

## Assessment of the Dermal Toxicity of *Hydnocarpus wightiana* Seeds Extract in Experimental Rats

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This investigation aims to determine the dermal safety dose range for the entire *Hydnocarpus wightiana* plant. The acute and subacute skin toxicity investigations were evaluated using the OECD criteria 402 and 410. In the acute dermal toxicity study, the extract dosages (2000 and 5000 mg/kg) were topically given in single doses. The overall behaviour, adverse effects, and death were noted for up to 72 hours. Subsequently, in sub-acute dermal research, the various concentrations of the extract were topically given at doses of 500, 1000, and 2000 mg/kg for 28 days, and any changes were recorded. In the two groups (i.e., 2000 and 5000 mg/kg extract treatment groups), no significant behavioural alterations, sleepiness, or drowsiness were seen, and only mild sedation and lethargy were noted. Overall, there were no discernible poisoning signs or symptoms. Giving extracts (2000 mg/kg) improved the liver function parameters considerably. It was discovered that no signs were observed on the dermal application of the extract.

**Keywords:** Acute toxicity study; Dermal Toxicity; Ethanolic extract of *Hydnocarpus wightiana* (EEHW); *Hydnocarpus wightiana*; Sub-acute toxicity study.

Plants are widely accepted, effective, affordable, safe, and low-cost, making them useful as alternative medicines for treating a variety of ailments. The belief that herbal formulations are safe and natural, enabling them to effectively cure a variety of ailments, has led to an increase in the public's use of these products<sup>1</sup>. Other researchers have reported that the production process of certain herbal treatments may entail the addition of metals (like cadmium) from the soil, or they may include heavy metals, aflatoxins, and hazardous bacteria<sup>2</sup>.

Herbal medicines do not have any of the negative or hazardous side effects linked to synthetic drugs used in traditional treatment because they are sourced from nature<sup>3</sup>. As with conventional orthodox therapies that are well researched and manufactured, the toxicity of traditional herbal medicines should be examined, although this is not frequently the case<sup>4</sup>. Rather, it should be done for legal and registered herbal medicinal items. As a result, those who use herbal medicine usually focus on the items' medical

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benefits rather than their harmful effects on various organs; nevertheless, toxicity research should also be highlighted.

The plant species known as Chaulmoogara (*Hydnocarpus wightiana*) belongs to the daisy family. Although it is sometimes referred to as a Chaulmurga, it is distinct from the popular blooming plant of the same name. Because of its calming and antibacterial qualities, calendula is applied topically to heal wounds and provides nourishing benefits for the skin. Additionally, it could help with radiation dermatitis, leg ulcers (venous and neuropathic), gingivitis, mucositis from radiation, vaginal candidiasis, episiotomy healing, and nappy dermatitis. It possesses cytotoxic, anticancer, and anti-inflammatory properties<sup>5</sup>. The result is the likelihood of toxicity having an impact on a whole organism, such as a plant, bacterium, or animal. It encompasses impacts on the substructure of the body, including hepatotoxicity (effects on the liver) and dermatotoxicity (effects on the skin, cells, and organs). These interactions can differ based on the cell membrane and the molecular characteristics of the toxicants,<sup>6</sup> since they might take place in the extracellular matrix, underneath tissues, on the cell surface, and inside the cell body. Furthermore, toxic effects must be seen before toxicants attach to vital organs such as the liver, spleen, or kidneys. Determining a drug's level of toxicity is important for public health since chemical exposure can be harmful to the body. The issue of medicinal plants is attracting the attention of several medical institutes around the globe. Furthermore, there is currently a dearth of studies on the relevant dosage toxicity of therapeutic plants. It should be stressed, therefore, that customary usage of any plant for biological reasons does not, under any circumstances, ensure the safety of the plants<sup>7</sup>.

Traditional medicine was used by the ancient Indians long before modern medicine was developed, and it is today an essential aspect of Indian culture. In-depth information about traditional Indian medicinal plants may be found in the books "Charaka Samhita" and "The Way of Ayurvedic Herbs". The daisy family of plants is well-known in India for having a multitude of bioactive substances that may be used to cure a wide range of ailments, and this book served as a useful starting point for phytochemicals and ethnobotanists who wanted to research and analyse

therapeutic plants. All of this study adds to our understanding of Indian medicinal herbs<sup>8</sup>. We concluded that the safe dose range of an ethanolic extract made from *Hydnocarpus wightiana* seeds is not well-established based on acute and subacute dermal toxicity tests conducted on laboratory animals. This was the result of an appropriate evaluation of the literature.

## MATERIALS AND METHODS

### Plant Materials: Collection and Authentication

In March 2024, the seeds of *Hydnocarpus wightiana* were harvested in the Garhwal region, specifically in the Chamoli, District of Uttarakhand. The seeds were washed and air-dried. Authentication was done by the Scientist in Charge, Forest Research Institute, Dehradun, India. A voucher specimen was submitted to the department (Authentication No: NIScPR/RHMD/Consult/2024/430-31).

### Preparation of seed extract

*Hydnocarpus wightiana* seeds were dried at room temperature, ground into a rough powder, and then sieved through sieve number 18. The powdered seeds were defatted using petroleum ether (40–60%) to get rid of fat and other pigments. The defatted dry seeds powder (Marc) was extracted using different solvents according to polarity like ethyl acetate, ethanol and water in that order using a Soxhlet system. In a rotatory evaporator, the extracted materials were concentrated until they were completely dry, eliminating any remaining solvent. The extracts were kept between 2 and 8 degrees Celsius in a refrigerator<sup>9</sup>.

### Animals

We obtained 180–250 g of healthy Wistar rats (Both Sexes) from the Moradabad animal house of IFTM University. The animals were housed in polypropylene cages with a 12:12 light/dark cycle, a temperature of 28°C, and a relative humidity of 60–70%. The animals were given unrestricted access to regular pellet meals during the trial. The animal may be given mineral water in exchange for a fee. The acute toxicity and pharmacology methods were approved by the Institutional Animal Ethics Committee of IFTM University in Moradabad (IAEC/2022/01).

### Skin preparation for dermal toxicity study

After applying hair removal cream and shaving with a hand razor, at least 10% of the rat's skin around the dorsal thoracic area was exposed, under OCED recommendations nos. 402 and 410. The entire procedure was carried out under anaesthesia using xylene (5 mg/kg) and ketamine (50 mg/kg). The rats' dorsal areas were treated topically with seed extract. (Figure no. 7.1) <sup>10</sup>.

### Acute Dermal Toxicity Study

The OECD guideline 402 was used to evaluate the acute dermal toxicity research. All of the study's rats were given unrestricted access to water and fasted for the entire night before the trial. Every animal was divided into four groups, with six non-pregnant or nulliparous rats in each group.



**Fig. 1.** After application of ointment

**Table 1.** Groups have been classified as follows

Sr. No.	Sample Treatment	Dose Regimen (mg/kg)
Group (I)	Blank	None
Group (II)	Simple Paraffin	5000
Group (III)	EEHW	2000
Group (IV)	EEHW	5000

EEHW: Ethanolic extract of *Hydnocarpus wightiana*

The acute dermal toxicity research employed a limited dosage, or 2000 mg/kg b.w., by OCED recommendations; this dose can be regarded as a lower dose.

An additional dose, 5000 mg/kg, is included for a better understanding of the toxicity; this dose is to be regarded as a larger dose. Conversely, the positive control group was given 10%w/w white soft paraffin at a dose of 5000 mg/kg. On the initial day of the investigation, each dosage was administered topically once, to the rats' dorsal side. For the first 24 hours, all of the rats were observed, and the first 6 hours received extra care. Following 14 days, all rats underwent rigorous weight assessment and were assessed for other criteria, including biochemical and haematological, by OCED recommendations <sup>11</sup>.



**Fig. 2.** After application of ointment

**Table 2.** Groups have been classified as follows

Sr. No.	Sample Treatment	Dose Regimen (mg/kg)
Group (I)	Blank	None
Group (II)	Simple paraffin	2000
Group (III)	EEHW	500
Group (IV)	EEHW	1000
Group (V)	EEHW	2000

EEHW: Ethanolic extract of *Hydnocarpus wightiana*

### Divisions of animals in different groups for acute toxicity study

#### Sub-acute dermal toxicity study

Sub-acute dermal toxicity studies were evaluated in accordance with OCED standard 410. For this investigation, the rats were divided into five groups, each consisting of five male and five female rats. In this investigation, the EEHW was administered topically once daily for 28 days, during which all animals were observed and under the guidance of a camera (Figure no. 2). Every

operation and observation was carried out under OECD guideline 410<sup>12</sup>.

#### Divisions of animals in different groups for sub-acute toxicity study

##### Termination of the experiment

Upon completion of the sub-acute dermal toxicity testing, each animal underwent an overnight fast. For euthanasia, a large dosage of ketamine HCl (80 mg/kg i.p.) was administered. The blood sample was taken via heart puncture and was thereafter put into bottles, both heparinized and

**Table 3.** Observation of general behaviours

Observation	Control Group Group-I	Simple Paraffin Group-II	2000mg/kg EEHW Group-III	5000mg/kg EEHW Group-IV
Body Weight	N	N	N	N
Temperature	N	N	N	N
Food intake	N	N	N	N
Urination	N	N	N	N
Respiration rate	N	N	N	N
Abnormal effect on the skin	No	No	No	No
Drowsiness	No	No	No	slight drowsiness
Sedation	No	No	No	No
Eye colour	No	No	No	No
Stool	N	N	N	N
General physique	N	N	N	N
Any lethal effect	No	No	No	No

N: No Change or Normally; No: None

**Table 4.** Rate of Mortality (%)

Groups	Drug	Dose	Mortality rate (%)
Group (I)	Blank	None	0
Group (II)	Simple Paraffin	2000mg/kg	0
Group (III)	EEHW	2000mg/kg	0
Group (IV)	EEHW	5000mg/kg	0

**Table 5.** Average organ weight (g) of rats

Organ	Control	Average organ weight (gram)			
		Paraffin	EEHW (500mg/kg)	EEHW (1000mg/kg)	EEHW (2000mg/kg)
Liver	5.145±0.122	5.535±0.451	5.456±0.202	5.134±0.531	5.125±0.333
Kidney	2.121±0.101	2.123±0.128	2.241±0.128	2.533±0.010	2.839±0.128
Heart	2.232±0.004	2.43±0.002	2.432±0.017	2.661±0.034	2.343±0.116
Lungs	6.112±0.211	6.421±0.652	6.454±0.345	6.435±0.129	6.903±0.017
Spleen	0.402±0.016	0.419±0.014	0.401±0.015	0.403±0.011	0.307±0.10

non-heparinized, for the measurement of various biochemical and haematological parameters. In addition, a sample of the kidney, liver, and skin was taken for analysis by pathologists. To determine the average organ weight in the final experiment, all of the rats' organs were removed<sup>13</sup>.

## RESULTS AND DISCUSSION

### Acute Dermal Toxicity Study

In the investigation on acute dermal toxicity, the seeds of *Hydnocarpus wightiana* were

applied topically at a dosage of 2000 mg/kg and 5000 mg/kg b.w. without causing any indications of dermal toxicity. Table No. 3 illustrates the overall conduct that was noticed throughout the first four hours. As a result, it was estimated that the LD50 would be more than 5000 mg/kg, and the EEHW appeared safe at 5000 mg/kg. After EEHW at a dose of 5 g/kg was administered, there were indications of less drowsiness compared to the control group. The characteristics recorded in the study on acute dermal toxicity after the injection of the seed extract were compared with those observed in the paraffin-treated groups and the control group.

**Table 6.** Effect of dermal application of EEHW on Hematological parameters of rats

Parameters	Effect of dermal <i>Hydnocarpus wightiana</i> extracts on haematological parameters.				
	Control	Paraffin treated	EEHW (500mg/kg)	EEHW (1000mg/kg)	EEHW (2000mg/kg)
Hb	11.13±0.23	11.12±0.27	11.40±0.54	11.24±0.25	11.03±0.04
Lymphocyte	51.96±2.005	44.42±0.23*	51.77±0.69	51.23±2.22	51.23±0.32
MCH	21.43±0.23	21.42±0.27	21.50±0.04	21.34±0.85	21.13±0.14
MCHC	41.22±0.93	41.42±0.81	40.50±0.29	40.34±0.42	40.13±0.31
MCV	81.42±0.23	88.72±0.27	79.40±0.04	78.34±0.21	75.13±0.24
Monocyte	6.16±0.30	6.03±0.24	6.80±0.18	6.27±0.32	6.27±0.14
Neutrophils	27.43±0.24	30.42±0.71	28.77±0.24	25.34±0.45	26.12±0.72
PCV	31.33±1.316	37.23±0.11	30.21±0.08	30.11±0.27	30.12±0.92
RBC	6.58±0.01	7.50±0.51	6.47±0.38	6.33±0.38	6.13±0.15
WBC	10.18±0.24	12.25±0.13	10.18±0.28	09.93±0.88	09.11±0.51

Data are expressed as mean ± S.E.M from six rats and analyzed by one-way ANOVA followed by Tukey tests.

\*P<0.05 as compared to control group animals

**Table 7.** Effect of dermal application of EEHW on Biochemical parameters of rats

Parameters	Effect of dermal <i>Hydnocarpus wightiana</i> extracts on biochemical parameters				
	Control	Paraffin treated	EEHW (500mg/kg)	EECHW (1000mg/kg)	EEHW (2000mg/kg)
A/G Ratio	0.80±0.10	1.11±0.08	0.617±0.90	0.93±0.07	0.83±0.21
Albumin	4.22±0.23	5.12±0.23	4.50±0.34	4.34±0.34	4.43±0.41
ALP	144.66±3.15	167.23±4.56	140.33±5.11	141.10±6.03	139.22±5.16
Bilirubin (TB)	0.53±0.23	0.12±0.27	0.71±0.01	0.54±0.04	0.56±0.04
Creatinine	0.312±0.04	0.182±0.32*	0.411±0.02	0.470±0.09	0.655±0.02
SGOT	31.12±1.36	38.23±0.11*	31.21±0.23	37.11±0.47*	38.12±0.22*
SGPT	55.70±0.98	59.35±0.28	59.03±0.623	64.18±0.84*	67.37±0.45*
Total Cholesterol (TC)	128.500±2.247	120.420±2.317	118.320±4.432	122.430±3.136	125.927±4.203
Total Protein (TB)	5.32±0.03	6.01±0.24	6.42±0.14	6.04±0.84	6.23±0.91
Urea	36.88±0.415	30.57±0.35	32.20±0.20	30.16±0.60	31.37±0.21
Uric acid	0.64±0.07	0.15±0.06*	0.50±0.09	0.45±0.06	0.54±0.03

Data are expressed as mean ± S.E.M from six rats and analyzed by one-way ANOVA followed by Tukey tests.

\*P<0.05 as compared to control group animals

**Sub-acute toxicity study**

The sub-acute dermal toxic impact of EEHW was evaluated by applying the OECD guideline 410 at a limit test dosage of 1000 mg/kg. Even after 28 days, topical application of

the EEHW at doses of 500, 1000, and 2000 mg/kg b.w. did not cause any treatment-related side effects or death. In contrast to the control group, the groups treated with extract did not experience any clinical impacts or adverse effects, according to the research.

**Effect of extract on relative organ body weight**

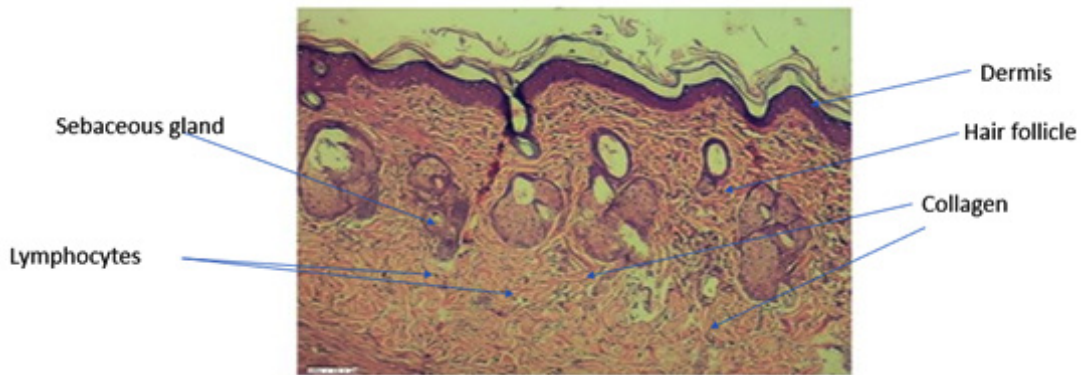
The average organ weight of the animals in the extract-treated groups did not change significantly over that experiment. The impact of EEHW on the weight of vital organs relative to body weight is seen in Figure 3 and Table No. 5. The findings also demonstrated that, among other vital organs, there are no discernible variations in the kidney, liver, heart, or lungs.

**Effect of extract on haematological parameters**

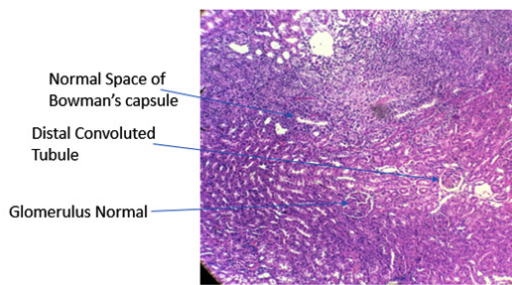
Table No. 6 presents the haematological test parameters findings. When compared to the control group, all of the haematological measurements were found to be within normal limits. There were no appreciable toxicity symptoms or alterations in the haematological



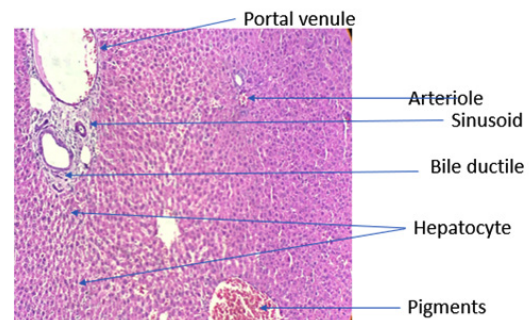
**Fig. 3.** Dissected vital organ after weighing



**Fig. 4A.** Skin



**Fig. 4B.** Kidney



**Fig. 4C.** Liver

**Fig. 4.** Photomicrographs of Histopathological slides of vital organs at 100X

parameters between the EEHW group data and the control groups.

#### **Effect of extract on biochemical parameters**

Table No. 7 presents the key biochemical test parameter results. Comparing the experimental group to the control group revealed that several biochemical measures, including TP, TB, Albumin, Globulin, Urea, Creatinine, and Uric Acid, were all within normal ranges. When compared to the control group, other biochemical markers such as SGOT and SGPT demonstrated a marginally significant difference at dosages of 1000 and 2000 mg/kg. There was no discernible toxicity in the results.

#### **Effect of seeds extract on Histopathological parameters**

The results of the histological analysis of skin slices from rats given extracts containing both normal and ethanolic bases are displayed in Figure 4 (Figure 4A). Every instance in the treatment and control groups had their histology evaluations completed for the current severe and sub-intense skin toxicity ponders. Overall, there was little impairment associated with the treatment in the rats fed ethanolic meals made from ground removal. The most amazing finding was that histological examinations showed no evidence of skin tissue damage, and the therapy had no effect on any metrics.

The histological analysis of a kidney slice treated with EEHW and a normal base is presented in Figure 4B. The rat kidneys' tubules and glomeruli remained intact. Rats administered 500 mg/kg body weight of EEHW did not show signs of tubular necrosis; however, rats administered 1000 and 2000 mg/kg body weight of an ethanolic extract derived from *Hydnocarpus wightiana* showed minimal signs of tubular necrosis and lymphocytic infiltration. Rats administered 2000 mg/kg b.w. of EEHW did not show signs of brain necrosis or tubular oedema.

Rat liver sections from rats receiving normal base and EEHW treatments are histologically examined (Figure 4C). Rats receiving 500 and 1000 mg/kg b.w. EEHW did not show signs of hepatic necrosis; rats receiving 2000 mg/kg b.w. EEHW showed normal hepatic plates and portal veins. EEHW did not show significant hepatocyte degeneration.

## **CONCLUSION**

Plant-based medicines are more widely used in healthcare due to their low occurrence of adverse effects and widespread usage in pharmaceuticals. Bioactive chemicals obtained from herbal plants are frequently utilised since they are thought to be safe and not negatively affect health<sup>14</sup>. Since plant-based medicines are recognised to be essential in the treatment of a variety of chronic ailments, researchers have favoured them over allopathic pharmaceutical therapies in the modern period<sup>15</sup>. Nevertheless, no scientific study has examined the toxicity or adverse consequences of these treatments. Therefore, the objectives of this study were to evaluate the *Hydnocarpus wightiana* seeds extract for acute and sub-acute toxicity assessments as well as to identify the dosage range that could be used as a suitable reference for future research.

Laboratory animals were given single doses of 2000 and 5000 mg/kg b.w. during the first four hours of treatment as part of the dermal acute toxicity evaluation of the seed extract. A 72-hour observation period was then observed to search for any adverse effects. There were no discernible changes in behaviour or mortality in either group. The LD50 is thought to be greater than 5000 mg/kg. Any pharmacological drug with an oral LD50 of more than 1000 mg/kg has the potential to be both safe and low harmful<sup>16</sup>. This suggests that the ethanolic extract of *Hydnocarpus wightiana* at a single dosage of 5000 mg/kg body weight is safe.

A sub-acute toxicity study utilising doses of 500, 1000, and 2000 mg/kg of extract was conducted according to OECD guidelines. Important organs like the liver, kidney, heart, pancreas, and spleen were found to have normal relative weights in both the treatment and control groups, showing no side effects and being statistically non-significant ( $P > 0.05$ ). There was no statistically significant difference ( $P > 0.05$ ) in the haematological parameters between the treatment group and the control group following a 28-day course of seed extract therapy.

The estimated blood biochemical parameters of the treated animals did not differ significantly ( $P > 0.05$ ) from those of the control group. The transaminase enzymes SGOT (AST) and SGPT (ALT), however, demonstrated positive



results for 500, 1000, and 2000 mg/kg extract, with a significant increase ( $P > 0.001$ ) in animals treated with extract in comparison to the corresponding control group. Liver damage has been associated with increased blood levels of hepatic enzymes and transaminases (SGPT and SGOT) in several investigations. Thus, the elevation in liver hepatic enzyme levels (SGPT and SGOT) after the administration of the ethanolic extract may have been caused by a phytochemical compound that, in larger quantities, may be dangerous in the liver and produce liver damage. However, it's conceivable that these changes have no discernible toxicological effects<sup>17</sup>. Important information about the acute and subacute dermal toxicity profiles of *Hydnocarpus wightiana* seeds' ethanolic extract is presented in this paper; this information will be helpful for future in vivo and clinical studies on this plant's potential medicinal uses. Examining the ethanolic extract of *Hydnocarpus wightiana* seed for dermal toxicity in laboratory animals revealed that it was nontoxic.

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#### Conflicts of Interest

The authors do not have any conflict of interest.

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