International Journal of Innovative Research and Review ISSN: 2347 – 4424 (Online)
An Online International Journal Available at http://www.cibtech.org/jirr.htm
2015 Vol. 3 (2) April-June, pp.76-82/Ezejindu et al.
Research Article

THE EFFECT OF AQUEOUS LEAF EXTRACT OF *SYMPHYTUM OFFICINALE* (COMMON COMFREY) ON THE LIVER OF ADULT WISTAR RATS

***Ezejindu D.N.[1], Udemezue O.O.[1], Anyabolu A.E.[1], Chukwujekwu I.E.[1], Anike L.C.[2], Obialor D.C.[2], Akingboye A.J.[3] and Ihim A.C.[4]**

[1]Department of Anatomy, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria
[2]Department of Physiology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria
[3]Department of Microbiology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
[4]Department of Medical Laboratory Science, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria
**Author for Correspondence*

ABSTRACT

The aim of this study is to investigate the effect of aqueous extract of *symphytum officinale* leaf (common comfrey) on the liver of adult wistar rats. Twenty adult wistar rats of an average weighing 219g were used for the study. They were apparently divided into four groups of five animals each. Group A served as the experimental control and were orally administered 0.3ml of distilled water; the experimental groups B, C & D were orally received 0.4ml, 0.6ml and 0.8ml of aqueous extract of *symphytum officinale* leafs for twenty eight (28) days respectively. Twenty four hours after the last administration, the animals were weighed and weights were recorded. The animals were sacrificed under the influence of chloroform vapour and dissected. The liver organ were harvested, weighed and trimmed down to a size of 3mm×3mm and fixed in 10% formalin for histological studies. The results of this study revealed that consumption of *symphytum officinale* in low dose or small amount had no effect on the histological appearance of the liver but when consumed in high dose or excessive induced mild distortion of histological liver appearance which includes mild central vein hypertrophy, increased cellularity and periportal fibrosis of liver cells.

Keywords: *Symphytum Officinale, Wistar Rats, Liver Weight, Distilled Water, Body Weight*

INTRODUCTION

The world Health Organization (WHO) estimates that 80 percent of the population of some Asian and African Countries presently uses herbal medicine for some aspect of primary health care. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine.

According to the world Health Organization approximately 25% of modern drugs in the United States have been derived from plants (World Health Organization, 2008).

At least 7000 medical compound in the modern pharmacopoeia are derived from plants (Interactive European Network for industrial Crops and their Application, 2000-2005). A number of herbs are thought to be likely cause adverse effects (Talalay, 2001).

Further, "adulteration, in appropriate formulation, or lack of understanding of plant and drug interactions have led to adverse reactions that are sometimes life threatening or lethal (Elvin-Levis, 2001).

Contemporary herbalists have a mixed view of comfrey, despite widespread historical use. Its traditional names of kritbone, boneset and the derivation of its latin name *symphytum* (from the Greek Symphis, Meaning growing together of bones, and phyton, a plant) speak to its long standings reputation as a therapeutic herb (Staiger, 2013; Yarnell, 1999).

The plant contains the small organic molecule allantonin, which is thought to stimulate cell growth and repair while also depressing inflammation (University of Maryland Medical Center, 2012).

Research Article

More than 900 drugs, toxins and herbs have been reported to cause liver injury. Attempts are made globally to get scientific evidence for these traditionally reported herbal drugs. This scenario provides a necessity to carry out research on hepatotoxicity (Jaeschke *et al*., 2002).
Therefore, this study aims at investigating the effect of aqueous leaf extract of *symphytum officinale* (common comfrey) on the liver of adult wistar rats.

MATERIALS AND METHODS

Breeding of Animals

Twenty four apparently healthy adult wistar rats were purchased from the animal house of Anatomy Department, University of Calabar, Cross River State, Nigeria and bred in the animal house of University of Uyo, Akwa Ibom State.

The rats were maintaining ad libitum on water and growers mash bought from Nkwo Nnewi Market Anambra State, they were acclimatized for two weeks under normal temperature (27℃-30℃) before the experiment. Ethical committee permission was gotten from Faculty of Bioscience Nnamdi Azikiwe University Ethical committee.

Drug Preparation

Common comfrey (*symphytum officinale*) leafs were plucked from Okitipupa in Ondo State. It was identified at herbarium unit, Botany Department, Nnamdi Azikiwe University, Anambra State. It was sun-dried and then milled to a powder. 300mg/kg body weight was dissolved in 10mls of distilled water and administered to the animals.

Experimental Protocols

The twenty adult wistar rats were weighed and allocated into four groups (A, B, C & D) of five animals each. Group A served as the control while the other groups (B, C & D) serve as the test groups. The animals were fed with grower mash; a product of livestock feed Nig. Ltd. Lagos, Nigeria.

The experiment lasted for twenty eight days (28) and throughout the duration of the experiment, Group A was fed with 72g of normal feed (grower mash without *symphytum officinale* extract) while test groups B, C and D were fed with 72g grower mash plus 0.4ml, 0.6ml & 0.8ml of aqueous extract of *symphytum officinale* leaf (common comfrey) respectively for each day.

Twenty four hours after the last administration, the animals were weighed and recorded. The animals were then sacrificed under the influence of chloroform vapour and dissected, liver organ were harvested and weighed.

Tissue Processing

For easy study of sections under microscope, the tissues passed through several processes of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining. Fixation was carried out in 10% formal saline for 10 hours.

After fixation, the tissue washed in stream tap water. Dehydration of fixed tissue was done using ascending grade of alcohol, 50%, 70%, 90% and 95% absolute alcohol.

The tissue was clear in xylene after which infiltration was done in a molten paraffin wax at 60℃ for two hours each in two changes. The embedding of the tissue was done in molten paraffin wax and was sectioned afterwards. Haematoxylin and eosine method was used in staining.

Statistical Analysis

The data of this study were analyzed using T-test of SPSS version 16 software packages and $P<0.005$ was considered as the level of significance.

RESULTS AND DISCUSSION

Physical Observation

The animals used during this experiment were thoroughly observed and all survived to the end, except the animals in groups D which appeared weak and less active at the later stage of the experiment.

Research Article

Morphometric Analysis of Body Weight

Table 1: The result obtained from calculation of initial, final and weight changes of the various groups are presented in the table below
(Mean ± SEM Given for Each Measurement)

Groups	Group A	Group B	Group C	Group D	F-Ratio	Prob. of Sig.
Initial Body Weight	177.40±3.10	180.70±1.10	186.30±3.00	191.30±3.30	60.010	<0.005
Final Body Weight	200.00±3.70	196.70±2.10	174.60±2.60	168.00±1.40	47.360	<0.005
Weight Changes	22.60±0.60	16.00±0.40	-11.70±0.10	-23.30±1.90	11.310	<0.005

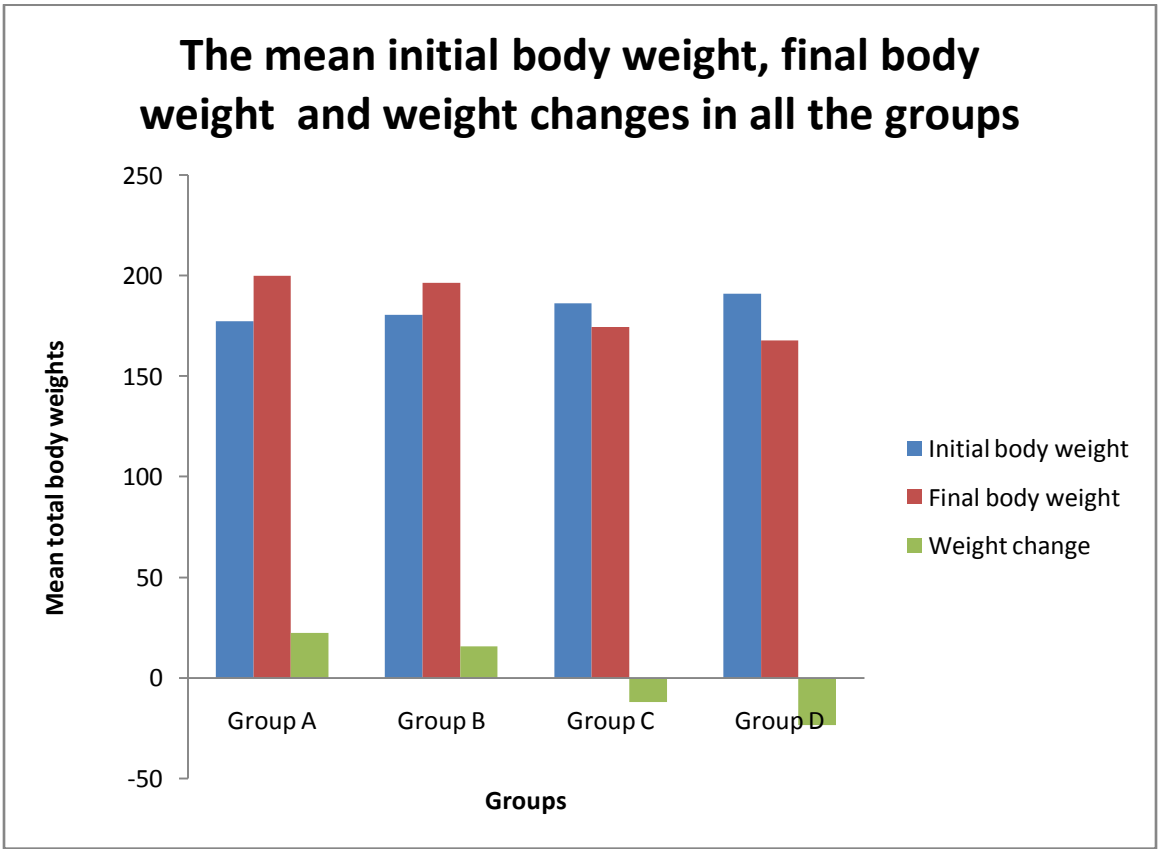


Figure 1: Bar chart showing the mean initial body weight, final body weight and weight changes in all the groups

Morphometric Analysis of Liver Weight

Table 2: Comparison of mean relative liver weight of all the groups (A, B, C & D). (Mean ± SEM Given for Each Measurement)

Groups	Group A	Group B	Group C	Group D	F-Ratio	Prob of Sign
Liver weight	5.28±0.310	5.31±0.316	5.47±0.140	5.64±0.561	41.30	<0.005

Research Article

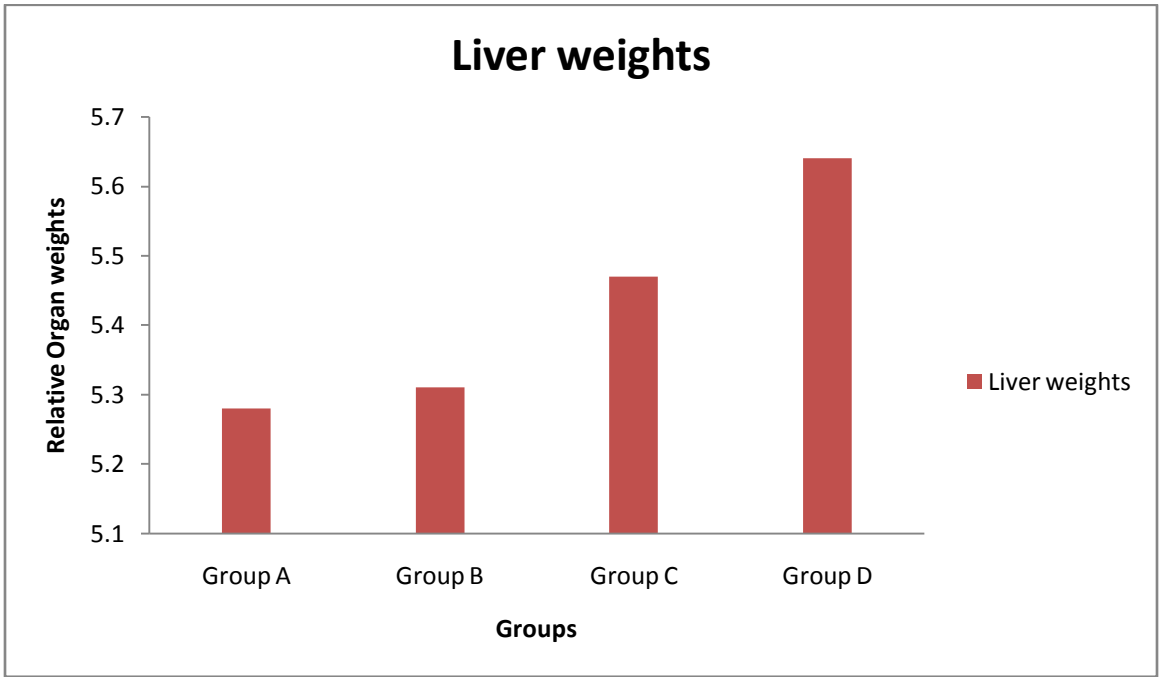
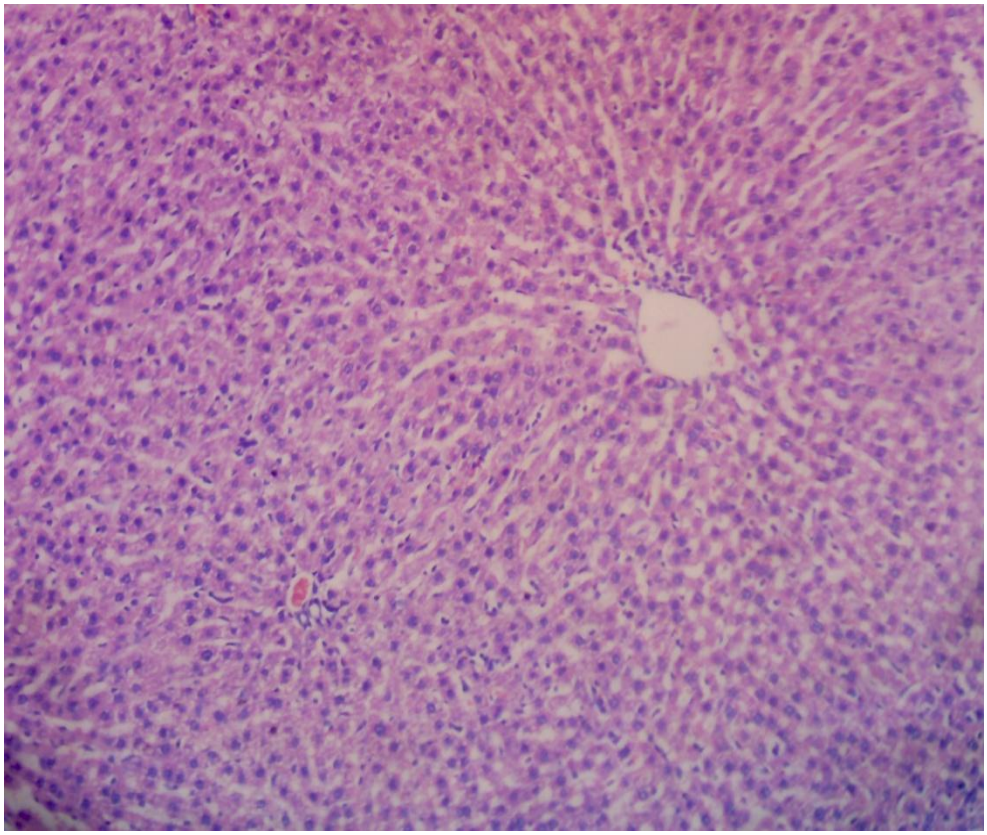
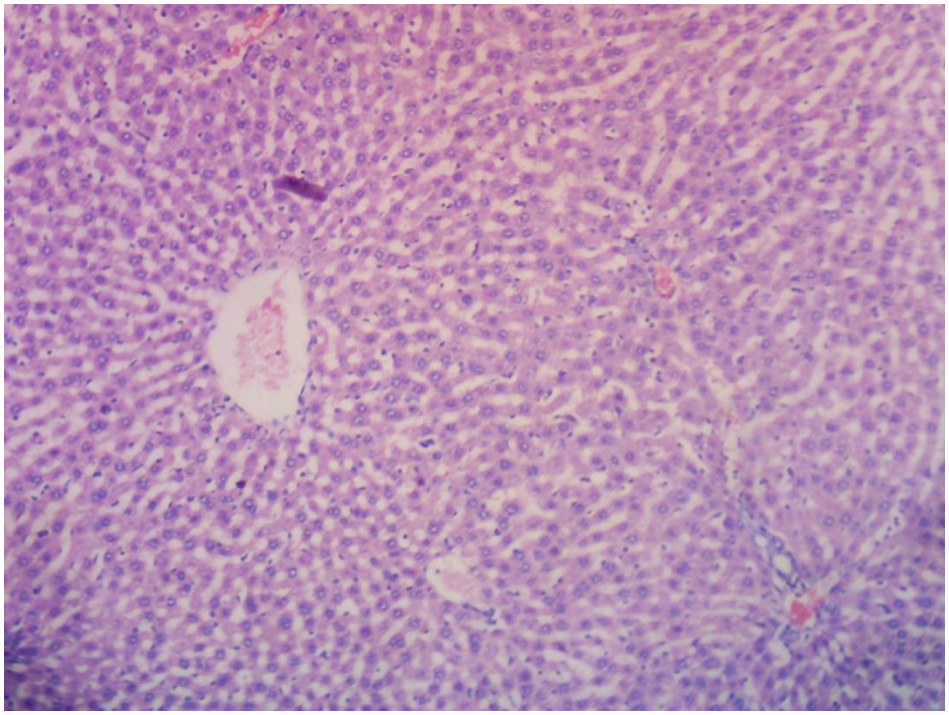


Figure 2: Bar chart showing the organ weights of all the groups

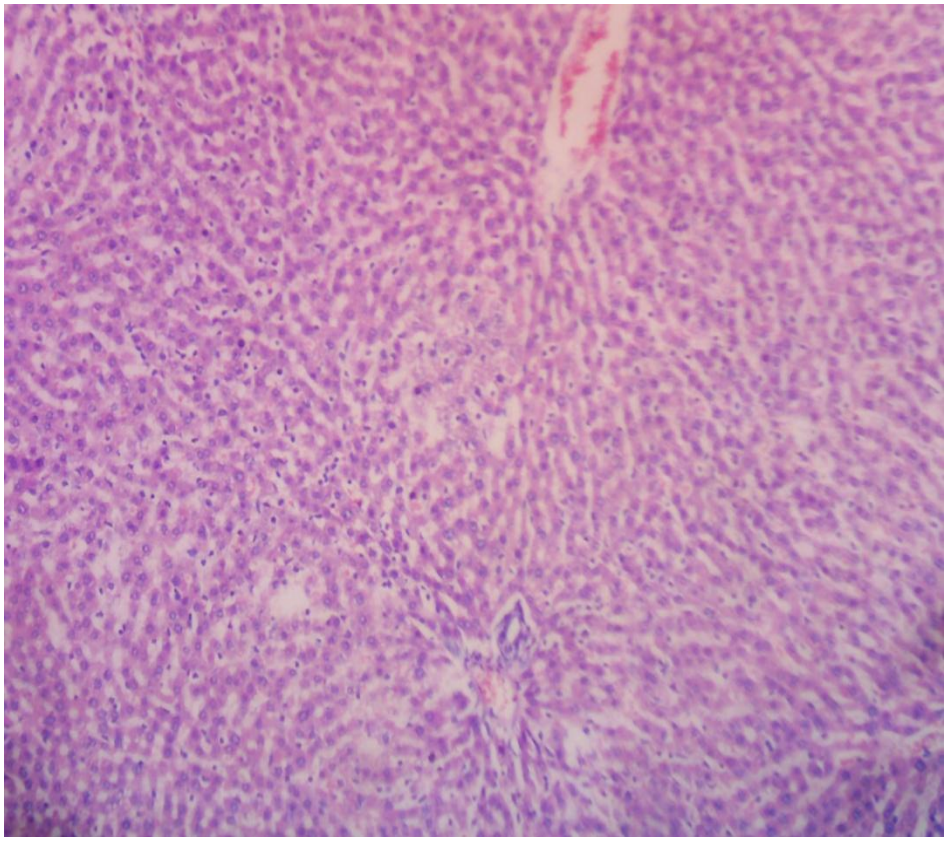
Histopathological Findings



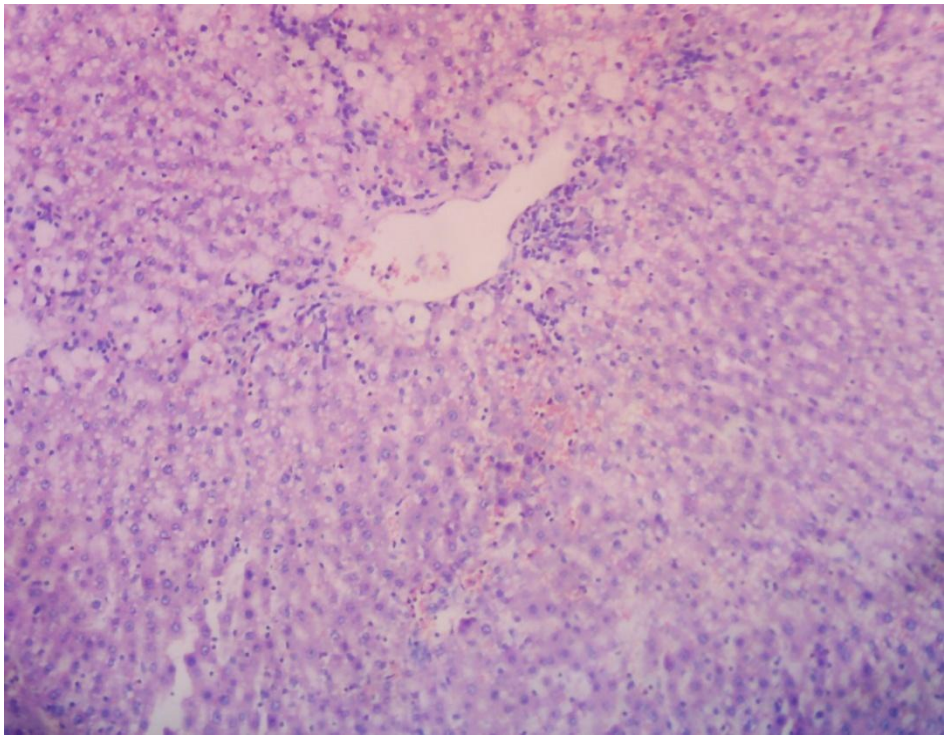
Photomicrograph 1: (Control group A) showing normal architectural structure of the liver-central vein surrounded by hepatocytes, with sinusoids



Photomicrograph 2: (Group B, treated with 0.4ml of aqueous extract of *symphytum officinale*) showing normal liver architecture, displaying normal central vein and hepatocytes



Photomicrograph 3: (Group C, treated with 0.6ml of aqueous extract of *symphytum officinale*) showing hypertrophy of central vein and increased cellularity



Photomicrograph 4: (Group D, treated with 0.8ml of aqueous extract of *symphytum officinale*) showing periportal fibrosis and hypertrophied hepatocytes of the liver tissues

Discussion

Comfrey was historically used to treat a wide variety of ailment ranging from bronchial problems, broken bones, sprams, arthritis, gastric and varicose ulcers, severe burns, acene and other skin conditions. It was reputed to have bone and teeth building properties in children, and have value in treating "many female disorders" comfrey plants constitute mucilage, steroidal saponins, tannins, pyrolizidine alkaloids, inulin and proteins (Nick, 2014).

Studies associating comfrey with veno-occlusive disease (VOD), do not differentiate between Russian and common comfrey, plants with different level of pyrrolizidine alkaloids (PAS). Veno-occlusive disease (VOD) can in turn lead to liver failure and comfrey has been implicated in at least one death, though type of comfrey being consumed, and other dietary, physiological and pharmacodynamic factors were not accounted for (Stallings, 2014).

In 2001, the United State Food and Drug Administration issued a ban of comfrey product marketed for internal use, and warning label for those intended for external use (FDA/CFSAN-FDA, 2009; Koll and Khingenburg, 2002). In additional to restriction on oral use, some expert recommended applying comfrey extracts no longer than 10days in a row, and no more than 4-6weeks a year (University of Maryland Medical Center, 2012; Stickel, 2000).

It has been reported that comfrey plants (*symphytum officinale*) contain pyrrolizidine alkaloids (Hirono and Mori, 1979); pyrrolizidine alkaloid poisoning causes a liver disorder in humans called hepatic veno-occlusive disease. The small and medium veins in the liver become obstructed, eventually leading to liver dysfunction, cirrhosis and death. Break down product of the pyrrolizidine alkaloids appear to bind to tissue in the liver, causing cell dysfunction and blockage of the veins by a proliferation of connective tissue (Maltocks, 1968).

In the present study, the final body weight of groups C and D decrease significantly ($P<0.005$) when compared with the control while group B Increase significantly ($P<0.005$) with the control group A.

The mean relative organ weight of groups C and D increase significantly when compared with the control while group B was statistically similar with control group A.

The histological findings revealed hepatocellular hypertrophy, periportal fibrosis and hepatrophied hepatocyte in the lever cells of groups C and D when compared with the control while group B showed normal cyto-architecture of the liver tissue.

Conclusion

From this present study, we therefore inferred that consumption of *symphytum officinale* (common comfrey) in low dose is not harmful to the liver but when consumed at high dose over a long period of time could cause histopathological lesions to the liver cells (hepatocytes).

REFERENCES

Elvin-Levis M (2001). Should we be concerned about herbal remedies. *Journal of Ethnopharmacology* **75**(2-3) 141-164.

FDA/CFSAN-FDA (2009). Advises Dietary Supplement Manufacturers to remove comfrey products from the Market. *PubMed* **4**(4) 123-134.

Hirono I and Mori H (1979). Induction of hepatic tumors in rats by senkirkine and symphytine. *Journal of the National Cancer Institute* **63**(2) 469-71.

Interactive European Network for industrial Crops and their Application (2000-2005). Summary Report for the European Union. QLKS-CT-2000-00111.

Jaeschke HGJ, Cederbaum AL, Hinson JA, Pessayre D and Lemastery JJ (2002). Mechanism of hepatotoxicity. *Toxicology Science* **65** 166-176.

Koll R and Khingenburg S (2002). Therapeutics characteristics and tolerance of topical comfrey preparations, Results of an observational study of patients. *Fortschr Med. Org* **120**(1) 1-9.

Maltocks AR (1968). Toxicity of pyrrolizidine alkaloids. *Nature* **217** 728-737.

Nick Jean (2014). Comfrey Power, Organic Gardening. *Journal of Pharmacology* **4**(5) 278-283.

Staiger C (2013). Comfrey root: from tradition to modern clinical trials. *Wiener Medizinische Wochenschrift* **163**(3-4) 58-64.

Stallings Ben (2014). Does comfrey Really Improve soil. *Permaculture News*, Permaculture Research institute **6**(1) 236-244.

Stickel F and Seitz HK (2000). The efficiency and safety of comfrey. *Public Health Nutrition* **3**(4(A)) 501-508.

Talalay P (2001). The importance of using scientific principles in the development of medicinal agents from plants. *Academic Medicine* **76**(3) 238-247.

University of Maryland Medical Center (2012). *Comfrey* (University Press) 101-109.

University of Maryland Medical Center (2012). *Comfrey* (University Press) 121-125.

World Health Organization (2008). Traditional medicine; Definition. *Journal of Pharmacology* **9**(6) 294-307.

Yarnell E (1999). Misunderstand "Toxic" Herbs. *Alternative & Complementary Therapias* **5** 6-11.