15, 1998. Institutional Animal Ethics Committee (IAEC) protocol 085/1222 (IDRAL/IAEC-3-2022) was approved on December 23, 2022.

2.3. Acute Oral Toxicity Study

Using a fixed dose method, the acute toxicity test was conducted in accordance with OECD guideline 423 [23]. According to OECD guidelines, testing one sex (often females) is enough. [22,25] Twelve 8–9-week-old female wistar rats weighing 180–189 g (Figure 1) were divided into one of four subgroups, labelled as G1, G2, G3 and G4. Three rats were in each group, with Group 1 (G1) serving as the control. Prior to the dose, all rats were fasted for the previous night and food was given 3 to 4 hours later. All the rats received an oral dose in a constant dosage volume of 10 ml/kg body weight

after the 2000 mg and 5000 mg test suspensions were produced in distilled water for obtaining 200 mg/ml and 500 mg/ml solutions, respectively. For 14 days, all the animals were monitored for any indications of acute oral toxicity. In line with the Globally Harmonised System (GHS) for chemical classification that causes acute toxicity, the Constac Plus granules will subsequently be graded and classified.

Figure 1 Female wistar rats before dose administration

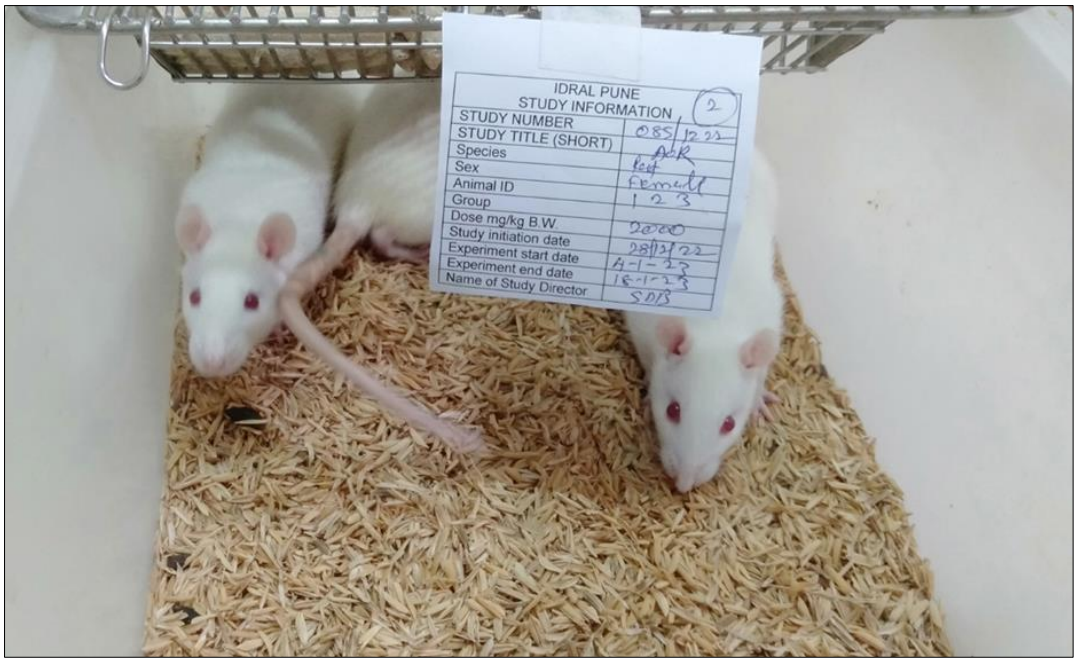

Figure 2 Female wistar rats after dose administration

2.4. Observational Parameters

All animals were closely monitored for treatment-related clinical symptoms, morbidity and mortality after oral administration of Constac Plus granules at various time intervals of 30 min, 1 hr, 2 hr, 4 hr, and 6 hr post-dosing on the first day and once daily thereafter for 14 days. (Figure 2)

2.5. Body weight

Body weight measurements were taken on the day before the dose (day 0), the treatment day (fasting body weight), at weekly intervals after that, and on the day of the mortality. On days 7 and 14 after dosing, the average body weights and weight gains for the group were calculated.

2.6. Necropsy and Gross Pathology

All of the remaining animals were humanely put to death by carbon dioxide asphyxiation at the end of the experiment. Gross pathological alterations in every animal used in the study were observed and documented.

3. Results

3.1. Acute oral toxicity

All animals were administered a single oral dose of 2000 mg/kg and 5000 mg/kg of Constac Plus granules. They all survived throughout the experimental period and did not show any signs of toxicity immediately after dosing and during the observation period of 14 days. The animals in the vehicle control group (G1) were completely normal, and no deaths were seen during the observation period of 14 days after the administration of the Constac Plus (Tables 2 and 3). Additionally, none of the animals in the control group (G1) (Table 4) exhibit any anomalies in their individual animal fate and necropsy findings. The body weight gain after 7 and 14 days was found to be 6.97% and 17.42% respectively.

Table 1 Overall Incidence of Mortality after receiving polyherbal formulation (Constac Plus granules) for 14-day observations in the acute oral toxicity study

Group	Dose (mg/kg)	Mortality	
		Females	
		Absolute	%
G1	0	0/3	0
G2	2000	0/3	0
G3	2000	0/3	0
G4	5000	0/3	0

Table 2 Individual Animal Clinical Signs & Mortality (G1) (Distilled water treatment)

Animal ID	Observation at: hrs.					Days												
	½	1	2	4	6	2	3	4	5	6	7	8	9	10	11	12	13	14
	Female wistar Rats																	
1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Total mortality	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mortality%	0																	

N-Normal

Table 3 The rat body weight (G1) (Distilled water treatment)

Group	Animal ID	Sex	Dose (mg/kg)	Body weight(g) before and after receiving Distilled water			Weight gain	
				Day 0	Day 7	Day 15	% Gain (Day 7)	% Gain (Day 15)
G1	1	Female	0	181	196	211	8.29	16.57
	2			182	195	218	7.14	19.78
	3			182	192	211	5.49	15.93
Mean ± SD				181.66 ± 0.57	194.33 ± 2.08	213.33 ± 4.04	6.97 ± 1.40	17.42 ± 2.06

The formula for the % weight gain is as follows

$$\% \text{ Weight Gain (Day 7 or 15)} = \frac{\text{Day 7 or 15 (Weight after 7 or 15 days)} \times 100}{\text{Day 0 (Initial weight)}}$$

% Weight Gain (Day 7 or 15) = Answer-100

Table 4 Individual animal fate and necropsy finding (G1) (Distilled water treatment)

Animal No.	Fate	Necropsy Findings	
		External Observations	Internal Observations
1	TS	NAD	NAD
2	TS	NAD	NAD
3	TS	NAD	NAD

NAD: No Abnormalities Detected; TS: Terminal Sacrifice

Animals in groups 2 and 3 (G2 and G3) received a dose of about 2000 mg/kg of Constac Plus granules. During the 14-day post-dosing observation period, no deaths were noted, and all of the animals were in good condition. (Tables 5, 6 and 7). Furthermore, neither the outcomes of the necropsies nor the individual animal fate for any of the animals in groups 2 or 3 showed any inconsistencies (Table 8).

Table 5 Individual Animal Clinical Signs & Mortality (G2) (2000 mg/kg)

Animal ID	Observation at: hrs.					Days												
	½	1	2	4	6	2	3	4	5	6	7	8	9	10	11	12	13	14
	Female wistar Rats																	
4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Total mortality	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mortality%	0																	

N-Normal

Table 6 Individual Animal Clinical Signs & Mortality (G3) (2000 mg/kg)

Animal ID	**Observation at: hrs.**					**Days**												
	½	**1**	**2**	**4**	**6**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
	Female wistar Rats																	
7	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
8	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Total mortality	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mortality%	0																	

N-Normal

Table 7 The rat body weight (G2 & G3) (2000 mg/kg)

Group	**Animal ID**	**Sex**	**Dose (mg/kg)**	**Body weight(g) before and after receiving Constac Plus granules**			**Weight gain**	
				Day 0	**Day 7**	**Day 15**	**% Gain (Day 7)**	**% Gain (Day 15)**
G2	4	Female	2000	183	191	211	4.37	15.30
	5			185	196	216	5.95	16.76
	6			190	199	217	4.74	14.21
Mean ± SD				186 ± 3.60	195.33 ± 4.04	214.66 ± 3.21	5.02 ± 0.82	15.42 ± 1.27
G3	7	Female	2000	184	192	212	4.35	15.22
	8			188	197	217	4.79	15.43
	9			187	196	218	4.81	16.58
Mean ± SD				186.33 ± 2.08	195 ± 2.64	215.66 ± 3.21	4.65 ± 0.26	15.74 ± 0.73

Table 8 Individual animal fate and necropsy finding (G2 & G3) (2000 mg/kg)

Animal No.	**Fate**	**Necropsy Findings**	
		External Observations	**Internal Observations**
4	TS	NAD	NAD
5	TS	NAD	NAD
6	TS	NAD	NAD
7	TS	NAD	NAD
8	TS	NAD	NAD
9	TS	NAD	NAD

NAD: No Abnormalities Detected; TS: Terminal Sacrifice

Animals in Group 4 (G4) received a dose of roughly 5000 mg/kg of Constac Plus granules. No mortality rates were noted throughout the 14-day post-dosing observation period, and all animals were healthy. Furthermore, neither the necropsy data nor the individual animal fate indicated any abnormalities in any of the Group 4 animals.

Table 9 Individual Animal Clinical Signs & Mortality (G4) (5000 mg/kg)

Animal ID	**Observation at: hrs.**					**Days**												
	½	**1**	**2**	**4**	**6**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
	Female wistar Rats																	
10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
12	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Total mortality	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mortality%	0																	

N-Normal

Table 10 The rat body weight (G4) (5000 mg/kg)

Group	**Animal ID**	**Sex**	**Dose (mg/kg)**	**Body weight(g) before and after receiving Constac Plus granules**			**Weight gain**	
				Day 0	**Day 7**	**Day 15**	**% Gain (Day 7)**	**% Gain (Day 15)**
G4	10	Female	5000	183	193	213	5.46	16.39
	11			189	195	218	3.17	15.34
	12			187	196	216	4.81	15.51
Mean ± SD				186.33 ± 3.05	194.66 ± 1.52	215.66 ± 2.51	4.48 ± 1.18	15.74 ± 0.56

Kruskal Wallis test was used at a 5% level of significance to check the percentage weight gain in wistar rats varies with the dose.

H0: The median percent weight gain across the three-dose level is equal. Vs. **H1:** At least one of the median percent weight gains is different from others

Where,
H0 = Null Hypothesis
H1= Alternative Hypothesis

P- value for the above test is 0.3377 (> 0.05), so we accept the null hypothesis at a 5% level of significance. i.e., group population medians are equal. It shows that the weight gain percentage in wistar rats does not differ significantly across the dose.

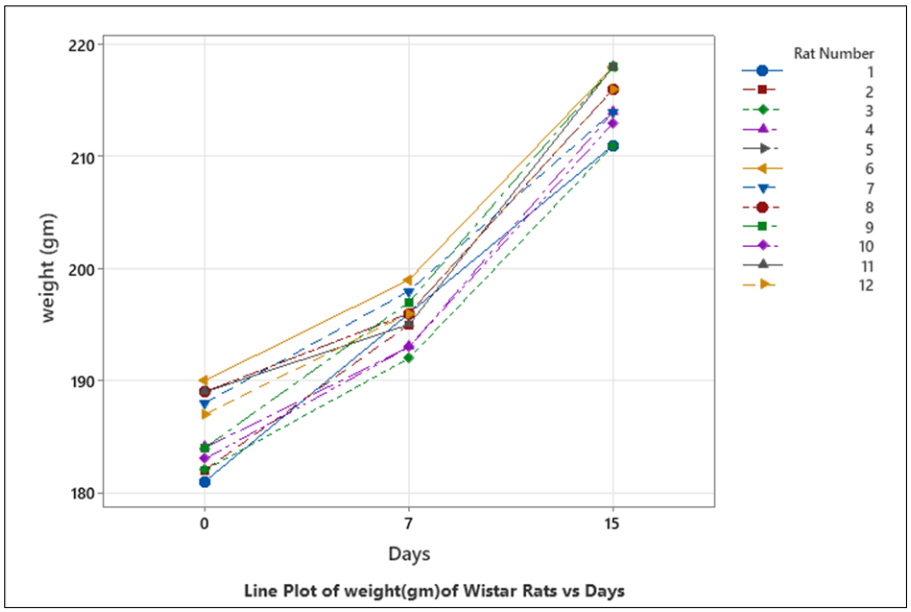

Figure 3 Line plot of Weight (gm) of wistar rats vs. Days

Table 11 Individual animal fate and necropsy finding (G4) (5000 mg/kg)

Animal No.	Fate	Necropsy Findings	
		External Observations	Internal Observations
10	TS	NAD	NAD
11	TS	NAD	NAD
12	TS	NAD	NAD

NAD: No Abnormalities Detected; TS: Terminal Sacrifice

Figure 4 Gross Pathology Image of Vital organs – Liver, kidney, Heart, Spleen, Adrenals, Sex organs & G.I

4. Discussion

Unsatisfactory defecation characterized by infrequent stools, difficulty stool passage or both are generally referred to as constipation [26, 27]. Most studies indicate that the prevalence of constipation in North America is between 12% to 19% [28]. Constipation can be treated in a variety of ways, including increasing fluid intake, eating more foods high in fiber, increasing physical activity, taking fiber supplements, prescribing osmotic or stimulant laxatives as well as even pelvic floor therapy [29]. The universe's natural resources have consistently provided chemotherapeutic medicines. As a result, natural products and their derivatives constitute the majority of the active ingredients in many pharmaceuticals currently used in clinical care [30]. Due to their relatively higher effectiveness, low cost and decreased invasiveness, natural remedies for constipation have received a lot of attention recently [31, 32].

Acute oral toxicity assessment of Constac Plus granules was conducted on healthy female wistar rats. The oral median lethal dosage (LD_{50}) of the formulation in rats was found to be greater than 5000 mg/kg body weight. This proves that the formulation is typically non-toxic when taken orally. The Constac Plus granules can be classified as category 5 GHS (Globally Harmonised System for Chemical Classification Substances and Mixtures) in accordance with 2001 OECD standards 423 of acute toxicity because the highest dose of 5,000 mg/kg body weight did not result in animal death. The principal findings, among a number of additional toxicity indicators, clinical signs and symptoms, show the harmful effects of medications on critical body organs [33]. The essential organs of our body—liver, kidney, heart, lungs, and spleen— (Figure 4) are the main metabolic targets of any toxic material [34]. There were no unusual weight changes and no fatalities in any of the groups. No anomalous or altered behaviour was seen in the treatment groups or the vehicle group in terms of clinical symptoms. Additionally, during the 14-day treatment period, no signs of mortality were seen, and the necropsy results and individual animal fates were both normal. During the final necropsy on day 14, there were no obvious gross pathological alterations in the organs of the treated rats.

5. Conclusion

Acute oral LD_{50} of Constac Plus granules in wistar rats is greater than 5000 mg/kg body weight, and the GHS classification category is 5 or Unclassified (>5000) based on the parameters of the current study and the results obtained. These results concluded that using Constac Plus granules orally is safe.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The experiments were carried out in accordance with IDRAL's standard operating procedures and the requirements established by the Committee for Control and Supervision of Experiments on Animals (CCSEA), as published in The Gazette of India on December 15, 1998. Institutional Animal Ethics Committee (IAEC) protocol 085/1222 (IDRAL/IAEC-3-2022) was approved on December 23, 2022.

References

[1] Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants (Basel). 2017;6(4):42.

[2] Pushpa VH, Jayanthi MK, Shashank PM, Ramu R. Pharmacological profile of Shiva Gutika: an uncharted and versatile polyherbal drug. All Life. 2021;14(1):215-219.

[3] Shivaraj Y, Kavri KR, Asia Nuzhat FB, Rajesh R. Acute oral toxicity study of "Polyherbal formulation (Rosmarinus officinals, Ashwagandha and Amla) in Wistar rats". 2021;9(4):16-27.

[4] David A, Enegide C. The importance of toxicity testing. Journal of Pharmaceutical and BioSciences. 2013;4:146-148.

[5] Riaz A, Khan RA, Ahmed S, Afroz S. Assessment of acute toxicity and reproductive capability of a herbal combination. Pak J Pharm Sci. 2010;23(3):291-294.

[6] Merlin LKM, Komlaga G, Arnold DF, Caleb F, Anning AK, Dickson RA. Toxicity and Safety Implications of Herbal Medicines Used in Africa. Herbal Medicine. 2019.

[7] WHO. The WHO Recommended Classification of Pesticides by Hazard, and Guidelines to Classification. Geneva: WHO Press, 2010:1-81.

[8] Ashwin P, Deepak K, Paresh G. Clinical evaluation for safety, efficacy and adverse drug reaction of Constac Plus an improved polyherbal formulation when used for chronic constipation. wjpmr. 2018;4(8):133-136.

[9] Ashwin P. Paresh G, Jaya M, Deepak K. The effect on Constac Plus in the management of severe chronic constipation: A Pilot Study. International Journal of Ayurveda and Pharma Research. 2016;4(2):1-4.

[10] Mukherjee P, Roy S, Bhattacharyya S, Debnath PK, Biswas TK, Jana U, et al. Clinical study of Triphala- a well known phytomedicine from India. Indian J Pharmcol Ther. 2006;5:51–54.

[11] Sheng Z, Yan X, Zhang R, Ni H, Cui Y, Ge J, et al. Assessment of the antidirrhoeal properties of the aqueous extract and its soluble fractions of Chebulae Fructus (Terminalia chebula fruits). Pharmaceut. Biol. 2016;54(9):1847-1856.

[12] Deb A, Barua S, Das B. Pharmacological activities of Baheda (Terminalia bellerica): A review. Journal of Pharmacognosy and Phytochemistry. 2016;5(1):194-197.

[13] Sharma R, Raizada S, Gautam A, Bhatia A. Phytochemical and Antibacterial Analysis of Terminalia chebula and Terminalia bellirica. Green Chemistry in Environmental Sustainability and Chemical Education. 2018;131-137.

[14] Mehmood MH, Rehman A, Rehman U, Gilan AH. Studies on Prokinetic, Laxative and Spasmodic Activities of Phyllanthus emblica in Experimental Animals. Phytotherapy research. 2012;27(7):1054-1060.

[15] Mohammad SAK. A Review on Senna: An Excellent Prophetic Herbal Medicine. wjpmr. 2020;6(7):113-118.

[16] Saxena S. Glycyrrhiza glabra: Medicine over the millennium. Natural Products Radiant. 2005;4(5):358-367.

[17] Sharma V, Agarwal RC. Glycyrrhiza glabra- A Plant for the Future. Mintage Journal of Pharmaceutical & Medical Sciences. 2013;2(3):15-20.

[18] Ranjan B, Manmohan S, Singh SR, Singh RB, Kumar TA. Medicinal Uses of Trachyspermum ammi: A Review. Pharmacologyonline. 2011;2:477-485.

[19] Badgujar SB, Patel VV, Bandivdekar AH. Foeniculum vulgare Mill: A Review of Its Botany, Phytochemistry, Pharmacology, Contemporary Application, and Toxicology. BioMed Research International. 2014.

[20] Anwar F, Abbas A, Alkharfy KM, Gilani AH. Cardamom (Elettaria cardamomum Maton) Oils. Essential Oils in Food Preservation, Flavor and Safety. 2016:295-301.

[21] Ahmed T, Husain MK, Tariq M, Siddiqui JI, Khalid M, Ahmed MW, et al. A Review on Operculina turpethum: A Potent Herb of Unani System of Medicine. Journal of Pharmacognosy and Phytochemistry. 2017;6(1):23-26.

[22] Jena J, Gupta AK. Ricinus Communis Linn: A Phytopharmacological Review. International Journal of Pharmacy and Pharmaceutical Science. 2012;4(4):25-29.

[23] Sushama D. A Review on Pharmaceutical and Therapeutic Aspects of Narikel Lavan-An Ayurveda Remedy for Digestive Disorder. IJAAR. 2019;IV(IV):361-366.

[24] OECD guidelines for testing of chemicals, No. 423, 'Acute Oral Toxicity – Acute Toxic Class Method'. The Organization for Economic Co-operation and Development (OECD) guidelines for the testing of Chemicals, adopted by the council on 17th December 2001.

[25] Ashwin DP, Gajanan BB, Pravin PK, Sudhir DB. Acute oral toxicity study of Acideem Plus Tablet: An Ayurvedic polyherbal formulation. International Journal of Science and Research Archive. 2023;9(2):332-341.

[26] Brandt LJ, Prather CM, Quigley EM, Schiller LR, Schoenfeld P, Talley NJ. Systematic review on the management of chronic constipation in North America. Am J Gastroenterol. 2005;100(1):S5-S21.

[27] Andrews CN, Storr M. The pathophysiology of chronic constipation. Can J Gastroenterol. 2011;25(B):16B-21B.

[28] Higgins PD, Johanson JF. Epidemiology of constipation in North America: a systematic review. Am J Gastroenterol. 2004;99(4):750-759.

[29] Mearin F, Ciriza C, Mínguez M, Rey E, Mascort JJ, Peña E, et al. Clinical Practice Guideline: Irritable bowel syndrome with constipation and functional constipation in the adult. Rev Esp Enferm Dig. 2016;108(6):332-63.

[30] Baker DD, Chu M, Oza U, Rajgarhia V. The value of natural products to future pharmaceutical discovery. Natural product reports. 2007;24(6):1225-44.

[31] Ashwin P, Gopal KC, Gajanan B, Ramesh B. Polyherbal formulation Anoac-H suppresses the expression of RANTES and VEGF for the management of bleeding hemorrhoids and fistula. Mol Med Rep. 2021;24(4):736.

[32] Porwal A, Kundu GC, Bhagwat G, Butti R. Herbal medicine AnoSpray suppresses proinflammatory cytokines COX-2 and RANTES in the management of hemorrhoids, acute anal fissures and perineal wounds. Exp Ther Med. 2022;23(1):86.

[33] Subramanion LJ, Zakaria Z, Chen Y, Lau YL, et al. Acute oral toxicity of methanolic seed extract of cassia fistula in mice. Molecules. 2011;16(6):5268-5282.

[34] Auletta CS. Acute, Subchronic and Chronic Toxicology. CRC Press, New York, 1995.