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University of Eldoret, Kenya, Mount Kenya University, Kenya, Chukwuemeka Odumegwu Ojukwu University, Nigeria, Kyambogo University, Uganda and University of Makeni, Sierra Leone

Evaluation of the Effect of Hydroethanol Extract of *Osmundastrum Cinnamomeum* on the Liver function of Castor Oil-Induced Diarrhea in Wistar Rats.

*Ugochi Vivian Chukwuemeka[1], Angelista Ojiugo Amadi.[1]Vivian Nneka Soronnadi[1], Valentine Chukwuebuka Ezeanyanwu[1], Andrew Chibuzo Nwaka,[2], Collins Bambode Lukong,[2] Angela Mma Ochia[3], Nneka Clare Onuoha[1].

[1]Science laboratory technology, Imo state polytechnic Omuma, Imo state.
[2]Department of Biochemistry, Chukwuemeka Odimegwu Ojukwu University Uli, Anambra state.
[3]Department of Biochemistry, University of Agriculture and environmental sciences Umuagwo, Imo state.

* Corresponding Email: guccinady@gmail.com

Abstract

The present study was carried out to evaluate the liver function effect of hydroethanol leaf extract of Osmundastrum cinnamomeum on castor oil –induced diarrhea in wistar rats. Thirty five wistar albino rats were divided into seven groups with five rats in each group. Rats in group A served as the normal control and was given water throughout the experiment, group B served as the negative control and was given only 0.5ml of castor oil by oral gavage, group C served as the positive control and was given 0.5ml of castor oil and 5mg/kg loperamide (standard drug), group D received 100mg/kg of extract before 0.5ml of castor oil (pre-treatment), group E received 300mg/kg of extract before 0.5ml of castor oil (pre-treatment), group F received 0.5ml of castor oil and 100mg/kg of extract (Post-treatment), group G received 0.5ml of castor oil and 300mg/kg of extract (Post-treatment). The biomarker enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct bilirubin (DB) and total bilirubin (TB) were investigated and their values for the above groups showed that there was no significant (P>0.05) difference on the values of the enzymes when compared with the standard range of accepted values for liver function tests which are: ALT (10-55µ/L), AST (10-40 µ/L), ALP (45-115 µ/L), DB (less than 5.1µmol/L) TB (1.71 -20.5 µmol/L). The study demonstrated that Osmundastrum cinnamomeum did not have any negative significant effect on the liver function and may be used in the management of liver diseases.

Keywords: *osmundastrum cinnamomeum, aspartate aminotransferase, alanine aminotransferase, castor oil, diarrhea., wistar rats*

Introduction

Background of the Study

In the beginning, the trial and error method was used to treat illnesses or even simply to feel better, and in this way, to distinguish useful plants with beneficial effects (Kunle *et. al.,* 2012). Medicinal plants have been used in virtually all cultures for the treatment of human diseases since time immemorial (Bako *et al*., 2005). Curative properties of medicinal plants have been attributed to the presence of secondary metabolites which includes alkaloids, sterols, tannin, saponin, phenol and flavonoid (Bero *et al*., 2009).

The Cinnamon Fern (*Osmundastrum cinnamomeum*) is a deciduous fern that grows in wet areas throughout the Adirondack Mountains and New York State. Cinnamon Ferns are among the first ferns to emerge in the spring. This fern is a member of the *Osmundaceae* family, which also includes the Interrupted Fern and Royal Fern (Boughton, 2005). The rhizome is said to have skin softening and slightly astringent properties and to be effective against diarrhea. This fern is easy to grow, especially in woodland gardens with moist soil. A decoction is used internally in the treatment of headaches, joint pain, rheumatism, colds etc., and also to promote the flow of milk in a nursing mother (Jud *et al.,* 2008).

The plant is harvested from the wild for local use as a food and a medicine, and is occasionally cultivated as a food crop. It is a source of 'osmunda fibre', which is used in potting mixes for growing orchids, and is also grown as an ornamental in

gardens. Although we have found no reports of toxicity for this species, a number of ferns contain carcinogens so some caution is advisable. Many ferns also contain thiaminase, an enzyme that robs the body of its vitamin B complex. In small quantities this enzyme will do no harm to people eating an adequate diet that is rich in vitamin B, though large quantities can cause severe health problems. The enzyme is destroyed by heat or thorough drying, so cooking the plant will remove the thiaminase (Benjamin *et al*., 2017).

Diarrhea is the passage of loose or watery stools occurring three or more times in a 24-hour period which means an increased frequency or decreased consistency of bowel movements, and it affects people of all ages. It is usually a symptom of an infection in the intestinal tract, which can be caused by a variety of bacterial, viral, and parasitic organisms (Amole *et al*., 2010).

The liver is one of the largest organs and is very important for various functions including metabolism and excretion of toxic and waste compounds from the body. All the compounds ingested are under the control of the liver for their safety and flow into the systemic circulation (Ozougwu & Eyo, 2014). It liver is one of the most heterogeneous organs, both functionally and structurally complex after the brain. The liver is involved in almost all vital metabolic functions including the uptake, metabolism, and excretion of carbohydrates, proteins, fats, cholesterol, fat-soluble vitamins, etc. (Ozougwu & Eyo, 2014).

Liver Function Tests (LFTs) are one of the most commonly-requested screening blood tests. Whether for the investigation of suspected liver disease, monitoring of disease activity, or simply as 'routine' blood analysis, these tests can provide a host of information on a range of disease processes. The title 'liver function tests' is, however, somewhat of a misnomer; only the bilirubin and albumin given in this panel offer information regarding the functional capacity of the liver (Benjamin *et al*., 2017).

Methodology

Samples

Osmundastrum cinnamomeum was obtained from a farm at Etioha in Ohaji Local Government Area, Imo State, Nigeria.

Apparatus:

Weighing balance, electric blender, dissecting set, dissecting board, 5ml syringes, test tubes, serum bottles, sample bottles, rat cage with plastic base, aluminium rat feeding trough, improvised rat plastic water bottles, nose mask, hand gloves, weighing balance, stopwatch, filter paper, masking tape, spatula, cotton wool, aluminium foil, muslin cloth, water bath, centrifuge, white paper, Pasteur pipette and micropipette.

Reagents:

Ethanol, Randox test kit, castor oil, distilled water

Method

Preparation of Extract

Osmundastrum cinnamomeum leaves were collected, dried at room temperature and ground to powder. Exactly 150 g of the pulverized sample was macerated in 1L of 70% ethanol and stirred at two hours interval for a period of 24 hrs for complete extraction. After 24 hrs the mixture was sieved using muslin cloth and filtered using whatmann fiter paper. The filtrate was concentrated at 50°C using water bath. The extract realized after concentration weighed 12.8 g. The extract was stoppered in a bottle and preserved in the refrigerator until use.

Experimental Design

A total of 35 rats was broadly divided into 7 groups of 5 rats each and used for the study. Thirty five male Wistar albino rats were randomized into five groups of five rats each. Group A: Normal control (given only food and water), Group B: Diarrhea untreated (Negative control) Group C: Diarrhea + standard drug (5mg/kg loperamide) (Positive control), Group D: Diarrhea + 100mg/kg of *Osmundastrum cinnamomeum* extract (Pre-treatment which means the group was taking the extract for 2 weeks before diarrhea was induced), Group E: Diarrhea +300mg/kg of *Osmundastrum cinnamomeum* extract (Pre-treatment which means the group was given the extract for 2 weeks before diarrhea was induced), Group F: Diarrhea + 100mg/kg of *Osmundastrum cinnamomeum* extract (Post-treatment which means that the group was given the extract after the diarrhea was induced), Group G: Diarrhea + 300mg/kg of *Osmundastrum cinnamomeum* extract (Post-treatment which means that the group was given the extract after the diarrhea was induced).

Induction of Diarrhea

The animals were fasted for 24hrs before the administration of extract and induction of diarrhea. However, the rats in groups D and E of each model were pretreated for a period of two weeks before 24hrs fasting and then treated before induction of diarrhea. One hour after the administration of the extract, diarrhea was induced using 0.5ml of castor oil. The castor oil was given to the animals orally.

Liver function test

Serum biochemical indices routinely estimated for liver functions were analysed. They include: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct and total bilirubin. The parameters were determined using Randox diagnostic test kits. The procedures used were according to the manufacturer's instruction.

Results

Effect of *Osmundastrum cinnamomeum* leaves on Liver Function Test

Effect of *Osmundastrum cinnamomeum* leaves on Liver Function Parameters of Castor Oil-induced diarrheal rat models.

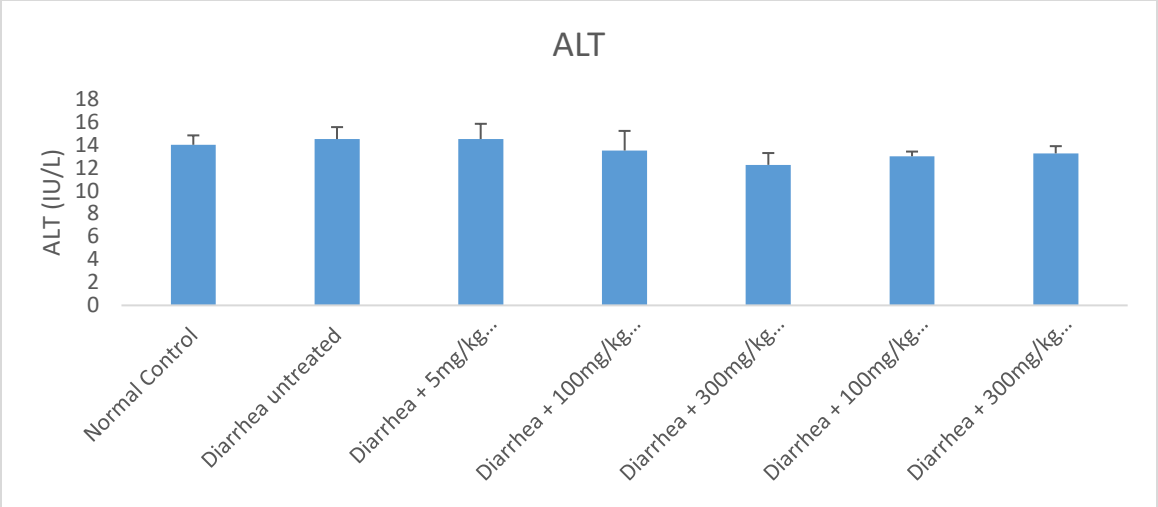

Figure 1: Effect of hydroethanol extract of *O. cinnamomeum* leaves on alanine transaminase of intestinal content of castor oil-induced diarrheal rat models

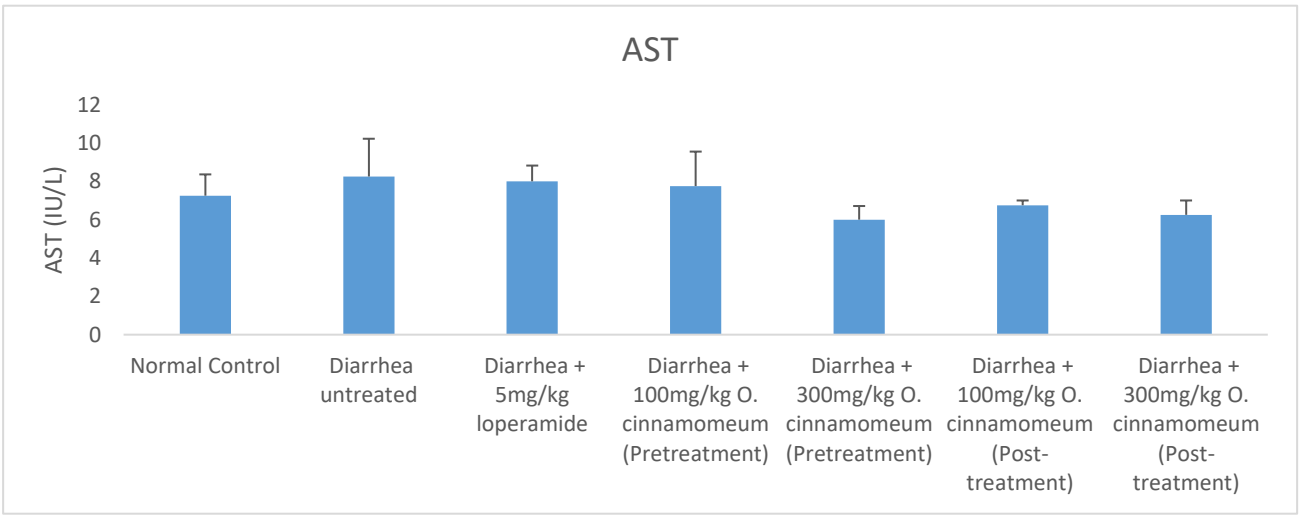

Figure 2: Effect of hydroethanol extract of *O. cinnamomeum* leaves on aspartate transaminase activity of intestinal content of castor oil-induced diarrheal rat models

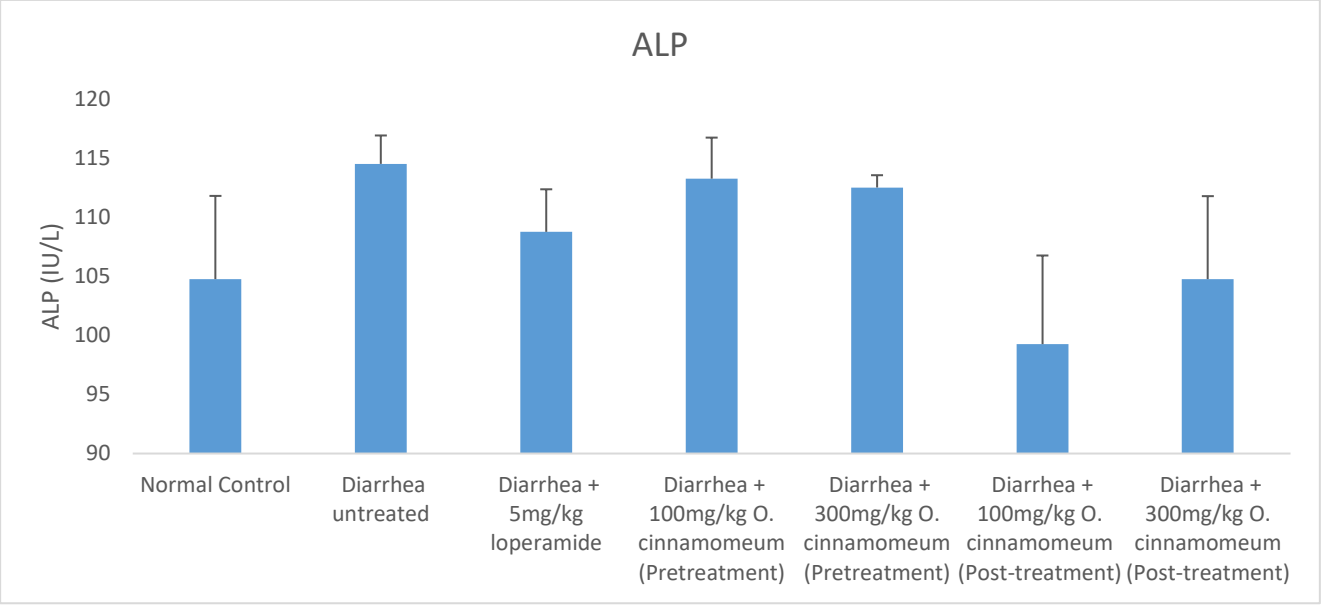

Figure 3: Effect of hydroethanol extract of *O. cinnamomeum* leaves on alkaline phosphatase activity of intestinal content of castor oil-induced diarrheal rat models

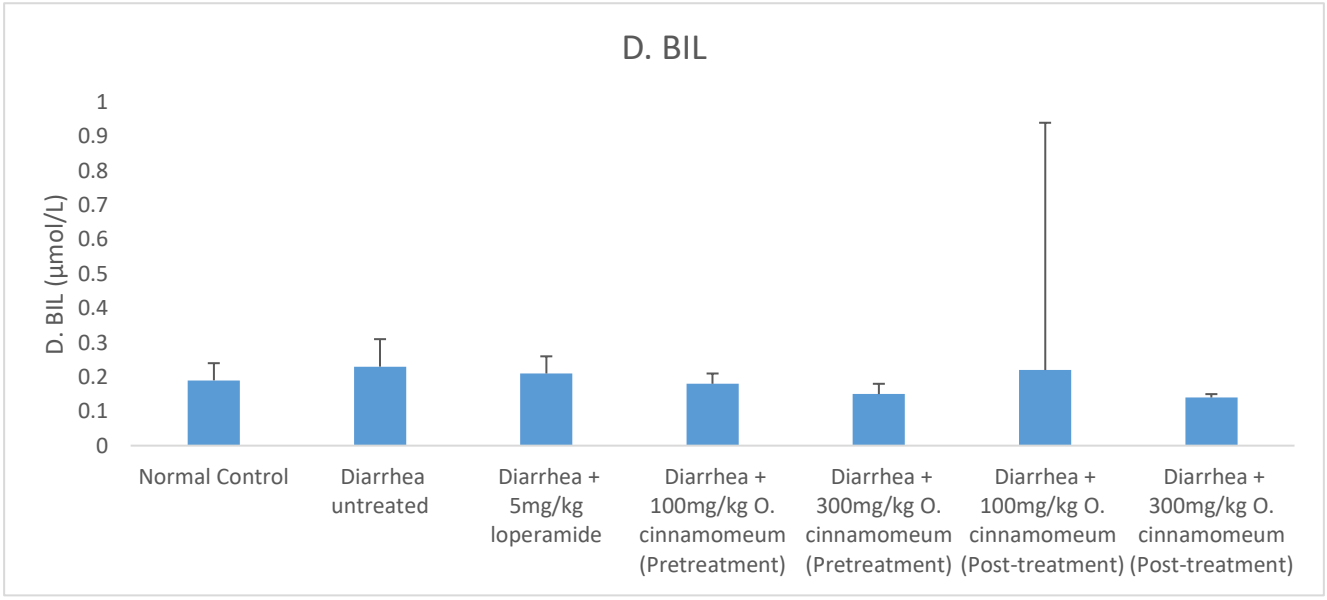

Figure 4: Effect of hydroethanol extract of *O. cinnamomeum* leaves on direct bilirubin activity of intestinal content of castor oil-induced diarrheal rat models.

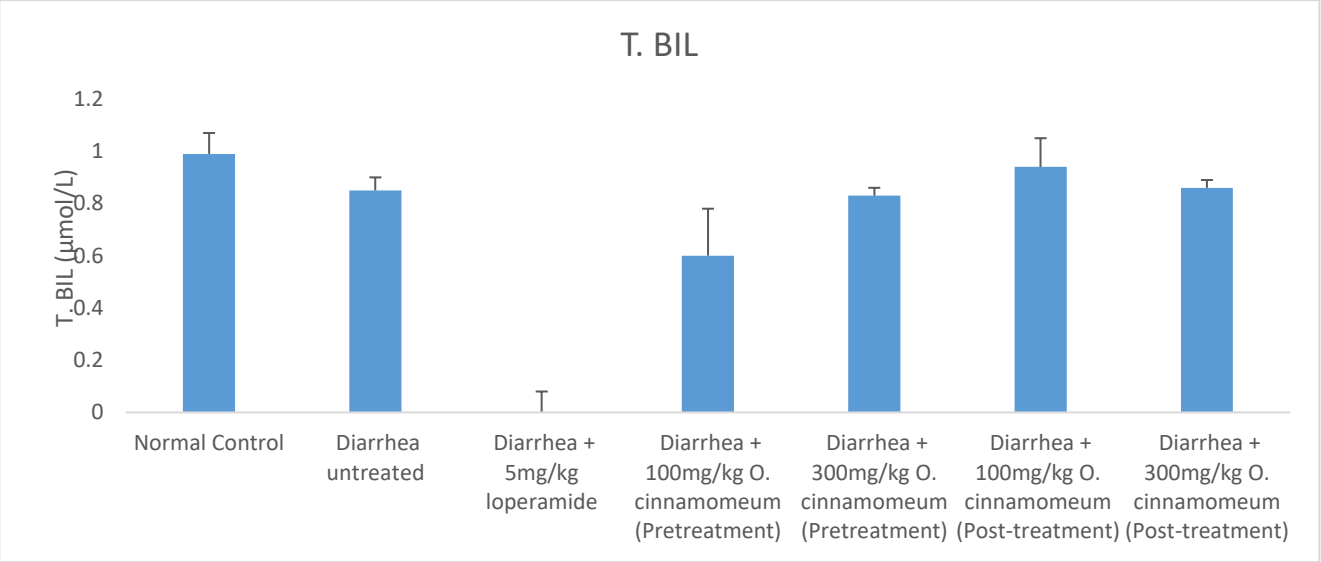

Figure 5 : Effect of hydroethanol extract of *O. cinnamomeum* leaves on total bilirubin activity of intestinal content of castor oil-induced diarrheal rat models

Effect of hydroethanolic extract of *O. cinnamomeum* leaves on liver function of castor oil-induced diarrheal rat models.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	D. BIL (U/L)	T. BIL (U/L)
Group A: Normal Control	14.00±0.82	7.25 ± 1.11	104.75 ± 7.04	0.19 ± 0.05	0.99 ± 0.08
Group B: Diarrhea untreated	14.50 ± 1.04	8.25 ± 1.97	114.50 ± 2.40	0.23 ± 0.08	0.85 ± 0.05
Group C: Diarrhea + 5mg/kg loperamide	14.50 ± 1.32	8.00 ±0.82	108.75 ± 3.60	0.21 ± 0.05	0.1. ± 0.08
Group D: Diarrhea + 100mg/kg *O. cinnamomeum* (Pretreatment)	13.50 ± 1.71	7.75 ± 1.80	113.25 ± 3.47	0.18 ± 0.03	0.60 ± 0.18 b
Group E: Diarrhea + 300mg/kg *O. cinnamomeum* (Pretreatment)	12.25 ± 1.30	6.00 ± 0.71	112.50 ± 1.04	0.15 ± 0.03	0.83 ± 0.03
Group F: Diarrhea + 100mg/kg *O. cinnamomeum* (Post-treatment)	13.00 ± 0.41	6.75 ± 0.25	99.25 ± 7.50 b	0.22 ± 0.72	0.94 ± 0.11
Group G: Diarrhea + 300mg/kg *O. cinnamomeum* (Post-treatment)	13.25 ± 0.63	6.25 ± 0.75	104.75 ± 7.02	0.14 ± 0.01	0.86 ± 0.030.

bSig. decrease with respect to group A

Discussion

Biochemical indices provide much needed parameters for determining the level of damage or effect of foreign compounds (plant materials) on the blood and tissues of living animals (Odeyemi, 2008). It has been established that there is a relationship between serum biochemical parameters and liver and kidney functions of experimental animals (albino rats) (Ileka *et al*., 2014 and Nwosu *et al*., 2017).

Liver function tests give information about the state of the liver, describing its functionality (albumin and lipid profile), cellular integrity (transaminases) and its link with biliary tract (ALP) (Ezejiofor *et al*., 2013). Thapa and Anuj (2007) had reported that standard range of accepted values for liver function tests, beyond which liver damage may be suspected is ALT (10 – 55 µ/L), AST (10 – 40 µ/L), and ALP (45 – 115 µ/L). Kamal and Hessah (2015) corroborated this when they reported that rise in AST, ALT and ALP values beyond this limits indicate early diagnosis of hepatotoxicity and tissue damage. It has been reported that liver toxicity is associated with increase in various serum liver enzymes resulting from damage to the hepatocytes.

This (castor oil-induced diarrhea rat model) study has shown that administration of *O. cinnamomeum* hydroethanol extract to feed each of these test animals at different doses did not change the ALT, AST, bilirubin, level of their serum indicating the plant did not induce changes in the activities of serum enzymes, protein synthesis and deamination when compared to the normal control, except the total bilirubin 100mg/kg pre-treatment and ALP 100mg/kg post-treatment group which reduced significantly when compared with the normal control group.

Higher values of these indices in the serum will have indicated severe toxicity; damage to tissues and cell membrane in the liver leading to release of the enzymes into the serum, hence their use as markers for toxicity (Odeyemi, 2008; Ileke *et al*., 2014; Nwosu *et al.,* 2017). ALT and AST are liver enzymes responsible for conversion of proteins and amino acids into energy for the liver cells.

The lack of significant alterations in the levels of ALT, AST, ALP, DB and TB are good indicators of liver functions, which suggests that the repeated administration of *O. cinnamomeum* do not have toxic effects on liver. The plant has been reported for the presence of phenolics as one of the important phytoconstituents. Plant phenolics are well known for their antioxidant activity. The phenolics in *O. cinnamomeum* may play a protective role against the oxidative damage to the liver cells by scavenging the free radicals (Ezejiofor *et al*., 2013).

Conclusion

Elevations in ALT and AST in out of proportion to ALP, and bilirubin denotes a hepatocellular disease. The study has clearly demonstrated that there was no hepato toxicity in the animal under investigation as seen in all the indices under study. In conclusion, *Osmundastrum cinnamomeum* was able to protect the liver against any liver damage.

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