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28 Days Repeated Dose Oral Toxicity Study of Hydro-Alcoholic Extract of *Celosea argentea* Seed in Wistar Rats

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Abstract

The aim of this study was to evaluate the subacute oral toxicity study of hydroalcoholic extract of *Celosea argentea* seed (HAECAS) in male and female wistar rats. 28 days oral toxicity study of *Celosea argentea* seed extract was performed at doses of 200, 500 and 1000mg/kg. During the study period all the animals were observed for weekly body weight, food consumption, behavior changes, any sign of morbidity and mortality. At the end of study, the animals were humanely sacrificed and assessed for biochemical, hematological analysis and histopathological examination. The results of the study indicated to increase in the body weight and food consumption at 500 and 1000 mg/kg. The results of biochemical and hematological examination revealed significant alteration in the MCH, neutrophils count, lymphocytes, albumin, total protein and creatinine level at higher dose of HAECAS. In the histopathology, animals treated with extract at 500 and 1000mg/kg body weight showed minimal to mild hepatocellular vacuolation in liver and tubular degeneration at high dose in kidney; however, heart and lungs did not show any toxicity when compared with control group animals. From results, it concludes that repeated administration of hydroalcoholic extract of *C. argentea* increases the body weight as well as food consumption indicates its role as dietary supplement. The hydroalcoholic extract of *C. argentea* was found to be safe in 28 days repeated dose toxicity study. While at higher dose i.e. 1000 mg/kg there was increased in neutrophil count, creatinine, albumin, total protein and decreases the lymphocytes count. There was also mild to moderate congestion, and tubular degeneration at higher dose of HAECAS.

Keywords
Celosea argentea, subacute oral toxicity, biochemical and hematological parameter, histopathological examination.

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INTRODUCTION

Herbal medicines are in used since ancient times for treatment of several ailments. Reports have shown that over 70% of Africans or Asians depend on natural product medicines [1]. Phytochemical interactions of prisons lead to injury or death of living tissues. Toxicology is like science and art like medicine. It includes observational data gathering and data utilization to predict outcome of exposure in human and animals. The ancient humans categorized some plants as harmful and some as safe [2]. Seen the risks of toxicity, studies on the safety and effectiveness of medicinal plants have become one of the main concerns to guarantee the use [3].

Celosia argentea (family-Amaranthaceae) grows as a weed during the rainy season throughout India and other tropical regions of the world, such as China, Srilanka, South Asia, Africa and America [4]. In India, it is found to be grown as a weed of bajra fields. It is an herbaceous, erect and branching plant [5]. The dried ripe seed of celosia argentea commonly called semen Celosiae for clinical use. The major constituents are consisting of saponins, steroids triterpenoids, proteins, and etc [6]. Pharmacological uses of Celosia argentea seeds are hepatoprotection, anti-tumor, anti-diarrhea, anti-diabetes, antioxidant etc. [7-10]. Amaranthacea family is widely used by the population and its importance towards this family result from fact that it has several properties namely: activities anti-diabetic and anti-cholesterol, which reduces the pains, regulates the fertility [11-13].

In the present investigation, our study focuses the safety evaluation of hydroalcoholic extract of *Celosea argentea* seeds in terms of 28 days repeated oral dose toxicity study in wistar. The study was performed in order to increase the confidence in their safety to humans to treat various ailments.

MATERIAL AND METHODS

1. Plant material

The seeds of *Celosea argentea* were collected from vita, district of Sangli, Maharashtra and authenticated by M/s. shamantak enterprises, Dr. Gautam, Botanist, pune, India. Certificate of Aunthentification number of celosia argentea is SE/ AC/ 2018/04.

2. Preparation of extract

The seeds were collected from the mature plants, shade dried and powdered (80 mesh). The powdered seed (253g) was defatted with petroleum ether and later extracted (soxhlet) using 90% ethanol. The solvent free alcoholic extract was dissolved in distilled water and employed for subacute toxicity.

3. Experimental Animals

The animals used was adult male and female rats (150-200g), from animal house of All India Shri Shivaji Memorial Society, College of Pharmacy, Pune. They were maintained at ambient temperature 25±2◦c, relative humidity of 45-55% and a photoperiod of 12 h light /dark cycle. During acclimatization, the rats were randomized into experimental and control groups individually in sanitized cages housed with sterile paddy husk as bedding. The animal had free access to food (Nutrivet life sciences, Pune) and water ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidelines of CPCSEA, Government of India (CPCSEA/IAEC/PC-09/01-2K18).

4. Subacute toxicity study

This study was performed according to the Organization of Economic Cooperation and Development [14-16]. Wistar rats of both sexes (150-200g) were divided into four (I-IV) groups (n=6; six males and six females per group), and their weights were recorded. All animals had free access to water and food throughout the experiment. Groups II-IV were administered the hydroalcoholic extract of *Celosea argentea* seed (HAECAS) at daily doses of 200mg/kg, 500mg/kg, 1000 mg/kg and Group I served as the control and received distilled water at the same volume for 28 days. During the period of administration, the animals were observed and weight daily and weekly respectively to detect any signs of toxicity. At the end of the 28 days of administration, blood samples were collected from each animal under light anesthesia for analysis of hematological parameters such RBC, WBC, Hb, Hematocrit, platelets, neutrophils, eosinophil's and basophils etc. while the biochemical parameters such as AST, ALT, creatinine, total protein, albumin etc. were analyzed. The rats were sacrificed by cervical dislocation and vital organs (heart, liver, kidney and lung) were collected for histopathological examination.

5. Hematological Analysis

Hematological parameter included white blood cells (WBCs) count, red blood cells (RBCs) count, hematocrit (%), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, neutrophils (%), Eosinophils (%), Lymphocytes (%), Basophils (%) and Monocyte (%). The hematological determinations were carried out using Nihon Cohden Celtac alpha.

6. Biochemical analysis

The serum was separated from the non-EDTA blood and was assayed biochemical parameters, includes

liver function markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), Total bilirubin and direct bilirubin, nephrotic markers (creatinine) and protein profile (albumin and total protein). The biochemical parameters were performed using Chariot Prince Biochemistry Analyzer.

7. Histopathological examinations

The various organs (heart, liver, kidney and lung) were dissected and weighed. Weight sections from each organ were removed and fixed in 10% concentrated formalin, these tissues were trimmed and processes routinely. Tissue processing was done to dehydrate in ascending grades of alcohol, clearing in xylene and embedded in paraffin wax. Paraffin wax embedded tissue blocks were sections at 3-5 micrometer thickness with the rotary Microtome. Slides were stained with Hematoxylin and eosin (H and E) stain. The prepared slides were examined under microscope.

8. Statistical analysis

Statistical analysis was carried out using the Graph Pad Prism 7. All of the data are shown as the mean ± standard error of the mean (S.E.M) and were analyzed using one-way analysis of variance (ANOVA). Significant differences between the control and experimental groups were determined using Tukey- Kramer's all comparison test, P<0.05 was considered significant

RESULTS

Effect on food consumption

The results of 28 days repeated oral administration of HAECAS demonstrated to produce significant (p<0.05) increase in the food consumption at higher doses i.e. 500 and 1000 mg/kg as compared to vehicle control group. Increased in the food consumption was observed in third week of study.

Table 1: Effect of HAECAS on average food intake per week

Treatment	Sex	7 Days	14 days	21 days	28 days
Control	Male	66.77±2.43	75.96±2.47	78.32±3.41	83.72±2.87
	Female	65.14±3.55	72.45±2.38	79.83±3.12	81.20±2.36
HAECAS 200mg/kg	Male	65.37±2.99	87.76±2.47	89.21±2.62	96.33±3.14
	Female	66.44±3.88	79.32±2.49	87.08±2.98	94.54±3.75
HAECAS 500mg/kg	Male	66.54±3.90	78.8±3.31	92.83±3.52*	103.44±3.65*
	Female	58.8±2.27	71.3±2.63	88.32±2.63	99.73±3.47*
HAECAS 1000mg/kg	Male	57.64±2.48	80.28±2.5	97.21±2.06*	109.38±2.95*
	Female	69.14±3.16	72.28±3.05	91.22±3.41*	103.43±3.69*

Values are given in average weekly food consumption (gm.) per group of animals. Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. * Significant at p<0.05

Effect on body weight

The result demonstrated the significant (P<0.05) increase in the body weight observed at 500 and 1000 mg/kg doses in female and male rats as compared to respective vehicle control group from second week of study. While no significant changes occurred at 200 mg/kg dose of HAECAS (Table 2).

Table 2: Effect of HAECAS on Body weights

Groups	Sex	Body weight (g)		
		Day 0	Day 14	Day 28
Control	Male	193.5±1.32	227.2±2.92	242.75±2.42
	Female	191.75±3.19	215.5±5.57	234±2.27
HAECAS 200mg/kg	Male	197.5±2.39	239.2±6.2	265.5±10.88
	Female	194.2±2.17	236.5±4.97	241.5±5.83
HAECAS 500mg/kg	Male	195.75±3.42	244±6.78*	280.25±2.84*
	Female	199.5±1.25	236±5.71*	255.2±4.4*
HAECAS 1000 mg/kg	Male	197±1.47	253±6.09*	267.2±3.5*
	Female	195.5±3.92	241±5.62*	259.25±2.95*

Results are expressed as mean of body weight (gms) ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. *P<0.05, significant difference.

Effect on Organ weights of Heart, Liver, Kidney and Lung

The effect of HAECAS on the weight of vital organs (heart, liver, kidney and lung) are shown in table 3. On the Heart, liver and kidney, the results showed significant (P>0.05) increase in weight of liver of both male and female rats at doses of 500 and 1000 mg/kg.

Table 3: Effect of HAECAS on organ weights of rats

Organ (g/100g bw)	Sex	Control	HAECAS 200	HAECAS 500	HAECAS1000
Heart	Male	0.46±0.054	0.43±0.03	0.47±0.03	0.49±0.023
	Female	0.43±0.002	0.44±0.008	0.46±0.004	0.45±0.0025
Liver	Male	3.64±0.091	3.85±0.18	4.12±0.09*	4.22±001*
	Female	3.5±0.041	3.58±0.043	3.76±0.047*	3.78±0.029*
Kidney	Male	0.61±0.008	0.62±0.0032	0.63±0.01	0.63±0.006
	Female	0.57±0.021	0.61±0.004	0.61±0.004	0.60±0.0025
Lung	Male	0.71±0.012	0.71±0.0026	0.69±0.008	0.72±0.006
	Female	0.72±0.002	0.73±0.0043	0.74±0.006	0.73±0.0006

Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. *P<0.05 significant difference.

Effect on hematological parameters

The effect of the HAECAS on hematological indices in male and female rats were examined at the end of treatments. The results of hematological indices in male rats demonstrated no significant difference on parameters such as WBC, RBC, HBG, HCT, MCV, Platelets, Monocyte and Basophil. While there was significant (P<0.01) increase in the neutrophils in all treatment group whereas MCV (P<0.05), lymphocytes (P<0.01) and eosinophils (P<0.05) were found increased at highest dose level (1000 mg/kg) as compared to control group (Table 4).

The results of hematological indices in female rats showed no significant difference on parameters such as WBC, RBCS, HB, HCT, MCHC, Platelet, Monocyte, Neutrophil, Eosinophil and Basophil in treatments group. While there was significant (P<0.01) decreased in the MCH (P<0.001) and lymphocytes (P<0.001) at 500 and 1000 mg/kg while increased in neutrophils count at 1000 mg/kg of HAECAS as compared to control group (Table 5).

Table 4: Effect of HAECAS on hematological parameters

Parameters	Sex	Control	HAECAS-1	HAECAS-2	HAECAS3
WBC (10^3/µL)	Male	12.6±0.25	12.5±0.23	11.9±0.08	13.06±0.04
	Female	11.53±0.11	11.81±0.36	12.23±12.23	11.62±0.15
RBC (10^6 /µL	Male	8.12±0.10	7.55±0.11	7.54±0.20	7.96±0.16
	Female	6.64±0.5	6.25±0.057	12.11±0.12	11.73±0.11
HB (g/dl)	Male	13.63±0.28	14.46±0.37	13.81±0.28	13.75±0.53
	Female	11.63±0.51	11.11±0.29	12.11±0.12	11.70±0.16
Hematocrit (%)	Male	42.55±0.42	42.9±0.89	43±0.85	44.33±0.84
	Female	38.2±0.15	37.75±0.99	37.26±0.08	37.41±0.12
MCV (fl)	Male	62.58±2.41	56±4.31	59.83±2.46	63.66±2.65*
	Female	59.46±0.38	58.01±0.43	57.63±0.49*	59.48±0.42
MCH (pg)	Male	16.43±0.18	16.4±0.19	16.9±0.44	17.8±0.38
	Female	19.23±0.27	18±0.10***	16.35±0.09***	15.51±0.17***
MCHC (g/dl)	Male	29.86±0.27	28.91±0.31	31.25±0.34	29.35±0.22
	Female	29.97±0.32	28.9±0.86	29.15±0.3	28.33±0.08
Platelets ($10^{3/}$ dl)	Male	720±10.34	704±14.96	730±6.78	658±16.33
	Female	801.5±2.01	812±9.85	778.83±7.21	779±1.35
Monocyte (%)	Male	6.33±0.55	4.33±0.84	5.66±0.42	5.16±0.47
	Female	6.33±0.21	5.16±0.40	5.33±0.42	5±0.25
Lymphocyte (%)	Male	82.66±0.33	82.16±0.47	80.5±0.42	79.83±0.47**
	Female	82±0.36	81.58±0.34	79.35±0.42***	79.9±0.18***
Neutrophil (%)	Male	17.5±0.22	16.33±0.21	22.5±0.84**	22.33±0.95*
	Female	14.5±0.56	14.66±0.49	15.5±0.34	16.5±0.22*

Eosinophil (%)	Male	1.33±0.21	1.16±0.16	1.83±0.3	2.5±0.34*
	Female	1.83±0.75	2.16±0.30	2.33±0.43	2.66±0.33
Basophil (%)	Male	0.5±0.22	0.5±0.22	0.66±0.21	0.83±0.16
	Female	0.33±0.21	0.5±22	0.66±0.21	0.66±0.21

Results are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. *P<0.05, **P<0.01, **P< 0.001; significant differences.

Effect on Serum biochemical parameters

The results of the biochemical parameters are recorded in table 5. The results showed no significant differences in AST, ALT, total protein, total bilirubin and direct bilirubin at all treatment doses in both sexes compared to the vehicle control group. On the other hand, albumin and creatinine recorded to increase in both male and female significantly ($p<0.05$; $p<0.01$) at 1000 mg/kg of HAECAS.

Table 5: Effect of HAECAS on biochemical parameters

Parameters	Sex	Control	HAECAS 200 mg/kg	HAECAS 500 mg/kg	HAECAS 1000 mg/kg
AST (U/L)	Male	97.95±0.22	95.52±0.35	100.05±0.57	95.87±0.71
	Female	96.85±0.65	96.07±0.43	97.3±0.76	99.55±0.29
ALT (U/L)	Male	39.07±0.36	41.75±0.92	40.42±0.19	37.9±0.22
	Female	36.67±0.24	35.97±0.13	35.35±0.45	38.15±0.40
Albumin (g/dl)	Male	3.048±0.23	2.77±0.13	2.96±0.35	3.88±0.20**
	Female	2.89±0.036	2.73±0.075	2.9±0.009	3.22±0.009**
Total Protein (g/dl)	Male	6.54±0.13	6.6±0.10	6.58±0.056	7.18±0.031*
	Female	6.8±0.072	7.04±0.06	6.98±0.037	7.31±0.06*
Creatinine (mg/dl)	Male	0.65±0.024	0.66±0.046	0.68±0.018	0.72±0.006**
	Female	0.65±0.024	0.66±0.046	0.67±0.07	0.71±0.04*
Total Bilirubin(mg/dl)	Male	0.16±0.002	0.17±0.005	0.16±0.002	0.18±0.002
	Female	0.13±0.002	0.12±0.002	0.12±0.0017	0.14±0.002
Direct Bilirubin (mg/dl)	Male	0.018±0.00036	0.019±0.0003	0.022±0.00022	0.021±0.00016
	Female	0.02±0.0007	0.018±0.0003	0.022±0.00042	0.022±0.0004

Results are expressed as mean ± SEM (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. *P<0.05, **P<0.01; Significant differences.

Histopathological Examination

The results of histopathological examination indicated minimal to mild hepatocellular vacuolation in the liver at 500 and 1000 mg/kg of HAECAS. The histopathological examination of kidney reveled the mild to moderate tubular degeneration at 1000 mg/kg of HAECAS. However, heart and lungs did not show any toxicity when compared with control group animals (Fig1and2)

Figure No.1 Histopathological observations in male rats

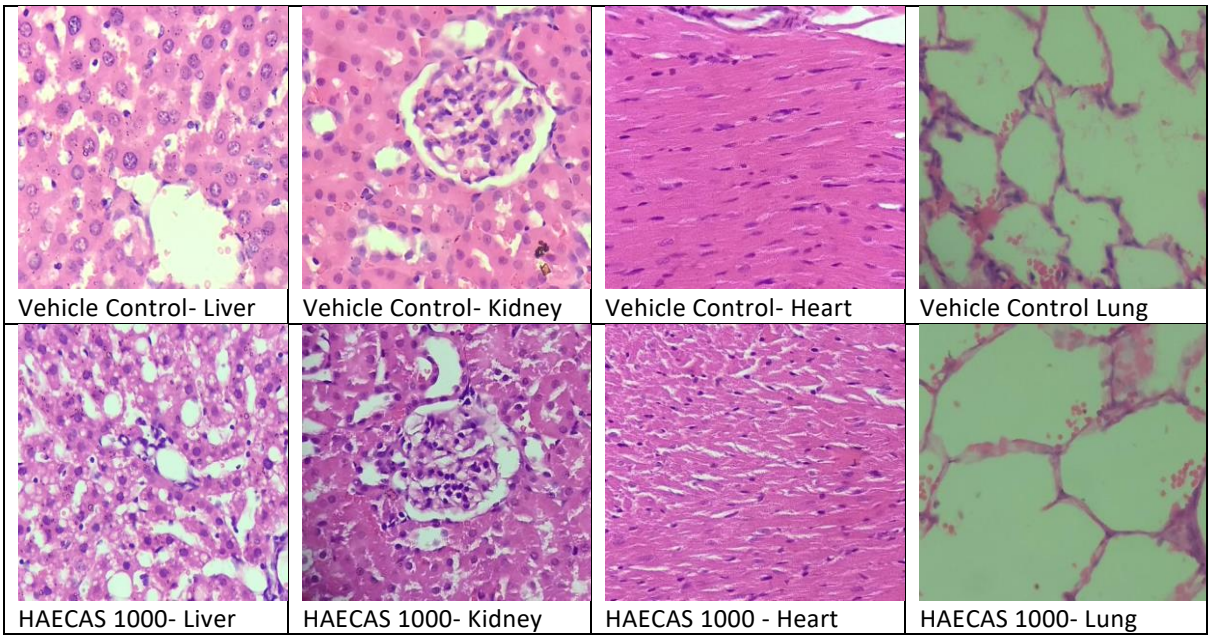
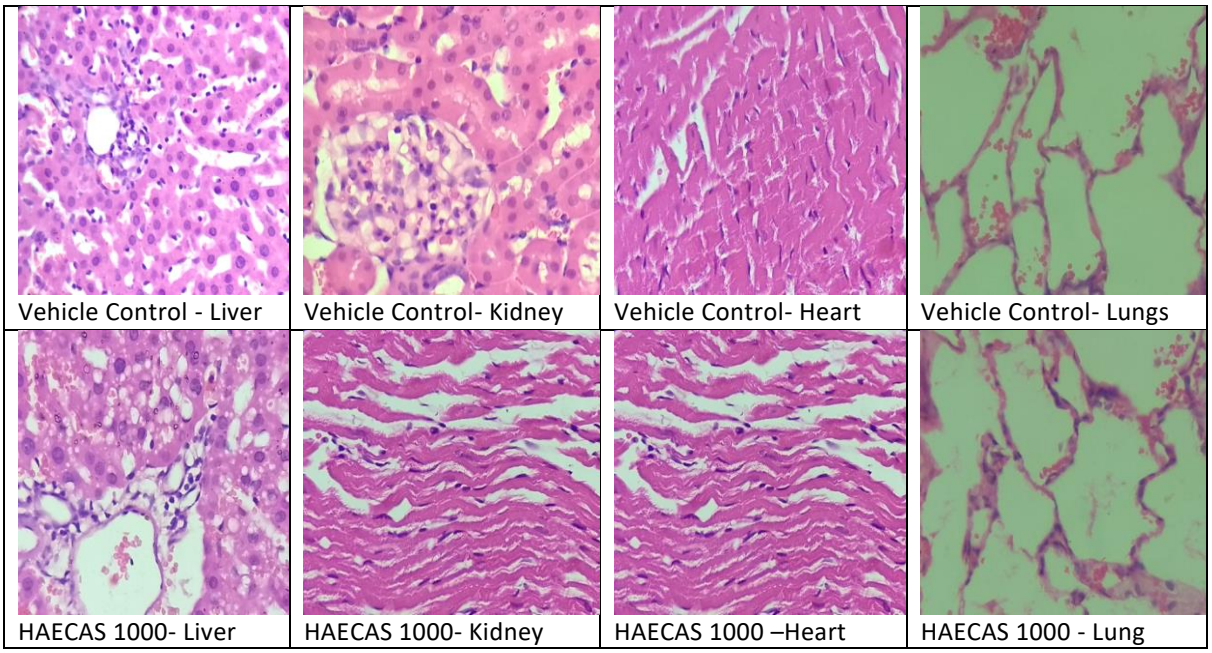


Fig. 2. Histopathological observations in female rats



DISCUSSION

The plants have therapeutics or toxic properties and they have crucial importance in folk medicine [17]. The use of any drug for treatments often based on long-term clinical experience. Medicinal plants play has an enormous importance in public health, especially for the low-income population, whose access to modern medicines is limited. There is limited scientific evidence regarding the safety and efficacy to support the continued therapeutic application of these herbal remedies [18]. In light of this in the present study, we have investigated the safety study of hydroalcoholic extract of the *Celosea argentea* seed in wistar rats.

The results of the present study demonstrated to significant increase in the food consumption and body weight of male and female rats at doses of 500 and 1000 mg/kg. It means that, at these oral doses, the HAECAS act as a dietary supplements and thereby increased the body weight. The weights of organ such as kidney, lung, heart, and spleen in the treated male and female rats did not differ significantly from those of the control group.

However, there was a significant increase in the weight of liver in 500 and 1000 mg/kg of HAECAS.

The evaluation of the hematological parameters is very important in the determination of the anomalies induced by a plant extract [19]. The hematological analysis of MCV, MCH, lymphocyte, neutrophil and eosinophil showed a significant difference in the treated group. The study of biochemical parameters are indicators of toxicity, raising the effectiveness on the vital organs. In this study, biochemical parameters such as, ALT, AST, total bilirubin, direct bilirubin haven't shown a significant difference between treated and controls. While there was increased in the albumin, total protein and creatinine level high dose of HAECAS. These result suggest that repeated oral administration of HAECAS at relatively high doses i.e. 1000 mg/kg shows some toxic sign that represented by increased in the level of neutrophils, total protein, creatinine, albumin level and decline in the lymphocytes.

In the histopathology, animals treated with extract at 500 and 1000mg/kg body weight showed minimal to mild hepatocellular vacuolation in liver at high dose; however, heart and lungs did not show any toxicity when compared with control group animals. In histopathological examination of kidney sections from rats treated with 1000 mg/kg showed tubular degeneration in kidneys when compared with control group. Thus, the significant elevation of creatinine as observed in biochemical estimation is confirmed by these histopathology finding.

CONCLUSION

From results, it concludes that repeated administration of hydroalcoholic extract of *C. argentea* increases the body weight as well as food consumption indicates its role as dietary supplement. The hydroalcoholic extract of *C. argentea* was found to be safe at 200 and 500 mg/kg dose in 28 days repeated dose oral toxicity study. While at higher dose i.e. 1000 mg/kg there was increased in neutrophil count, creatinine, albumin, total protein and decreases the lymphocytes count. In the present safety investigation, HAECAS exhibited mild nephrotoxic effect as indicated by increased in the creatinine level and mild to moderate tubular degeneration while the further toxicity studies are needed to investigate in details the toxic potential to complete the safety profile of *Celosea argentea* seed.

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