*Section: Research Paper*

# Acute oral toxicity evaluation of hydroethanolic extract of *Brassica Cretica* in wistar albino rats as per OECD 425

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**Abstract:**

The medicinal herb *Brassica Cretica* (Brassicaceae) has long been employed in traditional cuisine. The study was undertaken to confirm the plant's safety profile. A total of six female rats (n=6) were chosen at random. A hydroalcoholic extract of *Brassica Cretica (*HEBC) was given to a group of six rats at a dose of 2000 mg/kg body weight. Blood samples were taken at regular intervals to evaluate the hematological and biochemical markers. The vital organs were isolated and kept separate for histology examinations. The data suggests that, the extract's $LD_{50}$ is greater than 2000 mg/kg body weight. The body weight, behavior, renal function test, liver function test, and lipid profile remained unchanged.

**Key words:** Toxicity, Hematology, Organ Function Test

## Introduction:

Even though there have been advances in the manufacture of synthetic and semi-synthetic drugs for the treatment of various illnesses, traditional medicines still play a significant role in rural areas. This herbal medication's usefulness is clearly shifting in favour of overuse. [1] As the founder of toxicology, Paracelsus once observed, "There is nothing that is not a poison; all substances are poisons". The appropriate dosage is what distinguishes a treatment from a poison. Research on the therapeutic plants and their safety profiles is urgently needed. [2] Although the

toxicity of medications derived from plants is predicted to be relatively low, several medicinal plants used in traditional medicines have been found to have harmful effects.[3, 4] The majority of people in underdeveloped nations use traditional medicine made from natural ingredients to cure a variety of illnesses. These plants do, however, contain a variety of bioactive substances that have the potential to have negative effects. For the past three thousand years or so, "Ayurveda" has been widely practiced as a "alternative medicine" throughout the Indian subcontinent. Ayurveda has recently received increased attention in the realm of medicine due to its safety and consistency in providing results. Furthermore, it has been demonstrated to deliver superior therapeutic outcomes with fewer side effects than Western therapy. However, extensive toxicological knowledge is required before these conventional medicines may be developed into modern medications. The toxicological investigations of natural products have, however, been reported very infrequently in recent years. Therefore, it is necessary to undertake and record through safety investigations that look at these compound's potential harmful consequences.

*Brassica Cretica* is a member of the Brassicaceae family. Broccoli, a cabbage family vegetable of the genus Brassica, has been utilized as a vegetable from the ancient time. It's high in vitamin C, vitamin K, and vitamin A, as well as dietary fiber and minerals, but low in fat and energy. Broccoli is a good source of dietary selenium as well as Sulphoraphane, an isothiocyanate with anti-carcinogenic properties. In humans and animals, it is also recognized to be a natural inducer of phase-II enzymes such as haemoxygenase-1 (HO-1). Through the antioxidant-responsive element, Sulphoraphane can trigger the redox-regulated cardioprotective protein Trx. [6]

## Material and methods:

### Collection of plants

Brassica Cretica leaves were purchased from a local vendor in Pune, Maharashtra, India. A head (Botanist) of Botanical Survey of India Western regional Centre, 7-Koregaon road Pune-41044, verified the entire plant. The BSI herbarium has been deposited and voucher specimen number was obtained as BCRT1 Dated, 23 Sep 2021.

### Preparation of crude extract

The leaves were then air-dried in the shade at room temperature. The coarse powder of *Brassica Cretica* was macerated for the period of 48-hours at 80°C in a solvent of 70% ethanol. The filtrate was then subjected for drying in an oven at 40°C. Before being stored in the refrigerator, the heat-dried extract was kept in a desiccator.

### Preparation of dose

Brassica Cretica extract was suspended with 0.3% carboxymethyl cellulose (CMC) and prepared as a dose considering the body weight of animals.

**Preliminary phytochemical studies**

The extract was evaluated for the presence of several phytoconstituents such as tannins and phenolic compounds, cardiacglycosides, flavonoids, and saponins. [8]

**Animals and approval from animal ethical committee**

The parameters were conducted on healthy nulliparous and non-pregnant female Wistar Albino rats (200-220 gm). The animals were kept in the animal house of Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune, under standard housing conditions. The protocol was approved by IAEC (Institutional Animal Ethics Committee) with approval number (MCP/IAEC/07/2021).

**Acute toxicity assay**

The rats were selected at random and maintained in their home cages for at least 5-days prior to dosing to allow for adaptation to the laboratory conditions. Prior to dosing, the animals were fasted overnight with *ad. libitum* access to water. Acute oral toxicity of the extract was carried out using a limit test with a single dosage of 2000 mg/kg, p. o. The dose was calculated based on the animal's body weight. For the first 30-minutes, the animals were intensely observed, and then for the next 4 hours. After 1–2 hours after dosage, food was given. Following the survival of the treated animal, five more animals were given the same dose. For 6-hours, the group was intensively monitored, and then at regular intervals for 14 days. Other harmful consequences were identified in the rat who survived. The animal's weights were tracked from the start of the trial, and blood samples were collected via. Retro-orbital plexus under ether anesthesia, with serum separated for biochemical and hematological analysis. At the end, the animals were sacrificed by cervical dislocation and vital organs were removed, weighed, and stored in 10% formalin for histological assessment.

**Hematological analysis**

For hematological analysis, blood samples were collected from animals in EDTA-containing tubes. The following CBC parameters were determined: hemoglobin (Hb), total RBC, platelet count, white blood cells (WBC) count, neutrophils (N), lymphocytes (L), monocytes (M), and eosinophils (E).

**Biochemical analysis**

ERBA kits were used to measure various biochemical parameters in a semi-automated analyzer. The parameters for renal function test are creatinine and urea. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, albumin, and globulins are some of the liver function parameters that are measured. Cholesterol, Triglyceride, high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and the Cholesterol/HDL ratio were all measured.

Female Wistar Albino Rats

↓

1-animal will be treated with Hydroalcoholic extract of Brassica Cretica (2000 mg/kg)

↓

If lethality does not occur, further 4-animals will be treated with Hydroalcoholic extract of Brassica Cretica (2000 mg/kg)

**Histopathological study**

The essential organs (brain, heart, liver and kidney) of sacrificed rats were fixed in a 10% formalin solution.

**Results:**

**1. Phytochemical investigation**

Preliminary phytochemical analysis of Brassica Cretica extracts revealed the presence of several phytocompounds, which are shown in Table-1.

**Table 1-** Phytochemical investigation of Brassica Cretica extracts

| **Phytoconstituents** | **Test** | **Observation** |
|---|---|---|
| **Tannins and Phenolic compounds** | 5% $FeCl_3$ solution | Deep Blue-black color |
| | Bromine water | Discoloration of bromine water |
| | Dilute $HNO_3$ | Reddish to yellow color |
| **Cardiac Glycosides** | Legal's Test (to aq./alc. Extract add 1 ml of pyridine and sodium nitroprusside) | Pink to red color |
| | Keller- killiani Test (To extract add GAA 5%$fecl_3$ and conc. $H_2SO_4$ ) | Reddish brown color at junction and upper layer appears bluish green. |
| **Saponin Glycosides** | Foam Test (shake the drug extract with water) | Persistent stable foam observed |
| **Flavonoids** | Sulphuric acid Test (to extract add sulphuric acid) | Deep yellow solution |
| **Isothiocyanate Glycoside** | Sodium Picrate Test(drug placed in flask containing hot water a filter paper is soaked in sodium picrate solution) | Yellow color of the paper turns red |

## 2. Acute oral toxicity assay

The extract of Brassica Cretica showed no mortality in a limit test with a dose of 2000 mg/kg body weight of rats. The test animals were kept under observation for 30-minutes and then for four hours. The observations were kept for 14-days. There was no mortality in the entire course of observation.

## 3. Behavioral pattern and body weight

All of the animals' body weights were measured. After extract treatment, there was no significant change in body weight. During the investigation, all of the parameters were normal, including the eyes, fur, skin, fecal consistency, mucous membrane, breathing, salivation, Somatomotor activity, behavior pattern and urination.

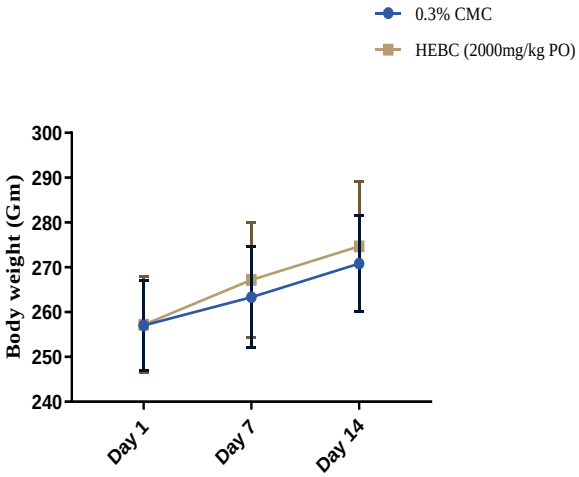



**Figure1-Effect of Brassica Cretica different extracts on body weight of rats in acute toxicity studies. HEBC-Hydroalcoholic extract of Brassica Cretica. Values are presented as mean ± SEM; N = 6**

**Table 2-**Effect of Brassica Cretica on behavioral pattern

| Treatment/ Parameters | 0.3% CMC | | | | | HEBC (2000mg/kg PO) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 Min | 4 hrs | 24 hrs | 48 hrs | 7 days | 14 days | 30 mins | 4 hrs | 24 hrs | 48 hrs | 7 days | 14 days |
| **Coma** | A | A | A | A | A | A | A | A | A | A | A | A |
| **Convulsions and Tremor** | A | A | A | A | A | A | A | A | A | A | A | A |
| **Eyes** | A | A | A | A | A | A | A | A | A | A | A | A |
| **Feces consistency** | N | N | N | N | N | N | N | N | N | N | N | N |
| **Fur And Skin** | N | N | N | N | N | N | N | N | N | N | N | N |
| **Itching** | N | N | N | N | N | N | N | N | N | N | N | N |
| **Mortality** | A | A | A | A | A | A | A | A | A | A | A | A |
| **Mucous membrane** | A | A | A | A | A | A | A | A | A | A | A | A |
| **Respiration** | N | N | N | N | N | N | N | N | N | N | N | N |
| **Salivation** | N | N | N | N | N | N | N | N | N | N | N | N |
| **Sleep** | N | N | N | N | N | N | N | N | N | N | N | N |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Somatomotor activity and behavior pattern** | N | N | N | N | N | N | N | N | N | N | N | N |
| **Urination (color)** | N | N | N | N | N | N | N | N | N | N | N | N |

**A-Absent; N- Normal**

## 4. Hematological analysis

The hematological analysis findings are shown in Fig. 2. When HEBC treated groups were compared to the control group; there was a substantial increase in Lymphocytes (*P<0.05), HCT count (**P<0.01), Total WBC count (*P<0.05), Platelet count (**P<0.01) and MCH (*P<0.05) were substantially higher in the HEBC treatment group than in the control group

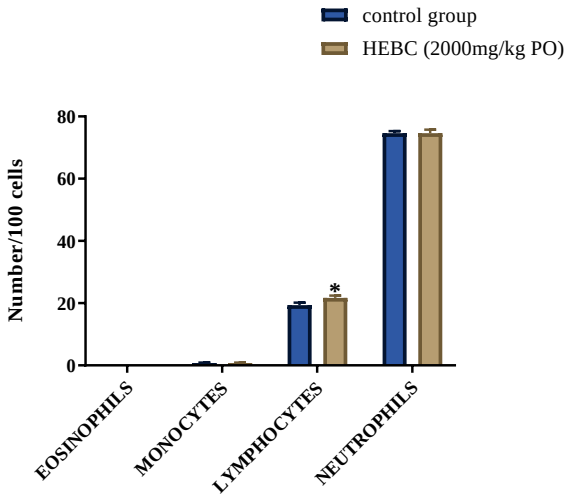



**Figure 2(a): Differential leukocyte count**

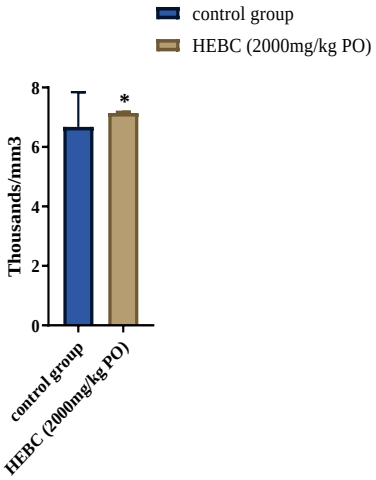



**Figure 2 (b) Effect on Total WBC count**

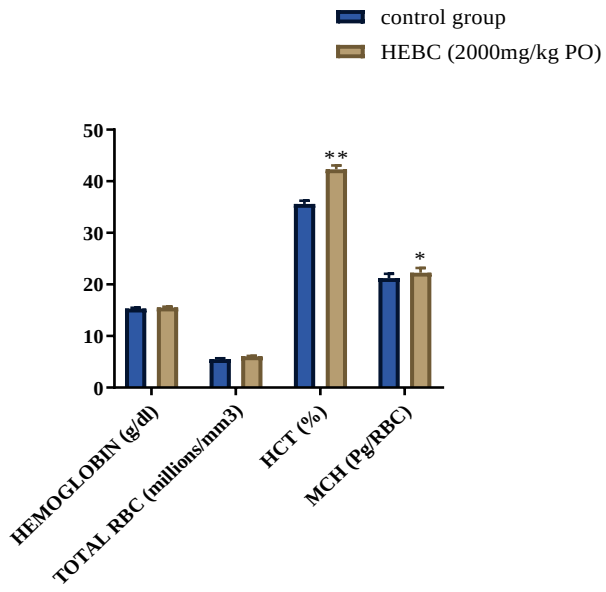



**Figure 2(c) Effect on hematological parameters**

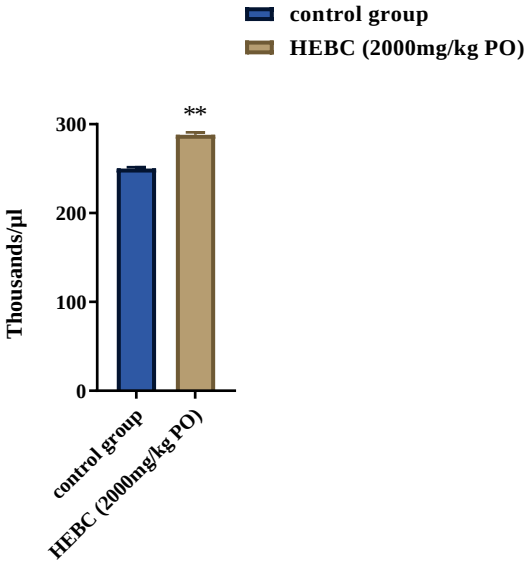
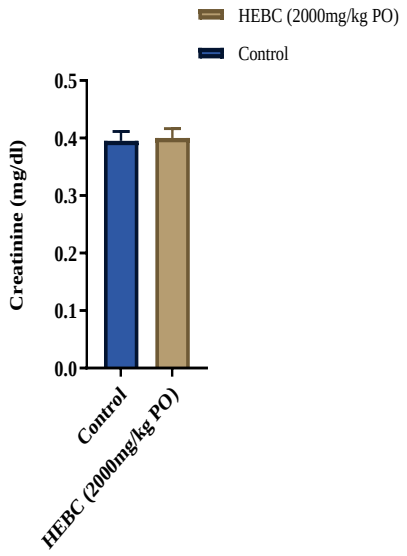



**Figure 2(d) Effect on platelet count**

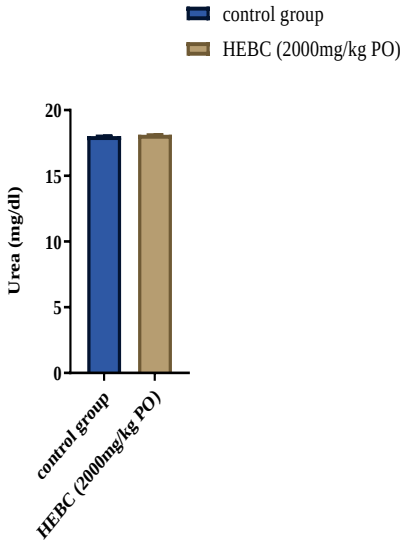
**Fig-2-Effect of extracts given at Limit dose (2000 mg/kg) in Haematological profile. Fig. 2a shows the effects of extract on differential WBC count, Fig. 2b shows Total leukocyte count, Fig. 2c shows effect on haematological parameters, Fig. 2d shows the effect of extracts on blood platelets. HEBC: Hydroalcoholic Extract of *Brassica Cretica*. Values are presented as mean ± SEM; N=6. *p<0.05 when compared with the vehicle group (0.3% Carboxymethyl cellulose gel).**

## 5. Biochemical analysis

There was no significant changes in the renal function test, liver function test and lipid profile following the limit dosage treatment.




**3(a): Effect on serum creatinine levels:**




**3(b): Effect on serum urea levels**

**Fig-3Effect of extracts given at Limit dose (2000 mg/kg body weight of rats) in renal function test. Fig 3a & Fig 3b shows the serum Creatinine level & Serum Urea level respectively; HEBC- Hydroalcoholic extract of Brassica Cretica; Values are presented as mean ± SEM; N = 6. *p < 0.05 when compared with the vehicle group (0.3% Carboxymethyl cellulose gel).**

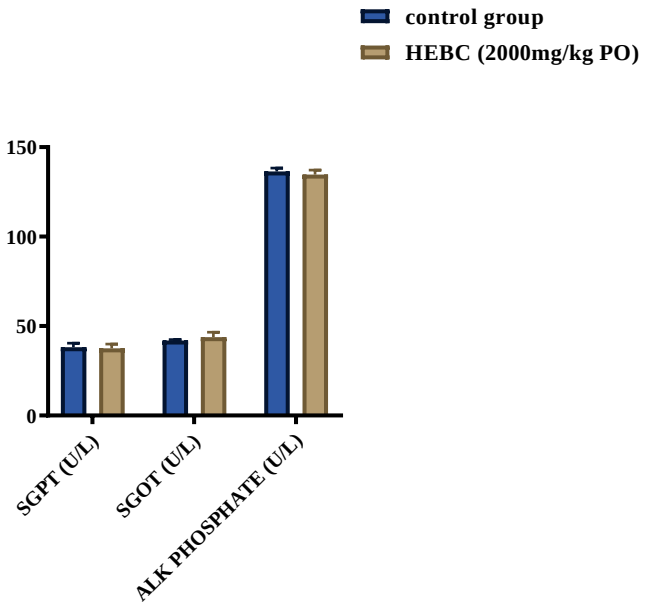



**Figure-4(a): Effect on serum biochemical parameters (Liver Function Test)**

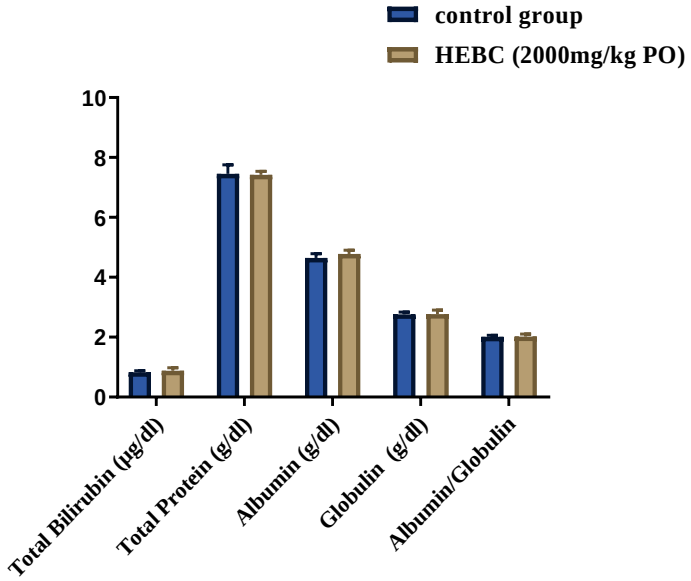



**Figure-4(b): Effect on serum biochemical parameters (Total Bilirubin, Total Protein, Albumin, Globulin and Albumin to Globulin ratio)**

**Fig-4 Effect of extracts given at Limit dose (2000 mg/kg body weight of rats) in Liver function test; Fig. 4a shows the effect of the extracts on SGPT, SGOT and Alkaline Phosphatase; Fig. 4b shows the effect of the extracts on Total Bilirubin, Total Protein, Albumin, Globulin and Albumin to Globulin ratio. HEBC-Hydroalcoholic extract of Brassica Cretica; Values are presented as mean ± SEM; N = 6. *p < 0.05 when compared with the vehicle group (0.3% Carboxymethyl cellulose gel).**

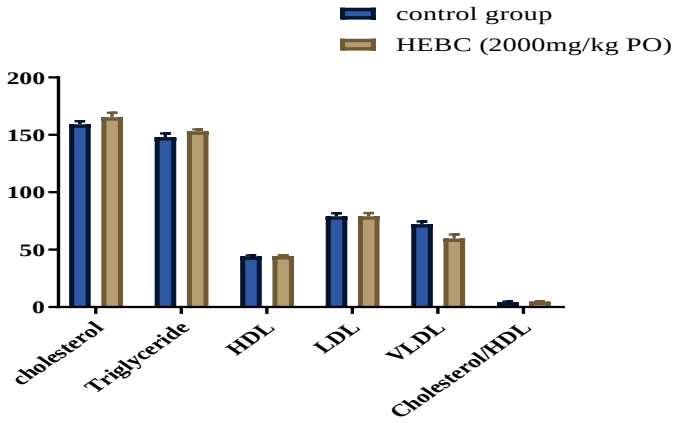



**Figure-5: Effect on serum biochemical parameters (Lipid Profile)**

- **Fig-5Effect of extracts given at Limit dose (2000 mg/kg body weight of rats) in Lipid profile. HEBC-Hydroalcoholic extract of Brassica Cretica; Values are presented as mean ± SEM; N = 6 *p < 0.05 when compared with the vehicle group (0.3% Carboxymethyl cellulose gel).**

## 6. Histopathological Studies

The histological analysis of all vital organs, including the brain, heart, liver, and kidney, revealed no differences between the treated and control groups.The isolated heart was fixed in 10% formalin. A specimen was taken for further histopathological examinations.

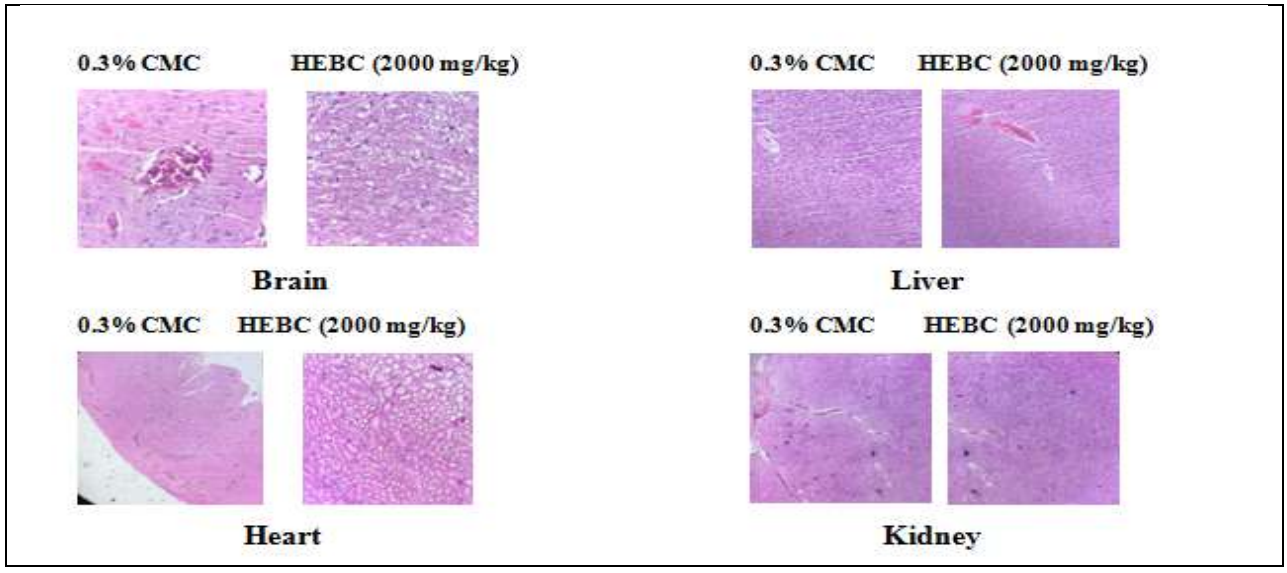



**Fig-6: Effect of extracts given at Limit dose (2000 mg/kg body weight of rats) on the Histology of the vital organs. HEBC: Hydro-alcoholic extract of Brassica Cretica.**

## 7. Discussion:

This experiment was performed to determine the plant's toxicity via the oral route using 425 toxicity criteria. Clinical signs and symptoms, as well as other toxicity indicators, can be used to assess toxic effects. [9] After the administration of a limit dose of 2000 mg/kg body weight of rat, no mortality was reported. During this trial, no significant changes in body weight were observed. It was indicated that all nutrients, including carbohydrates, proteins, and fats, are digested properly within the body because they are the nutrients that play a vital part in physiological function. [10-12]

The hazardous material affects the brain, liver, kidneys, and heart, which are the body's major critical organs. [13]In comparison to the vehicle control group, no lesions were discovered on histological inspection of the brain, heart, kidney, or liver when the animals were slaughtered at the end of the trial. As a result, the extract of Brassica Cretica belongs to group-5 (LD50 > 2000 mg/kg), which is the lowest toxicity category. [14]

Other biological measures, such as serum biomarker measurements, can be used to assess the body's health status. The SGPT, SGOT, albumin, globulin, and total proteins did not alter significantly after treatment with extract. [15-18]

Multiple hyperlipidemias are almost always caused by a combination of causes, including medications.[19] Our results suggested that there was no significant change in lipid profile following administration of a limit dose of 2000 mg/kg of *Brassica Cretica* extract, implying that it has no effect on lipid profile. [20]

Elevated serum creatinine and urea levels indicate renal impairment. There was no significant change in serum creatinine and urea in this study, which could indicate that this is a fairly safe plant for usage in traditional cuisine. In response to any toxic stress or environmental pollution, hematological parameters are sensory markers of physiological alterations in animals.[21]In hemostasis, blood platelets are extremely important.[22]There is no statistically significant change in WBC count or differential WBC count, implying that there is no infection.

## 5. Conclusion

Based on the results of acute toxicity studies conducted in accordance with 425, it can be stated that all of the plant extracts utilized in the study are relatively safe, with LD50 > 2000 mg/kg (Group-5 of toxicity class as per GHS).

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