In Vivo Acute Toxicity Evaluation of Desmidorchis Indica Stem Extract in Albino Rats

T. Vijayalakshmi[1], Usharani Boopathy[2], Shobana Chandrasekar[2], Rohini Durairaj[3*]

[1]*Research Scholar, Department of Biochemistry, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu*
[2]*Associate Professor, Department of Biochemistry, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu*
[3]*Assistant Professor, Department of Biochemistry, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu*
Corresponding Author: Rohini Durairaj, Email: drrohinidurairaj@gmail.com

KEYWORDS
Desmidorchis indica stem extract, Acute and subacute toxicity; Biochemical analysis; Hematological parameters; Histopathology.

ABSTRACT
Throughout human history, traditional herbal methods for treating diseases have been used without considering dose effects. The purpose of this study is to assess the acute toxicity of hydro-ethanolic extract of Desmidorchis indica (Family: Apocynaceae) in albino rats. Acute toxicity was achieved when a dose limit of 2000 mg/kg body weight was used. Observations were made, recorded 24 hours a day, and once a day for another 14 days. Rats were weighed and various observations were made once a day during this time, including signs of death, behavior, injury, and illness. At the end of each study, hematological analysis and biochemical parameters were evaluated. To detect histopathological examinations of the animal's vital organs (liver and kidneys) were performed compared to controls. No significant differences ($p > 0.05$) were observed in relative organ, weight, hematological, biochemical parameters, macroscopic abnormalities compared to controls. No mortality rate was recorded. Therefore, analysis of the results may lead to the conclusion that medium-term oral administration of Desmidorchis indica stem extract does not cause toxicity.

1. Introduction

Traditionally, humans have used herbal medicines for remedying various diseases since immemorial times. Uses of medicinal plants as herbal medicines have been increasing worldwide due to the philosophy of low cost, effectiveness, availability and fewer side effects compared to synthetic petrochemical-derived drugs (Ha et al., 2018; Gurib-Fakim, 2006). This philosophy coincides with the fact that medicinal plants have played a great role in drug discovery and contributed more than 25% of the available pharmaceuticals (Dewick, 2009). Principally, the potentiality of medicinal plants can be attributed to their ability to synthesize voluminous metabolites such as flavonoids, stilbenoids, alkaloids, steroids, quinones, glycosides, and terpenoids. These secondary metabolites have demonstrated significant bioactivities against diseases inflicting humans, animals, and plants (Tom, 2018). Notably, the ethnopharmacological reports have shown that the chemical functionalities and bioactivities of the metabolites from the herbal plants vary due to geographical factors. For example, there is evidence that harsh tropical climate influences herbal plants to synthesize more metabolites to counter to the stimuli than in less-harsh climatic conditions (Ha et al., 2018; Gurib-Fakim, 2006). They synthesize secondary metabolites in order to either to respond against the stress caused by the harsh conditions or generate defensive chemicals against the predators supported by conditions on the given geographic orientations (Dewick, 2009). Thus, such plants become potential herbal plants of varying abilities to produce herbal medicines to treat diseases.

Nevertheless, some studies have questioned the bioactivities of traditionally used medicinal plants because of their side effects they cause once administered to humans and animals (Nath et al., 2015; Bello et al., 2016). This questioning is valid because the toxicity effects of secondary metabolites from herbal medicines may be lethal or non-lethal. There is enough evidence that shows that herbal medicines can have negative effects on animals and humans (Toghueo et al., 2019; Jatsa et al., 2019; Kilonzo et al., 2016). Thus, the common belief that those concoctions from medicinal plants are natural and generally regarded as safe (grass) does not always hold true. Therefore, evaluating the toxicities of herbal bioactivities is necessary particularly when the bioactives are amenable to the development of drugs for treating human ailments. The evaluation of bioactive compounds

of Desmidorchis indica stem was carried out in our earlier studies (Thiagalingam V et al., 2023)

Administration of drugs in humans can be studied by observing cumulative effects and doses that cause toxicity such as mutagenicity, carcinogenicity, and teratogenicity (OECD, 2008). Toxicity testing is essential for estimating the level of damage caused by compounds to biological and non-biological materials. This test is typically performed on potential products to develop new drugs and determine the therapeutic potential of drug molecules. Toxicity tests are generally aimed at determining the side effects of drugs, especially in cases of cancer, heart disease, skin or eye inflammation (OECD, 2008a).The aim of the present study is to evaluate the acute toxicity of the hydro-ethanolic extract of Desmidorchis indica stem.

2. MATERIALS AND METHODS

Animals

Acute toxicity study carried out accordance with The Organization for Economic Cooperation and Development (OECD) guidelines for the Testing of Chemicals. Male albino rats of Wistar strain approximately weighing 180-200gms were used in this study. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2ºC and 12 hrs light / dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water ad libitum. They were acclimatized to the environment for 1 week prior to experimental use. All the animal experimental protocols were approved (Approval number: SU/CLATR/IAEC/XXIII/37/2024) by the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Acute toxicity studies

Albino rats were randomly assigned into two groups of each six rats. All animals were maintained on standard laboratory diets with water ad libitum. Group 1 is control group, fed daily with only normal laboratory diet and water. Group 2 treated with hyrdro-ethanol extract of Desmidorchis indica. Each rat was given a single dose of 2000 mg/kg body weight. Before dose administration, the body weight of each animal was determined and the dose was calculated according to the body weight. The animals were observed for any toxic effect for first 4 h after the treatment period. Further animals were investigated for a period of 14 days for any toxic effect. Behavioral changes and other parameters such as spontaneous activity, irritability, corneal refex, tremors, convulsion, salivation, diarrhea, lethargy if any, and also monitored for any mortality during the course of toxicity study.

Biochemical analysis

Haemoglobin was estimated by Cyanmethaemoglobin method (Dacie and Lewis, 1968) (Beacon Diagnostic Kit). RBC count and PCV by the method of Ochei and Kolhatkar, (2000). Protein was estimated by the method of Lowry et al. (1951). Albumin was estimated by the method of Rodkey (1965). The serum total bilirubin was estimated by the method of Malloy and Evenlyn (1937). The serum SGOT and SGPT were estimated by the method of Reitman and Frankel (1957). The serum alkaline phosphatase activity was estimated by the method of Kind and King's (1954). Urea was estimated by the method of Natelson (1957). Serum creatinine was carried out by alkaline picrate method of Boneses and Taussy (1954).Cholesterol and HDL were assayed by Allain et al (1974). Triglyceride was assaed by Werner et al (1981) method. HDL cholesterol was determined by the method of Allain et al. (1974). LDL cholesterol was calculated as per Friedewald's (1972) equation. Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). Superoxide dismutase activity was assayed by the procedure of Kakkar et al (1984). The activity of catalase was determined by the method of Beers and Sizer (1952). Reduced glutathione was determined by method of Moron et al (1979). The activity of glutathione peroxidase was estimated by the method of Rotruck et al (1973). The level of ascorbic acid was assayed by the method of Omaye et al (1979). α-tocopherol was estimated by the method of Baker et al (1980). Serum sodium was estimated by colorimetric method of Maruna and Trinders (1958). Serum potassium was estimated by method of Maruna (1957).

Histopathological studies

The organs, namely liver and kidney were carefully excised and weighed. These organs were preserved in a fixation medium of 10% buffered formalin for histopathological study Histological studies carried out by the method of Ochei and Kolhatkar, (2000). Slides were viewed on a photographic microscope to find out the

histological changes in liver and kidney.

Statistical analysis

Values are expressed as Mean ± SD for six rats. Data was calculated by student t-Test (Independent sample, P value two tail) using SPSS ver. 20. Statistically significant level 0.05. *P<0.05 significant and NS non-significant (^{NS}P>0.05).

3. RESULTS AND DISCUSSION

The consumption of natural substances to cure and prevent diseases is old and universal; it plays an important role in access to basic health care for populations. Research on the therapeutic potential of plants has flown over the years with a wealth of scientifically proven information, showing the considerable power of plants in the treatment of a wide range of diseases. All parts of the plant that is the subject of this work especially stems are traditionally recognized in the treatment of various diseases. The stem of Desmidorchis indica investigated for acute toxicity study and the results were presented below

General appearance and behavioral observations

Acute toxicity tests evaluate the potential of a substance to cause acute toxicity in a test organism on exposure to a single dose of a substance especially at high doses and within a short period. It also helps in the investigation of the therapeutic index of drugs and xenobiotic. The clinical signs and symptoms exerted by drugs on vital body organs are considered as principal observations among toxicity indicators. On the 14 days treatment of hydro-ethanolic extract of Desmidorchis indica stem stem, the rats were survived throughout the entire study period. No treatment-related toxic symptoms or mortality were observed after oral administration of tested extract. None of these rats had shown any abnormal behavioral responses in any dose range. There was no change in behavioral responses, spontaneous activity, irritability, corneal refex, tremors, convulsion, salivation, diarrhea and lethargy if any when compared to control group (Table 1).

Table 1: Acute toxicity study of extracts in wellness parameters of rats

	Animal group	
Observations	Control rat	Extract (2,000 mg/kg body wt)
Consciousness	+	+
Grooming	-	-
Touch response	+	+
Sleeping duration	+	+
Movement	+	+
Gripping strength	+	+
Righting re flex	+	+
Food intake	+	+
Water consumption	+	+
Tremors	-	-
Diarrhea	-	-
Hyper activity	-	-
Pinna reflex	+	+
Corneal reflex	+	+
Salivation	+	+
Skin color	+	+
Lethargy	-	-
Convulsion	-	-
Morbidity	-	-
Sound response	+	+

Note: + indicate normal - indicate absent

There were generally no significant differences observed in the relative body weights in this study (Table 1). From the present study it was seen that there was no significant change in the haematological and biochemical parameters in the Desmidorchis indica extract treated group compared to the normal control group (Tables 2 to 7). Gross examination at autopsy and histopathological evaluations of liver and kidney organs stained with haematoxylin and eosin revealed no significant differences (Plate 1). Acute oral toxicity effects of hydro-ethanolic extract of Desmidorchis indica extract on rats were studied and no animal deaths in rats receiving 2000 mg/kg of extract. No sign of toxicity was observed in the wellness parameters during the 14-days observation period. Therefore, the approximate acute lethal dose (LD_{50}) of Desmidorchis indica extract in rat was estimated to be higher than 2000 mg/kg.

Table 2: Effect of Desmidorchis indica on animal and organ weight of control and experimental rats (Acute toxicity)

Parameters	Group I (Normal)	Group II (2000mg/kg)	P Value
Initial day (gm)	187.66±4.21	188.33±3.52	^{NS}P>0.05
Final day (gm)	192.55±3.07	193.41±4.88	^{NS}P>0.05
Liver weight (gm)	5.62±0.28	5.57±0.15	^{NS}P>0.05
Kidney weight (gm)	1.54±0.11	1.49±0.13	^{NS}P>0.05
Acute Oral Toxicity Effects (N = 6)			
Animal live (Nos.)	6±0	6±0	^{NS}P>0.05
Animal dead (Nos.)	Nil	Nil	
% of Mortality	Nil	Nil	

Values are expressed as Mean ± SD for six rats. Data was calculated by student t-Test (Independent sample, P value two tail) using SPSS ver. 20. Statistically significant level 0.05. *P<0.05 significant and NS non-significant (^{NS}P>0.05).

Biochemical Analysis

The effects of acute administration of DISE on biochemical parameters are presented in Table 3 and 4. No statistically significant differences in the liver function parameters like alanine aminotranferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were observed. The kidney function parameters, like urea, and creatinine, did not reveal any significant changes. Additionally, no relevant changes were found in total protein, albumin, and globulin content. . The DSIE had no effect on serum electrolytes (Na and K).

Table 3: Effect of Desmidorchis indica on Liver profile of control and experimental rats (Acute toxicity)

Parameters	Group I (Normal)	Group II (2000mg/kg)	P Value
Protein (mg/dl)	7.41±0.18	7.43±0.13	^{NS}P>0.05
Albumin (mg/dl)	4.34±0.07	4.29±0.08	^{NS}P>0.05
Bilirubin (mg/dl)	0.74±0.02	0.75±0.02	^{NS}P>0.05
ALT (IU/L)	26.62±0.60	26.86±0.80	^{NS}P>0.05
AST (IU/L)	46.78±1.35	47.33±1.56	^{NS}P>0.05
ALP (IU/L)	52.10±1.67	53.25±1.39	^{NS}P>0.05

Values are expressed as Mean ± SD for six rats. Data was calculated by student t-Test (Independent sample, P value two tail) using SPSS ver. 20. Statistically significant level 0.05. *P<0.05 significant and NS non-significant (^{NS}P>0.05).

Table 4: Effect of Desmidorchis indica on kidney profile of control and experimental rats (Acute toxicity)

Parameters	Group I (Normal)	Group II (2000mg/kg)	P Value
Creatinine (mg/dl)	0.92±0.02	0.93±0.02	^{NS}P>0.05
Urea (mg/dl)	25.45±1.55	25.37±1.40	^{NS}P>0.05
Sodium (Meq/L)	155.24±3.91	154.68±3.63	^{NS}P>0.05
Potassium (Meq/L)	4.47±0.23	4.52±0.16	^{NS}P>0.05

Values are expressed as Mean ± SD for six rats. Data was calculated by student t-Test (Independent sample, P value two tail) using SPSS ver. 20. Statistically significant level 0.05. *P<0.05 significant and NS non-significant (^{NS}P>0.05).

Hematological Analysis

The effects of acute administration of DISE on haematological parameters (Hb, RBC, WBC, PCV, MCV, MCH and MCHC) are shown in Table 4. Administration of DISE (2000mg/kg) did not cause any significant difference in most of the hematological parameters when compared with the control group.

Table 5: Effect of Desmidorchis indica on hematology profile of control and experimental rats (Acute toxicity)

Parameters	Group I (Normal)	Group II (2000mg/kg)	P Value
Hb (gm/dl)	13.30±0.35	13.27±0.52	^{NS}P>0.05
RBC ($\times 10^6$/mm^3)	4.56±0.21	4.59±0.13	^{NS}P>0.05
WBC ($\times 10^3$/mm^3)	7.55±0.22	7.57±0.14	^{NS}P>0.05
PCV (%)	22.92±1.53	23.34±1.47	^{NS}P>0.05
MCV (famato litre)	50.41±5.06	50.83±2.87	^{NS}P>0.05
MCH (pico gram)	29.21±1.37	28.93±1.74	^{NS}P>0.05

MCHC (%)	58.31±4.70	57.0±4.51	^{NS}P>0.05

Values are expressed as Mean ± SD for six rats. Data was calculated by student t-Test (Independent sample, P value two tail) using SPSS ver. 20. Statistically significant level 0.05. *P<0.05 significant and NS non-significant (^{NS}P>0.05).

Oxidative stress markers

The effects of acute administration of DISE on oxidative stress parameters (MDA, SOD, Catalase, GPx, GSH, Vitamin C and E) are shown in Table 6. Administration of DISE (2000mg/kg) did not cause any significant difference in all of the oxidative stress parameters when compared with the control group.

Table 6: Effect of Desmidorchis indica on oxidative stress profile of control and experimental rats (Acute toxicity)

Parameters	Group I (Normal)	Group II (2000mg/kg)	P Value
MDA (nmol of MDA formed/L)	7.52±0.17	7.54±0.22	^{NS}P>0.05
SOD (U/ml)	4.51±0.26	4.58±0.17	^{NS}P>0.05
CAT (U/ml)	6.44±0.20	6.47±0.19	^{NS}P>0.05
GPx (U/ml)	8.61±0.27	8.59±0.26	^{NS}P>0.05
GSH (mg/dl)	4.52±0.18	4.47±0.18	^{NS}P>0.05
Vit-C (µg/dl)	3.51±0.18	3.56±0.24	^{NS}P>0.05
Vit-E (µg/dl)	2.50±0.17	2.45±0.13	^{NS}P>0.05

Values are expressed as Mean ± SD for six rats. Data was calculated by student t-Test (Independent sample, P value two tail) using SPSS ver. 20. Statistically significant level 0.05. *P<0.05 significant and NS non-significant (^{NS}P>0.05).

Table 7 showed the effect of Desmidorchis indica stem extract on lipids profile of control and experimental rats. There is no significant (P>0.05) changes were observed lipid profile as cholesterol, triglyceride, HDL and LDL on acute administration of DISE.

Table 7: Effect of Desmidorchis indica on lipids profile of control and experimental rats (Acute toxicity)

Parameters	Group I (Normal)	Group II (2000mg/kg)	P Value
Cholesterol (mg/dl)	91.50±2.89	92.06±2.92	^{NS}P>0.05
Triglyceride (mg/dl)	117.63±6.37	116.44±5.51	^{NS}P>0.05
HDL (mg/dl)	35.06±2.16	34.91±1.81	^{NS}P>0.05
LDL (mg/dl)	32.91±3.02	33.86±4.69	^{NS}P>0.05

Values are expressed as Mean ± SD for six rats. Data was calculated by student t-Test (Independent sample, P value two tail) using SPSS ver. 20. Statistically significant level 0.05. *P<0.05 significant and NS non-significant (^{NS}P>0.05).

Histological observation

The microscopic examination revealed that none of the organs from the Desmidorchis indica stem extract treated rats showed no alteration in cell structure or any unfavourable effects when viewed under the light microscope using magnification (10x40x) powers. No pathologies were recorded in the histological sections of the vital organs (liver and kidney) of the experimental group (Plate 1) and similar to the control group.

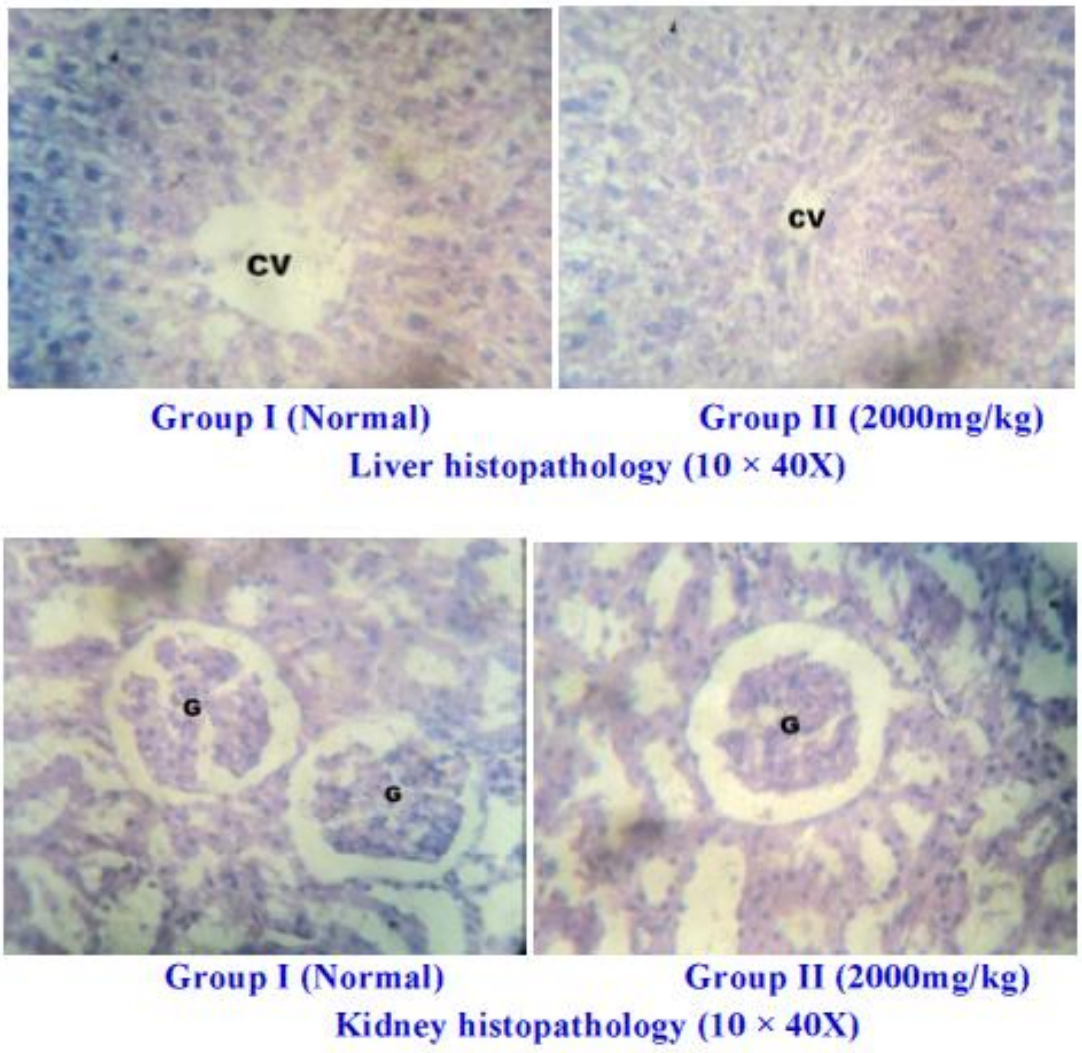

Plate 1: Histology of liver and kidney in control and Desmidorchis indica stem extract treated animal shows normal architecture

CV: Central vein

G: Glomerular: Bowman's capsule surrounds the glomerular capillary loops and participates in the filtration of blood from the glomerular capillaries

DISCUSSION

According to the World Health Organization, 80% of the remote area population rely on traditional medicine and the history of medicinal plants used by human as a medicine is about 60 000 year ol (Kifayatullah et al., 2014). The uses of medicinal plants as a source of drugs in primary health care have become popular universally, particularly in developing countries as a safe because of natural source. The bioactive compound isolated from herbal plants are believe to be harmless without causing any side effect on health, and thus is widely used as Over-the-counter (OTC) medication (Vaghasiya et al., 2011). Plant origin drugs are known to play a vital role in the management various chronic diseases and have received a great preference by researcher as alternatives source to allopathic pharmaceutical drugs in recent times (Mythilypriya et al., 2007) The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. However, there is a lack of proven scientific studies on the toxicity and adverse effect of these treatments.

For periods, herbal medicines and their preparations have been considered safe and effective with few side effects. This hypothesis may have had a significant impact on the indiscriminate use of these preparations among rural people. These formulations are usually administered over a long period of time without proper dosage monitoring by a specialist and without recognizing the toxic effects that may result from such long-term use (Eran et al., 2016). Therefore, scientific knowledge about oral toxicity is essential, which not only identifies

doses that can be used later, but also helps clarify the clinical signs that may be caused by the studied drug. Regardless of the pharmacological benefits of the Desmidorchis indica stem, Hence, the current study was undertaken to evaluate and focus on the acute toxicity of Desmidorchis indica stem in an animal model.

Acute toxicity testing provides initial information about the toxic mechanism of action of a drug, serves as the basis for classification and labeling, and helps determine the dose of a new compound in animal studies. Additionally, if high doses (e.g. 2000 mg/kg) are found to be viable, no further acute tests are performed (NRC, 2006)). In this study, Desmidorchis indica stem at a dose of 2000 mg/kg had no adverse effect on the treated rats in up to 14 days of observation. There were no significant changes in the weight and the organs of the rats. The bone marrow is a major location for novel blood cell manufacture and a vulnerable tissue targeted by toxic compounds in the hematopoietic system (Kifayatullah et al., 2015). Haematological parameters between the control and treatment groups showed that the extract was non-toxic to the hematopoietic system. Liver biomarkers are a specific tool for testing hepatotoxicity during drug biotransformation (Mukinda and Syce, 2007).

Additionally, most biochemical parameters remain unchanged. ALT, AST, ALP, and creatinine levels showed no associated changes, which are good indicators of liver and kidney function. No macroscopic lesions were found during histopathological examination. Kidney disease can be detected by measuring kidney indices such as creatinine, uric acid, urea, potassium, sodium, and chloride, and normal levels reflect a reduced likelihood of kidney problems (Dalle et al., 2006). No statistically significant differences were observed in liver function parameters such as ALT, AST, and ALP. In this study, there were no significant changes in ALT, AST, ALP, creatinine, urea, potassium and sodium. Additionally, no changes related to total protein, albumin, and globulin levels were observed in Desmidorchis indica stem extract fed rats when compared to the control. This indicates that the functional integrity of the kidney was not compromised after treatment with graded doses of the extract. Similarly, Desmidorchis indica stem extract oral administration non-significant changes ($p>0.05$) in total cholesterol (TC), serum triglyceride (TG), HDL and LDL levels were observed.

Effects of Desmidorchis indica stem extract on lipid peroxidation were evaluated by measuring MDA, SOD, Catalase, GPx, GSH, Vitamin C and E enzymatic and non-enzymatic activities. Elevation in oxidative stress in biological entities thereby interfering with the system's antioxidant defence mechanisms (Pajero et al., 2002). However, in this study, Desmidorchis indica stem administration at 2000 mg/kg bw did not cause any significant difference in all of the oxidative stress parameters when compared with the control group. Since no toxic stress were found during the acute toxicity study, further study was conducted to evaluate the subacute toxicity of Desmidorchis indica stem extract up to 14 days to prepare inclusive toxicological records on this plant.

4. Conclusion

Assessing the toxicity of medicinal plants is essential to ensuring the safety of the plants products used thereafter. The study concluded that Desmidorchis indica stem stem extract was not toxic at the test doses studied here and did not cause any obvious symptoms in acute oral toxicity studies. All hematological parameters, oxidative stress, lipid profile and biochemical parameters were not modified during the experimental period. Histological tests showed no significant changes in internal organs such as the kidneys or liver of treated rats. These results indicate that the unobserved side effect level (NOAEL) of Desmidorchis indica stem stem extract exceeded 2000 mg/kg/day. Additionally, these results contribute to the toxicity profile of Desmidorchis indica stem, which may be useful for practitioners using it as a herbal medicine. Further subacute studies are required to confirm the effective dose.

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