Pharmacogn J. 2024; 16(1): 76-87
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

*Original Article*

# The In-Vivo Assessment of the Effect of Traditionally Used Asparagus laricinus Extracts for Anticancer on the Kidney, Liver, and Spleen of Rats

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**History**

- Submission Date: 01-12-2023;
- Review completed: 17-01-2024;
- Accepted Date: 28-01-2024.

**DOI : 10.5530/pj.2024.16.11**

**Article Available online**

http://www.phcogj.com/v16/i1



## ABSTRACT

**Introduction:** The plants has been a fertile source of revealing novel molecules discovered by sophisticated techniques for drug discovery. The present research was triggered by the increase in the use of *Asparagus laricinus* as home remedy, with a lot of studies done invitro on the plant evaluating possible toxic effects of the dried roots extracts using Sprague Dawley rats as animal models was needed. The objectives of the study was to investigate deviations effects in haematology and histology parameters, on the liver, kidneys and spleen tissues of animals exposed to aqueous and ethanolic extracts of *Asparagus laricinus* roots. **Methods:** Interfaculty Animal Ethics Committee approval was obtained from the Faculty of Health Sciences at the University of the Free State. All experimental work was performed in Animal Research Unit at the University of the Free State, Bloemfontein, South Africa. The supernatant of dried plants was filtered, and the ethanol removed completely under vacuum. The aqueous sample was lyophilized to obtain dried powdered material. The powdered plant material was dissolved in distilled water to prepare 2%, 10% and 20% concentration. 54 Sprague Dawley rats (180g and 250g), both male and female, were divided into two groups of 24 and 30 rats for aqueous and ethanolic extracts respectively. The aqueous group was further divided into four subgroups of 6 rats which were exposed to 2%, 10% and 20% extracts and the final group were controls (unexposed). The ethanolic group was divided into five subgroups of 6 rats which were exposed to increasing doses of 50, 100, 200 and 400mg/kg/day extracts and the last group were controls (unexposed). The aqueous extracts were administered to the three subgroups for eight weeks ad libitum while the control group was exposed to tap water. Ethanol extracts were administered daily over a period of two weeks through gavage and the control group was administered water through gavage as well. Blood samples were collected, animals were sacrificed, and organs/tissues excised for histological assessment. **Results:** Haematological tests were selected as indicators of the damage to the tissue of organs, including the liver, kidney, and spleen. Comparison of treatment groups (n=6) and controls (n=6) across all ethanol extracts showed significant differences in the starting median change in weight at the 200g/kg/day dosage, as well as the median termination weight at 400g/kg/day. There were no statistical differences between the treatment groups and controls with regard to the rest of haematological variables. Comparison of the controls (n=6) and treatment groups (n=6) revealed an average median change in weight of slightly above 50g over the entire eight-week period of experimentation with aqueous extracts. The Histological evaluation could not reveal any pathological changes in both the aqueous and ethanolic extracts across all levels of dosage. **Discussion and conclusion:** Haematological results could not show any patterns in abnormalities although we observed statistically significant results on few parameters. Histologically, no pathological changes were observed. In conclusion, we summarize that the toxicological evaluation of *Asparagus laricinus* extracts may be considered relatively free of toxicity when given orally, as it did not cause death, damage, or inflammation to the tissues, nor produced any remarkable haematological adverse effects in both the male and female Sprague Dawley rats.

**Keywords:** In vivo, Asparagus Laricinus, Sprague Dawley rats, Haematology, Histology.

## BACKGROUND

Traditional medicine is a conceptual based on indigenous knowledge, acquired skills, and cultural practices reflecting of the theories, beliefs, and experiences by the indigenous diverse cultures, whether explicable or not[1,2]. This practice has been used to maintain health as well as in the diagnosis, treatment, and prevention the different types of illness[1,2]. The Chinese and Indians are the world leaning developed nations having a very sophisticated systems such as acupuncture and ayurvedic medicine. They lead by showing that traditional systems has to meet the needs of the local communities, and this was maintained for many centuries. This type of practice is generally accessible, cheap, and generally used in large parts of Africa, Asia, and Latin America.

For decades indigenous plants have been a source of medicine, nutrients and vitamins, or subsistence income available to the poorest in society[3]. Almost 65% of the world population rely on medicinal plants for primary health needs and around 80% utilize plants as their primary source of medicinal agents[4-7]. Even the sub-Saharan countries including South African with almost two third of its populations subscribing to traditional medicine[8]. Traditional medicine is an integral part of the South African cultural life, a position that is unlikely to change to any significant degree in years to come[9]. The is more than 300 000 traditional healers contributing a multi-million-dollar hidden economy[10-12] which even extends beyond the boards in SADC (Southern African Democratic countries) neighbouring countries[13]. In SADC with no restriction on the

**Cite this article:** Mokgawa SD, Makhoahle PM, Mashele S. The In-Vivo Assessment of the Effect of Traditionally Used *Asparagus laricinus* Extracts for Anticancer on the Kidney, Liver, and Spleen of Rats. Pharmacogn J. 2024;16(1): 76-87.

use and trade of traditional medicine some twinships like in Soweto, near Johannesburg, approximately 85% of the population consult traditional healers regularly[14,15]. The largest populated from in South Africa Kwazulu-Natal almost 8/10 people seeks medical advice from traditional healers, in preference or in addition to modern medicine[15]. Simultaneously, the enthusiasm for medicinal herbs and natural products have increased among the general public. There may be some safety concerns with traditional remedies, although they have been utilized in practice for hundreds of years.

The assumption that these plants have being used and are safe warrant scientific validation[16], and wrongly considered to have no side effects because they are 'natural'[17]. This safety assumption is based on their long-term usage in the treatment of diseases according to knowledge accumulated over centuries. This concept is largely incidental, and it is important to determine the toxicology of plant extracts, especially those that are used repeatedly over long periods. Perverse to views and general beliefs regarding the safety of medicinal plants, recent scientific research has shown that many plants used as food, or in traditional medicine, are potentially toxic, mutagenic, and carcinogenic[18-24].

### Asparagus laricinus

The genus *Asparagus* comprises approximately 100 species and consists of herbs, shrubs, and vines. It belongs to the family of Asparagaceae, a monocot and a member of the order Asparagales, and possesses great diversity throughout Africa, especially in South Africa[25]. *Asparagus laricinus* is part of the traditional medicine used in many parts of South Africa for the treatment of several ailments. The roots are used for the treatment of tuberculosis, and its use as a diuretic in the Khoisan and Cape Dutch ethnobotany[26]. Among the Batswana speaking people in theNorth West province, the roots are used for treatment of sores, redwater, urinary infections, umblical cord inflammation and general ailments[27]. The leaves and stem are medicinally used in Southwest parts of Gauteng for the treatment of different illness[28].

*Asparagus laricinus,* have not yet been scientifically assessed for their efficacy or safety to tissue or organs of recipients *in vivo*. Mashele *et al*., (2011) reported that *Asparagus laricinus* polyphenol extract exhibited a dose-dependent antimutagenic ability[29]. Furthermore, this extract showed no mutagenic effect on all tested *Salmonella typhimurium* bacteria strains *in vitro*. Previous *in vitro* studies on *Asparagus laricinus* extracts have demonstrated anticancer activity against three human cell lines namely, breast MCF7, renal TK10 and melanoma UACC62[30,31].

### Previous toxicology studies on medicinal plants

The is no scientific literature reporting the toxicological studies for *Asparagus laricinus*, even though more interest focused on other medicinal plants which are also used for the treatment of cancer. Toxicological studies on *Moringa oleifera* have indicated an absence of severe hepatotoxicity and organ damage, except in very high doses. The acute lethality ($LD_{50}$) test has been found to be relatively safe with subchronic toxicity studies, eliciting no significant difference in sperm quality, haematological in the treated rats, as compared to the controls[32-34]. Both studies engaged the use of laboratory experimental animal models to evaluate the toxicology of plant extracts.

## PROBLEM STATEMENT

Even though drugs targets at tissues and organs in order to reduce morbidity and avoid mortality in the population, in most cases complications and adverse effects are reported, more especially when used extensively. Majority of modern treatments have some side-effects which might be detrimental to the health of patients. These include, amongst others, chemotherapy, radiation therapy and surgery for the treatment of cancer.

Previous *in vitro* studies on *Asparagus laricinus* extracts have demonstrated anticancer activity against three human cell lines, namely, breast MCF7, renal TK10 and melanoma UACC62[30,31]. In this project *Asparagus laricinus* extracts was utilized to assess the safety and toxicology in vivo.

### The aim and objectives

The aim of the present work was to evaluate the possible toxic effects of the dried roots of *Asparagus laricinus* extracts using Sprague-Dawley rats as animal models.

### The objectives of this project

To investigate the toxicity of *Asparagus Laricinus* extracts by:

1. Investigating variations in haematological parameters after exposing the rats to different concentrations in different solvents.
2. Assessing histopathological variations of the liver, kidney and spleen tissues of rats exposed to different concentrations in different solvents.
3. Analysing the correlation between the haematological parameters and the histopathological results

## RESEARCH DESIGN AND METHODOLOGY

Toxicology is the study of how chemical substances interact with living systems and affect normal processes. The information obtained from toxicological studies is utilized to predict safe exposure levels of chemicals. Although *in vitro* analysis (e.g., tissue cultures and organs) of toxicity is preferred, animal models are necessary to validate the results of non-living or *in vitro* tests. The *in vitro* tests are performed as the last protective step before exposure of humans and animals to potentially dangerous substances. The advantage of animal testing is to evaluate the effect when the entire system is involved, which cannot be evaluated by *in vitro tests*; as hormones, enzymes and other systemic influences are not available.

### Study design

A case control study that involved experimental animals, where cases were exposed to different concentrations of *Asparagus laricinus* extracts utilising water, dichloromethane, and ethanol as solvents. Controls were not exposed to the extract but supplied with water, which served as placebo.

### Study population

Sprague Dawley rats of either sex were reared at the Animal Research Unit of the University of the Free State, Bloemfontein, South Africa. The rats were three months old; weighed between 180g and 250g at the beginning of the experiment; and were fed with standard pelleted food.

### Sample size

A total of 78 Sprague Dawley rats, both male and female, obtained from the Animal Research Unit at the University of the Free State in 2013, were divided into 3 major groups which were exposed to water, ethanol, and dichloromethane extracts of *Asparagus laricinus.* The three groups consisted of 24 rats for the water extract, 30 rats for ethanol extract and 24 rats for dichloromethane extract. A total of 12 rats were used as controls and they were included in the numbers supplied for the water and ethanol extracts. The six used for the water extract were utilised for the entire 2 months, while the other six controls were used for two weeks of exposure to ethanol and dichloromethane extracts.

The dichloromethane extract was discontinued when two rats died due to adverse effect after administration of the extract. This supports the reason that invitro testing should be performed to establish the safe

standard of concentration prior to exposing animals to the extracts. Discontinuing of the dichloromethane group (24 rats) resulted in only 54 rats being used for the entire experiment. As explained by Guptas et al., (2023) alternative methods should be established so as to avoid unnecessary scarifies of animals[35].

### Inclusion and exclusion criteria

Rats weighing between 150g and 250g were included in the study to ensure homogeneity amongst the study population.

Rats weighing above or below the above-mentioned range were excluded.

## MATERIALS

The plant was authenticated by scientists at the National Botanical Gardens in Bloemfontein, Free State, South Africa. Voucher specimen number MASH 200 was allocated to the plant.

### Plant Extraction Methods

The plant material (*A. laricinus*) was authenticated by scientists at the National Botanical Gardens in Bloemfontein, South Africa. The collected root materials were dried at room temperature, pulverised by a Macsalab Mill (Model 200, LAB), and weighed. The powder was then stored at room temperature until analysis. Plant material (10 grams of the dried roots) was soaked in a volume of 500 ml of ethanol, dichloromethane, or purified water for 72 hours under shaking conditions (120 rpms). The supernatant was filtered passively through a Whatman® filter paper, 11 cm in diameter. The solvent (ethanol) was removed completely under vacuum, by using a speed evaporator (Univapo 100H) at 50°C.The aqueous sample was lyophilised for 72 hours in the VIRTIS 5 L freeze dryer (VIRTIS New York, USA) to obtain a dried powdered plant extract. The dried samples were then reconstituted in either water or ethanol.

### Plant extracts administration method

Seventy-eight Sprague- Dawley rats were divided into three groups: 24 for the water extract, 30 for ethanol and 24 for dichloromethane extracts.

### Group 1: Water extract

Consisted of 24 rats which were further subgrouped into 4 groups of 6 rats per subgroup. Although we had six rats per group, they were further grouped into two rats per cage (see Figure 1) as per rules and regulations governing the use of laboratory animals. Water extracts were diluted to prepare 2%, 10% and 20% concentrations. The different concentrations were administered to three of the four groups *ad libitum*.

**Figure 1**: Setup for different water extracts and control group (each column represents a group, and each cage contains two rats).

The last group served as controls, where tap water was administered instead of the extract. The extracts were administered over a period of 2 months, using a 200ml feeding bottle per cage. Bottles were cleaned and replaced with a fresh extract after every two days.

### Group 2: Ethanol extract

This group was made of 30 rats which were further subgrouped into 5 groups of 6 rats per subgroup. Although we had six rats per group, they were further grouped into two rats per cage as per rules and regulations governing the use of laboratory animals. Ethanol extracts were administered once per day for two weeks. The extracts were administered through gavage by varying the volumes, resulting in the amounts as indicated in the table below:

Although the controls had water *ad libitum*, water was administered by gavage every time the exposed groups were administered with the extracts.

### Group 3: Dichloromethane extract

According to the proposal, different amounts as per table 2, below were supposed to be administered intramuscularly. Due to the nature of pain and ulcerations resulting from repetitive intramuscular injections on the tails of rats over a two-week period, scientists at the Animal Research Unit recommended a switch over to gavage feeding. The process was immediately stopped due to the death of two rats within a few minutes after administration of the extract through gavage. Administration of dichloromethane was discontinued, and this group was completely excluded from the research project.

### Sample collection

The rats were placed in a desiccator with Halothane (Safeline Pharmaceuticals) soaked in cotton wool for anaesthetic purposes until they were completely unconscious. Blood samples were collected in EDTA anticoagulated tubes (haematological analysis) and non-anticoagulated tubes (clotted blood for biochemical analysis) through insertion of the needle into the heart. The rats were administered with further halothane for euthanization. The blood collection process was done by scientist employed by the Animal Research Unit at the University of the Free State. Liver, spleen, and kidneys were excised (by the researcher) and immediately preserved with 10% Neutral Buffered Formalin for histological investigations.

### Laboratory investigations

Plant extracts ingested like medication or fluids are absorbed in the gastrointestinal tract. Absorbed substances are transported to the liver via the portal vein for detoxification and further processing and distribution throughout the body tissues and organs via the vascular system (blood). Blood passes through the kidneys where it is filtered in the nephrons, and where unwanted and waste products are excreted in urine.

The presence of toxic substances in ingested material may cause damage to tissues and organs such as the liver, kidneys, and blood cells in circulation. The extent of the damage may also be associated with the period of exposure and the amount/ concentration of substances in circulation.

Haematological tests would display destruction to the blood cells (red blood cells, white blood cells and platelets) because these cells would be exposed to toxic substances during transportation in the vascular system. Apart from the above-mentioned analytes, histological investigation of the organs/ tissues would then show the damage caused by such substances, as well as the degree of the damage.

Haematology samples were analysed within five hours using the ABX Pentra 60. All three levels of quality control samples were run in conjunction with the rats' blood for every batch of samples.

**Table 1: Animal groups and doses of ethanol extracts per day.**

| **Extract** dose(mg/kg/day) | 50 | 100 | 200 | 400 | **Controls** |
|---|---|---|---|---|---|
| **Number** of rats per group | 6 | 6 | 6 | 6 | 6 |

**Table 2: Animal groups and doses of dichloromethane extracts per day.**

| **Amount** of extract per day | 50mg/kg | 100mg/kg | 200mg/kg | 400mg/kg |
|---|---|---|---|---|
| **Number** of rats per group | 6 | 6 | 6 | 6 |

## Haematological parameters

An ABX Pentra 60 analyzer was used to determine the haematological parameters. Haematological parameters determined in this study included Red Blood Cell count (RBC), Haemoglobin (Hb), Haematocrit (Hct), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Cell count (WBC), differential count and platelet count (Plt).

The analyzer uses the multi distribution sampling system (MDSS) where one cycle is distributed into 3 blood samples for RBC/Plt, BASO/WBC and LMNE matrix which are channelled to the three respective chambers with reagents. The different full blood count parameters were analysed using current impedance changes; spectrophotometry; double hydrodynamic sleeving coupled with cytochemistry; and measuring of transmitted light; to measure the different parameters of the full blood count[36].

## Principle for the detection of RBC and Platelets

The analyzer uses the measurement of impedance variation generated by the passage of cells through a calibrated micro aperture. The specimen is diluted in electrolytic diluents (good conductor of current) and pulled through the calibrated micro-aperture. Two electrodes are placed on either side of the aperture. Electric current passes through the electrodes continuously. When the cell passes through the aperture, electric resistance between the two electrodes increases proportionately with the cell volume.

## Principle for the detection of Haemoglobin

Red blood cells are lysed, and the haemoglobin released combines with potassium cyanide to form a chromogenous cyanmethaemoglobin compound. The compound is measured through the optical part of the first dilution chamber using a spectrophotometric technique at a wavelength of 550nm. The absorbance obtained is multiplied by the coefficient of calibration in order to get the actual concentration in g/100ml.

Twenty-five (25)µl of whole blood was delivered to the LMNE chamber in a flow of EOSINOFIX. This reagent lyses the RBC, stabilizes the WBC in their native forms and stains the eosinophil nuclei with specific colouration. The solution was then stabilized and transferred to the measuring chamber. Each cell was measured both in absorbance (cytochemistry) and resistivity (volume).

# RESULTS

A matrix is drawn up with the volumes on the X-axis and optical transmission on the Y-axis from the above measurements. The study of the matrix image permits the clear differentiation of 4 out of the 5-leukocyte population.

## Histological Investigations

The liver, spleen and kidneys are prime targets for histological investigations because they are involved in the degradation and excretion of a myriad of chemical compounds. Renal damage has been associated with the use of the medicinal plants in the treatment of different disorders, including diabetes mellitus[37].

## Preservation/fixation

Excised liver, kidney and spleen tissues were immediately placed in 10% formalin for fixation. They were stored in separate specimen bottles at room temperature until processed.

## Processing

The samples were sliced into smaller tissues, stored in labelled histology cassettes before processing. They were processed with a microwave technique[38], using a H2500 microwave processor supplied by Energy Beam Sciences, Inc.

Tissue samples were immersed in a 100% ethanol container and placed in the microwave at 67°C for 16 minutes. Ethanol was discarded and the procedure repeated for a further 16 minutes. This was followed by immersion of the samples in 100% isopropanol and incubation at 60°C for 15 minutes. The step was repeated with fresh 100% isopropanol for a further 15 minutes. In all steps mentioned above, the container was loosely covered and agitated at all times. In the next two steps of the procedure, tissue samples were immersed in paraffin wax for 15 minutes each and the temperature was set at 65°C and 80°C respectively.

## Embedding and tissue slicing

The samples were embedded in paraffin wax using the Shandon HistoCentre, distributed by Optolabor (PTY) (LTD) in South Africa, according to the following procedure:

The cassettes containing processed tissues were removed from the wax container in the last processing step and placed on the heat section of the embedding instrument. The metal mould was heated slightly for each tissue, filled with molten paraffin wax and the tissue positioned in the molten wax using forceps. The labelled plastic mould of the cassette was placed onto the metal mould, filled completely with molten paraffin wax, and placed on the cold plate for the wax to harden.

Samples were cut into thin slices (5µm) using Leica™ microtome, floated in a water bath and picked with slides and labelled according to the plastic mould. The wax was allowed to melt in an oven set at 57°C for 30 minutes and the slides were stained.

## Staining

The slides were stained with Hematoxylin and Eosin (H&E) procedure as depicted in Table 3 below.

Cases and controls were analysed microscopically at 40X magnification by a pathologist at the Universitas Academic Complex, Bloemfontein. A total of 486 slides were microscopically evaluated because three tissues were sliced from different anatomical regions of the organs. The pathologist specifically undertook macrosopic and microscopic investigations for variations on the tissues excised.

Macroscopically, the pathologist investigated the presence of abnormal architecture of the tissue parenchyma on all specimens. Microscopically, any signs of dysplasia in terms of cellular changes, viz, nuclear pleomorphism, high nuclei-cytoplasm ratio, nuclear hyperchromasia, necrosis and abnormalities observed during mitosis were investigated by the pathologist. Including signs of inflammation were also investigated microscopically.

## Statistical analysis

Data was captured in Microsoft Excel® by the researcher. Any further analysis was done by a statistician using SAS Version 9.2. Descriptive statistics, namely frequencies and percentages, were calculated for categorical and medians, and percentiles were calculated for numerical

**Table 3: Hematoxylin and Eosin stain.**

| Step | Process | Solution | Timing |
|---|---|---|---|
| 1 | De-wax | Xylene | 5 minutes |
| 2 | De-wax | Xylene | 3 minutes |
| 3 | Hydrate | 100% ethanol | 1 minutes |
| 4 | Hydrate | 95% ethanol | 30 seconds |
| 5 | Hydrate | 70% ethanol | 30 seconds |
| 6 | Hydrate | water | 30 seconds |
| 7 | Nuclear stain | Mayer's Haematoxilyn | 5 minutes |
| 8 | Rinse | Water | 30 seconds |
| 9 | Differentiate | 1% acid alcohol | 30 seconds |
| 10 | Blueing | Scott's tap water | 1 minute |
| 11 | Cytoplasmic stain | Eosin | 5 minutes |
| 12 | Dehydrate | 70% ethanol | 30 seconds |
| 13 | Dehydrate | 90% ethanol | 30 seconds |
| 14 | Dehydrate | 100% ethanol | 1 minute |
| 15 | Clearing | Xylene | 1 minute |
| 16 | Clearing | Xylene | 1 minute |

Slides were mounted with a coverslip using Entellan.

**Table 4: Median change in weight of the controls (unexposed) and experimental (exposed) rats.**

| | | Median | IQR | Min – Max | p-value |
|---|---|---|---|---|---|
| Start mass (g) | Control | 180.0 | 178.0-188.5 | 158.0-201.0 | 0.252 |
| | Experimental | 188.0 | 179.0-193.0 | 164.0-204.0 | |
| End mass (g) | Control | 216.5 | 211.5- 238.0 | 209.0-271.0 | 0.102 |
| | Experimental | 225.0 | 220.0-244.0 | 201.0-265 | |
| Change in Mass | Control | 41.5 | 33.0-54.5 | 19.0-70.0 | 0.700 |
| | Experimental | 44.0 | 33.0-56.0 | 7.0-74.0 | |

IQR (Interquartile Range), Min (Minimum), Max (Maximum) g (Grams)

data. The normality of the numerical variables were tested using Shapiro-Wilk's test, and if a variable was skewed ($p < 0.05$) the median and inter-quartile range (IQR) was reported. Analytical statistics, namely the Kruskal-Walli's test, was used to compare median values between the control and experimental groups, as well as to compare the median values between the different extract concentrations. A significance level of 0.05 was used.

# RESULTS

## Mass

The median weight of all controls (n=12) and the experimental group combined (n=42) were statistically compared using Kruskal-Walli's test. No significant differences ($p>0.05$) between the median weights of unexposed and exposed rats at the start and termination of the experiment was observed, as illustrated by Table 4.

## Haematological parameters

Comparison studies for full blood count parameters between controls (n=12) and combined experimental group (n=42) were performed. There were no significant differences ($p>0.05$) between combined controls and experimental animals regarding haematological parameters of the combined ethanol and aqueous extracts at different levels/doses (Table 5 & 6).

*Comparative study of the control group versus the various ethanol extract group*

In this section, the statistical analysis for comparing controls and treatment groups of ethanoic groups, at increasing dosages, are presented. This was done to determine the effects of lower and higher ethanol extract concentration on the parameters.

## Mass

Table 7 below represents the statistical analysis of the comparison of the weight of rats (in grams) for the control (n=6) and treatment groups, at increasing dosages of ethanoic extracts ranging from 50mg/kg/day to 400mg/kg/day. Significant differences were observed for the initial mass of rats exposed to 200mg/kg/day ($p=0.0100$), and also for the termination mass of rats exposed to 400mg/kg/day ($p=0.0303$). There was no difference in the change of mass for the rest of the dosages administered.

Figure 2 below illustrates the graphical presentation of the differences between the initial and termination median masses at increasing ethanol extract doses. These clearly illustrates that the median change in weight over the two weeks experimental period is slightly below 50mg. It is also observed that rats exposed to 200mg/kg/day dose had the least weight change, while those exposed to a 400mg/kg/day dose had the most. These changes were not statistically significant.

## Haematological parameters

Full blood counts were determined for the rats treated with ethanol extracts at different doses ranging from 50mg/kg/day to 400mg/kg/day. A Kruskal-Walli's test for comparing median results was performed and the results showed no significant difference between the control (n=6) and treatment groups (n=6) across most levels of ethanoic extracts.

Table 8 illustrates significant differences ($p=0.0106$) in the median results of platelets at the dosage of 50mg/kg/day. It must be noted that the percentage change from the control median (730 x $10^9$/L) to 50mg/kg (850 x $10^9$/L), was 16%, while the human refence range is 150 – 410 x$10^9$/L (Bates & Lewis, 2011). The human range spans over a range of 63%. Furthermore, it must be noted that the 400mg/kg group's platelet median was 839 x $10^9$/L, which showed a 13% change with no statistically significant difference. The remaining parameters in both Tables 8 and 9 could not demonstrate any significant differences statistically ($p>0.05$).

It is of utmost importance to emphasize that the results for full blood count parameters are expressed in varying units, and therefore fewer figures for comparison of their medians would not be practical at this stage.

*Comparative study of the control group versus various aqueous extracts group*

## Mass

The median change in mass for both treated (experimental) and untreated (control) groups over the experimental period (8 weeks), were recorded. Results for controls and experimental groups were

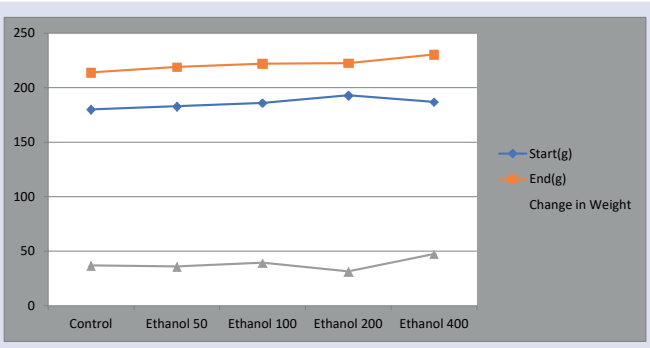


**Figure 2**: Graphical representation of the median change in mass for the rats on ethanol extracts.

**Table 5: Comparison of RBC parameters between combined controls and experimental groups.**

| | | RBC (x10$^9$ /l) | HB (g/dl) | HCT (%) | MCV (fl) | MCH (pg) | MCHC (g/dl) |
|---|---|---|---|---|---|---|---|
| Median | Control | 7.67 | 15.10 | 42.20 | 56.00 | 19.80 | 35.40 |
| | Exposed | 7.87 | 15.5 | 43.60 | 56.00 | 19.80 | 35.65 |
| IQR | Control | 7.5-8.1 | 14.8-15.3 | 41.4-43.6 | 54.0-56.0 | 19.0-19.9 | 35.3-35.9 |
| | Exposed | 7.4-8.1 | 14.9-16.0 | 41.5-45.0 | 55.0-56.0 | 19.6-20.1 | 35.4-35.8 |
| p-value | | 0.820 | 0.183 | 0.320 | 0.537 | 0.655 | 0.516 |

IQR (Interquartile Range), RBC (Red Blood Cells), HB (Haemoglobin g/dl), HCT (Haemoglobin Content %), MCV (Mean Cell Volume) fl (Femtoliters), MCH (Mean Cell Haemoglobin), pg (Picograms), MCHC (Mean Cell Haemoglobin Content g/dl)

**Table 6: Comparison of white cell parameters and platelet between combined controls and experimental groups.**

| | | WBC | LYM | MON | NEU | EOS | BAS | PLT |
|---|---|---|---|---|---|---|---|---|
| Median | Control | 3.70 | 2.83 | 0.32 | 0.48 | 0.03 | 0.03 | 734.0 |
| | Exposed | 4.25 | 3.45 | 0.36 | 0.54 | 0.02 | 0.03 | 795.0 |
| IQR | Control | 2.6-5.1 | 1.91 -3.80 | 0.16 -0.45 | 0.37 –0.57 | 0.02 -0.05 | 0.02 -0.04 | 669.0-753.0 |
| | Exposed | 3.2-5.8 | 2.60 -4.47 | 0.17 -0.43 | 0.35 -0.84 | 0.01 -0.09 | 0.02 -0.03 | 690.0-826.0 |
| p-value | | 0.436 | 0.443 | 0.848 | 0.429 | 0.737 | 0.3500 | 0.057 |

**WBC** (White Blood Cells x10$^9$ /l), **LYM** (Lymphocytes x10$^9$ /l), **MON** (Monocytes x10$^9$ /l), **NEU** (Neutrophils x10$^9$ /l), **EOS** (Eosinophils x10$^9$ /l), **BAS** (Basophils x10$^9$ /l), **PLT** (Platelets x10$^9$ /l)

**Table 7: Median weight of controls (unexposed) and experimental (exposed) rats at different levels of ethanol extract.**

| | | Control | Amount of ethanol extract (mg/kg/day) | | | |
|---|---|---|---|---|---|---|
| | | | 50 | 100 | 200 | 400 |
| Start mass | Median | 180.0 | 183.0 | 186.0 | 193.0 | 187 |
| | IQR | 179.0-183.0 | 173.0-191.0 | 184.0-189.0 | 190.0-197.0 | 183.0 -189.0 |
| | p-value | | 0.8717 | 0.1978 | 0.0100* | 0.2265 |
| End mass | Median | 214.0 | 219 | 222.0 | 222.5 | 230.50 |
| | IQR | 211.0-217.0 | 217.0-220.0 | 220.0-224.0 | 217.0-225.0 | 223.0-244.0 |
| | p-value | | 0.2281 | 0.0782 | 0.921 | 0.0303* |
| Change in mass | Median | 33.0 | 36.0 | 39.5 | 31.5 | 47.5 |
| | IQR | 30.0-37.0 | 29.0-44.0 | 33.0-43.0 | 24.0-33.0 | 34.0-55.0 |
| | p-value | | 0.873 | 0.333 | 0.420 | 0.078 |

*(P<0.05) statistically significant.

**Table 8: Comparison of WBC and PLT between controls and rats exposed to ethanol extracts across all doses.**

| | | WBC | LYM | MON | NEU | EOS | BAS | PLT |
|---|---|---|---|---|---|---|---|---|
| **Median** | Control | 3.80 | 3.01 | 0.40 | 0.46 | 0.03 | 0.04 | 730.00 |
| | Exposed 50mg/kg | 4.20 | 3.43 | 0.36 | 0.53 | 0.02 | 0.03 | 850.00 |
| **p-value** | | 0.233 | 0.144 | 0.465 | 0.584 | 0.465 | 0.097 | 0.011* |
| | Exposed 100mg/kg | 3.60 | 2.78 | 0.33 | 0.42 | 0.01 | 0.03 | 762.00 |
| **p-value** | | 0.410 | 0.855 | 0.584 | 0.170 | 0.462 | 0.139 | 0.201 |
| | Exposed 200mg/kg | 4.95 | 4.03 | 0.33 | 0.58 | 0.02 | 0.03 | 783.00 |
| **p-value** | | 0.394 | 0.201 | 0.394 | 1.00 | 1.00 | 0.159 | 0.394 |
| | Exposed 400mg/kg | 5.75 | 3.93 | 0.40 | 0.60 | 0.02 | 0.03 | 839.00 |
| **p-value** | | 0.575 | 0.748 | 0.200 | 1.00 | 0.872 | 0.059 | 0.0547 |

*(P<0.05) statistically significant; **WBC** (White blood cells x10$^9$ /l), **LYM** (Lymphocytes x10$^9$ /l), **MON** (Monocytes x10$^9$ /l), **NEU** (Neutrophils x10$^9$ /l), **EOS** (Eosinophils x10$^9$ /l), **BAS** (Basophils x10$^9$ /l), **PLT** (Platelets x10$^9$ /l)

compared using a Kruskal-Walli's test. There was no significant difference (p>0.05) in the median mass between the controls (n=6) and all experimental groups (n=18), as illustrated in Table 10.

Furthermore, the change in median weights throughout the entire experimental period was approximately 50g, as illustrated by Figure 3 below. The change in weight was similar in all groups, with 2% aqueous extract having a slightly higher mass, which was not significant.

## Haematological parameters

Tables 11 and 12, that compare the controls (n=6) and experimental groups (n=6) at different concentrations of aqueous extracts, specifically with regard to full blood count parameters and p-values, were prepared. There were no significant differences in all concentrations ranging from 2% to 10% (p>0.05) in FBC parameters. A significant difference was observed for haemoglobin (p=0.0353) in subjects exposed to 20% aqueous extracts, as illustrated in Table 11. It must be noted that the percentage change from control median haemoglobin concentration (15.1g/dl) to 20% aqueous (15.85g/dl) was 5%, while the human refence range for males is 13 – 17g/dl (Bates & Lewis, 2011). The human range spans over a range of 24%. Furthermore, it must be noted that 10% of the aqueous group's haemoglobin concentration was 15.95g/dl, which showed a 6% percentage change with no statistically significant difference.

**Table 9: Comparison of red blood cells parameters between unexposed and exposed rats for ethanol extracts across all doses.**

| | | RBC | HB | HCT | MCV | MCH | MCHC |
|---|---|---|---|---|---|---|---|
| Median | Control | 7.50 | 15.05 | 42.10 | 56.00 | 19.85 | 35.70 |
| | Exposed 50mg/kg | 7.95 | 15.70 | 44.10 | 56.00 | 19.80 | 35.80 |
| p-value | | 0.273 | 0.1190 | 0.143 | 0.827 | 0.700 | 0.855 |
| Median | Control | 7.50 | 15.05 | 42.10 | 56.00 | 19.85 | 35.70 |
| | Exposed100mg/kg | 7.06 | 14.40 | 40.10 | 56.00 | 20.10 | 35.80 |
| p-value | | 0.273 | 0.583 | 0.464 | 0.392 | 0.573 | 0.855 |
| Median | Control | 7.50 | 15.05 | 42.10 | 56.00 | 19.85 | 35.70 |
| | Exposed 200mg/kg | 7.57 | 15.15 | 42.40 | 56.00 | 20.10 | 35.80 |
| p-value | | 0.831 | 0.915 | 1.00 | 0.221 | 0.051 | 0.669 |
| Median | Control | 7.50 | 15.05 | 42.10 | 56.00 | 19.85 | 35.70 |
| | Exposed 400mg/kg | 7.59 | 14.95 | 41.45 | 55.00 | 19.80 | 35.60 |
| p-value | | 0.749 | 0.810 | 0.748 | 0.212 | 0.315 | 0.809 |

**IQR** (Interquartile range), **RBC** (Red blood cells x10$^9$ /l), **HB** (Haemoglobin g/dl), **HCT** (Haemoglobin content %), **MCV** (Mean Cell Volume in microtoliters), **MCH** (Mean Cell Haemoglobin Picograms), **MCHC** (Mean Cell Haemoglobin Content g/dl)

**Table 10. Median weight of controls (unexposed) and experimental (exposed) rats at different levels of aqueous extracts.**

| | | Control | Concentration of the aqueous extract | | |
|---|---|---|---|---|---|
| | | | 2% | 10% | 20% |
| Start mass | Median | 181.0 | 188.0 | 189.0 | 181.5 |
| | IQR | 176.0-197.0 | 182.0-188.0 | 168.0-192.0 | 173.0-197.0 |
| | p-value | | 0.5189 | 0.8728 | 0.9358 |
| End mass | Median | 235.5 | 246.5 | 236 | 236.0 |
| | IQR | 215.0-251.0 | 245.0-252.0 | 220-242.0 | 220.0-251.0 |
| | p-value | | 0.5218 | 1.000 | 0.8099 |
| Change in mass | Median | 54.5 | 60.0 | 49.5 | 51.0 |
| | IQR | 46.0-66.0 | 59.0-63.0 | 48.0-56.0 | 44.0-54.0 |
| | p-value | | 0.336 | 0.631 | 0.748 |

**Table 11: Comparison of RBC parameters between controls and exposed rats across all aqueous extracts.**

| | | RBC | HB | HCT | MCV | MCH | MCHC |
|---|---|---|---|---|---|---|---|
| Median | Control | 8.04 | 15.10 | 42.60 | 54.00 | 19.00 | 35.30 |
| | Exposed 2% | 7.99 | 15.45 | 43.75 | 55.50 | 19.70 | 35.55 |
| p-value | | 0.784 | 0.234 | 0.314 | 0.453 | 0.357 | 0.409 |
| | Exposed 10% | 8.08 | 15.95 | 45.20 | 56.00 | 19.65 | 35.55 |
| p-value | | 0.715 | 0.361 | 0.465 | 0.344 | 0.359 | 0.4059 |
| | Exposed 20% | 7.98 | 15.85 | 44.70 | 55.50 | 19.60 | 35.40 |
| p-value | | 0.521 | 0.035* | 0.082 | 0.396 | 0.356 | 0.348 |

**RBC** (Red Blood Cells x10$^9$ /l), **HB** (Haemoglobin g/dl), **HCT** (Haemoglobin Content %), **MCV** (Mean Cell Volume in Microtoliters), **MCH** (Mean Cell Haemoglobin picograms), **MCHC** (Mean Cell Haemoglobin Content g/dl), *(P<0.05) statistically significant

**Table 12: Comparison of WBC and PLT parameters between unexposed and exposed rats across all aqueous extracts.**

| | | WBC | LYM | MON | NEU | EOS | BAS | PLT |
|---|---|---|---|---|---|---|---|---|
| Median | Control | 3.40 | 2.3 | 0.17 | 0.52 | 0.02 | 0.02 | 734 |
| | Exposed 2% | 3.40 | 2.63 | 0.26 | 0.48 | 0.01 | 0.02 | 721.50 |
| p-value | | 0.855 | 0.855 | 0.359 | 1.000 | 0.645 | 0.130 | 0.465 |
| | Exposed 10% | 3.90 | 2.83 | 0.29 | 0.60 | 0.03 | 0.02 | 710.50 |
| p-value | | 0.715 | 0.144 | 0.464 | 0.143 | 0.578 | 0.082 | 0.855 |
| | Exposed 20% | 5.25 | 4.11 | 0.33 | 0.66 | 0.05 | 0.03 | 724.00 |
| p-value | | 0.201 | 0.855 | 0.714 | 0.583 | 0.782 | 0.121 | 1.000 |

**WBC** (White Blood Cells x10$^9$), **LYM** (Lymphocytes x10$^9$ /l), **MON** (Monocytes x10$^9$ /l), **NEU** (Neutrophils x10$^9$ /l), **EOS** (Eosinophils x10$^9$ /l), **BAS** (Basophils x10$^9$ /l), **PLT** (Platelets x10$^9$ /l)

## Histological investigations

Macroscopic examination of the kidney, liver and spleen tissue could not demonstrate any pathological variations between the treatment groups and controls in both aqueous and ethanol extracts, as assessed by the pathologist. A histopathological study of the rats' kidney, liver and spleen tissue indicated a normal architecture of tissue in both the controls and treatment groups, as illustrated in Figures 4 to 6, respectively.

Comparison of the controls (n=6) and treatment groups (n=6) revealed an average median change in weight of slightly above 50g over the entire eight-week period of experimentation with aqueous extracts. A significant difference (p<0.05) was observed for both haemoglobin

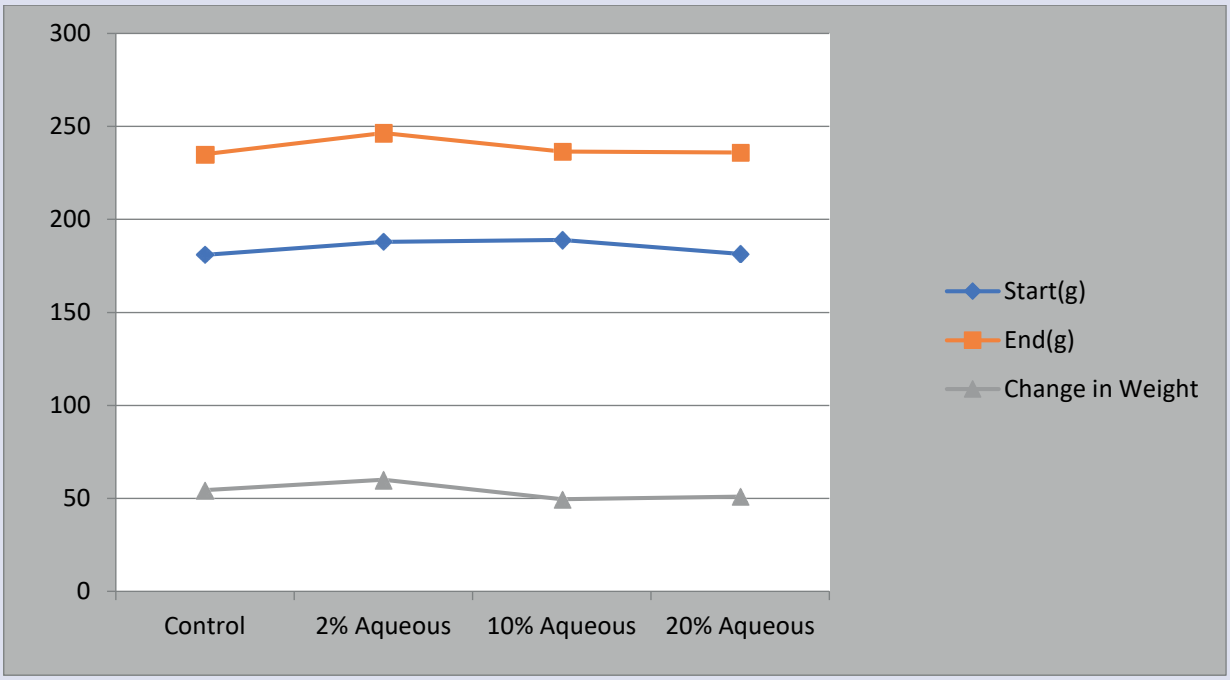


**Figure 3:** Graphical representation of the median change in mass of the rats treated with aqueous extracts over a period of eight weeks.

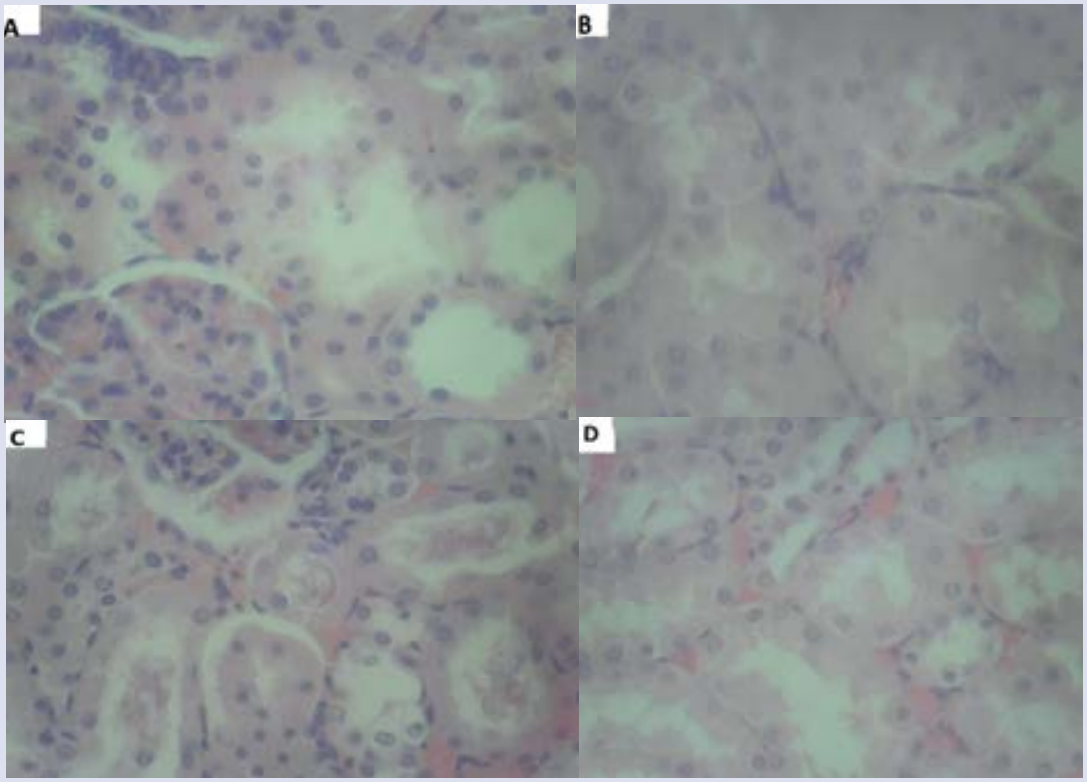


**Figure 4**: Photomicrographs of the longitudinal sections of the renal cortex region of control and treated animals. (A) Renal cortex of control (aqueous extract) rats showing glomeruli (40×). (B) Treated with 2% aqueous extract of *Asparagus laricinus*. (40×). (C) Renal cortex of a control (ethanol extract) rat showing glomeruli (40×). (D) Treated with 50mg/kg/day ethanol extract (40×).

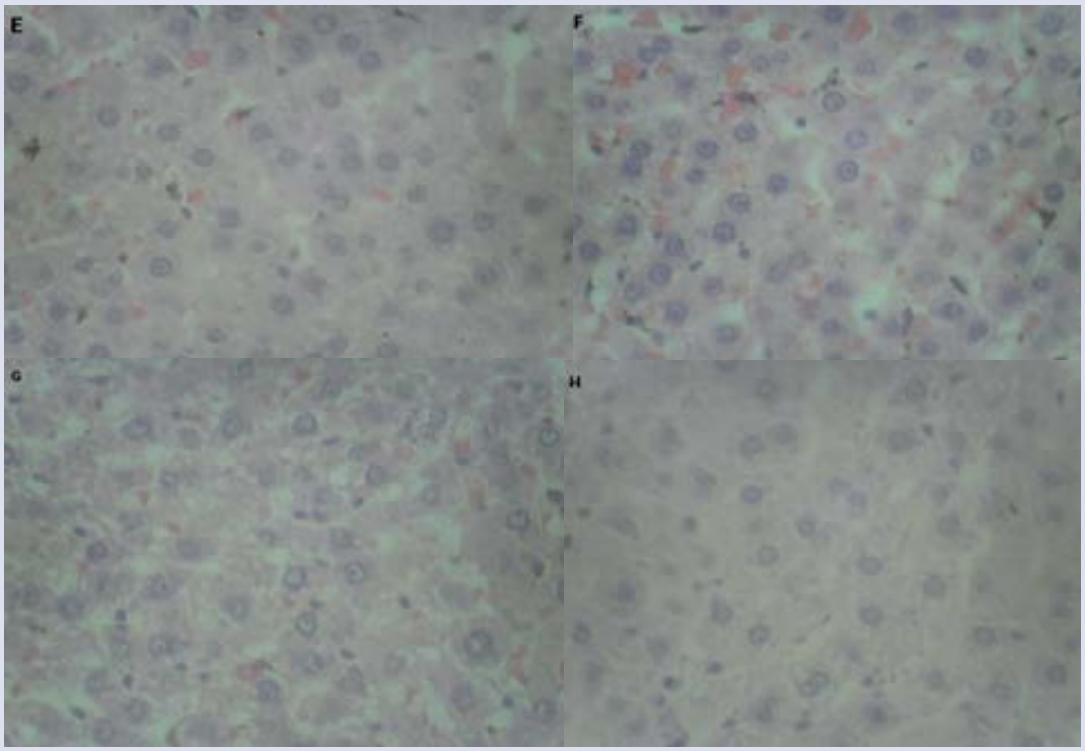


**Figure 5**: Effect of aqueous and ethanol extracts of *Asparagus laricinus* on the liver tissue of Sprague Dawley rats. (E) Control tissue for aqueous extracts (40×). (F) Treated with 10% aqueous extract. (40×). (G) Control rats for ethanol extract (40×). (H) Treated with 100mg/kg/day ethanol extract (40×).

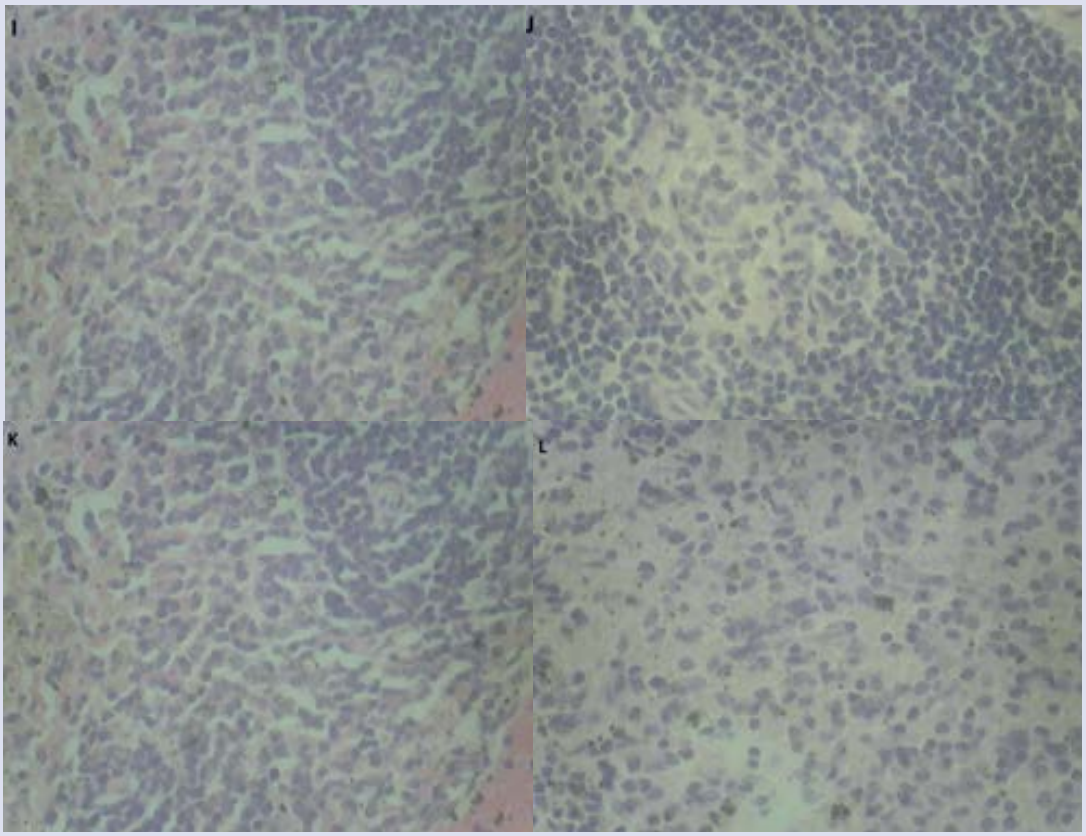


**Figure 6**: Photomicrographs of the spleen for control and treated animals. (I) Control tissue for aqueous extracts (40×). (J) Treated with 10% aqueous extract of *Asparagus laricinus*. (40×). (K) Control rats for ethanol extract (40×). (L) Treated with 200mg/kg/day ethanol extract (40×).

and BUN results with the 20% water extract. There were no statistical differences between the treatment and control groups with regard to the rest of haematological variables and selected biochemical tests. Histological evaluation could not reveal any pathological changes in both the aqueous and ethanolic extracts across all levels of dosage.

## DISCUSSION AND CONCLUSION

**Comparison of haematological parameter of control and different concentration of all extracts**

Full blood count results could not point in the direction of toxicity, adverse effects, or hazards, as indicated by statistically similar results between the exposed and unexposed groups, using both aqueous and ethanol extracts at different concentrations. However, as indicated in Table 8, significant differences were noted with ethanolic extracts for platelets at a dose of 50mg/kg/day (p=0.011). It must be noted that this statistically significant change produced a 15% increase in the median concentration, in comparison with the control. Although it seems significant, it must be noted that the reference range for platelets in humans spans 63%, and the 400mg/kg/day group showed a 13% increase without a statistically significant change. Therefore, it seems that even though a statistically significant change was obtained, it does not translate to a clinically significant change.

In the aqueous extracts, statistically significant results were observed for 20% of the extracts for Hb, as indicated in Table 11. This increase in the haemoglobin concentration produced a 5% change, while the human range spans 37% and the 10% aqueous extracts had a 6% change without a statistically significant change. Based on these facts it seems that the statistically significant change does not translate to a clinically significant change.

### Comparison of histological changes between control and different concentration of all extracts

There were no traces of damage or inflammation of the tissue excised from the kidney, liver and spleen of the rats exposed or unexposed to *Asparagus laricinus* extracts. Figures 4 to 6 compare haematoxilyn and eosin stains (H&E) from both exposed and unexposed tissue.

Studies do not reveal any toxicological studies on *Asparagus laricinus*; however, similar studies were conducted for anticancer plants using either mice, rats, or rabbits. There are reports on acute toxicity studies on *Hyptis suaveolens* Poit. (Lamiaceae) leaves; *Moringa oleifera* Lam. (Moringaceae) (leaves[32,34, 39,40,41]; *Newbbouldia laevis (Bignoniaceae)* (stem leaves and roots)[42] and *Nigella sativa (Ranunculaceae)* (seeds)[43,44] To cite but a few.

The histological assessment has proven that both aqueous and ethanolic extracts of *Asparagus laricinus* had no detrimental or adverse effects on the vital organs of the Sprague Dawley rats. Tissue damage, lesions or inflammation were not observed on the kidney, liver or spleen of the treatment groups as compared to the control group. The pattern was observed across increasing doses of aqueous and ethanolic extracts.

In summary the study shadowed that the toxicological evaluation of *Asparagus laricinus* extracts may be considered relatively free of toxicity when given orally, as it did not cause death, damage, or inflammation to the tissues, nor produced any remarkable biochemical and haematological adverse effects in both the male and female Sprague Dawley rats. Further studies may also be conducted to demonstrate *in vivo* efficacy against cancer, because thus far studies were conducted using cell lines (*in vitro* studies).

## LIMITATIONS

Although the collection and analysis of blood and tissue samples needs ample time, the actual experimental time was conducted over a maximum period of eight weeks, which perhaps was not sufficient for the tissue damage to occur. The number of rats was limited as a result of the of laws and policies governing the use of experimental animals for research purposes. We currently do not have the reference or normal ranges for biochemical and haematological parameters for rats, and therefore relied on comparing the results to those of the control group.

## RECOMMENDATIONS

The period of experimentation should be increased and motivation for a bigger sample size would also be necessary, for the researcher to improve the quality of the results. Normal ranges for the haematological and biochemical tests should be established.

## ACKNOWLEDGMENTS

None.

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## NOTES

Competing Interests: None

Funding: CUT staff bursary from the research office

Ethics approval: Interfaculty Animal Ethics Committee (Ethics number 16/2012 – Faculty of Health Sciences at the University of the Free State).

Guarantor: Pakiso Makhoahle, Sekobane Mokgawa and Samson Mashele

Contributorship: Pakiso Makhoahle, Sekobane Mokgawa and Samson Mashele conceived and wrote the article.

Provenance: Not commissioned; editorial review.

## REFERENCES

1. World Health Organization. Promoting the Role of Traditional Medicine in Health Systems: A Strategy for the African Region. WHO Regional Office for Africa, Temporary location, Harare, Zimbabwe. Document AFR/RC50/9 and Resolution AFR/RC50/R3. 2001. [cited 2023 Augst 12]. Accessed from: https://www.afro.who.int/sites/default/files/2017-06/ahm-special-issue-14.pdf
2. World Health Organization. WHO traditional medicine strategy 2002-2005. Geneva: WHO report. 2002. [cited 2023 Augst 12]. Available from: http://apps.who.int/medicinedocs/en/d/Js2297e/
3. Shackleton SE, Stadler JJ, Jeenes KA, Pollard SR, and Gear JSS. Adaptive strategies of the poor in arid and semi-arid lands – In search of sustainable livelihoods: a case study of the Bushbuckridge District, eastern Transvaal, South Africa. Wits Rural Facility, Acornhoek.199. [ cited 2023 Augst 20]. Available from: https://www.iisd.org/system/files/publications/participatory_research.pdf
4. Shanley P, and Luz L. The impacts of forest degradation on medicinal plant use and implication for health care in Eastern Amazonia. BioScience. 2003 June 01;53 (6): 573– 584.
5. Calixt JB. Twenty-five years of research on medicinal plants in Latin America: a personal view. J of Ethnopharma. 2005 August 22; 100: 131-134.
6. Kalantari H, Rezaei M, Mahdavinia M, Jahangirnejad R, Varnaseri G. Determination of the Mutagenicity Potential of Sankol Herbal Medicine by Single Cell Gel Electrophoresis in Rat Hepatocytes in Comparison with H2O2. Jundishapur J Nat Pharm Prod. 2012 August 25;7(3):123-126.
7. Addae-Mensah I. Towards a Rational Scientific Basis for Herbal Medicine – a Phytochemist's Two-Decade Contribution. Accra: Ghana Universities Press. 1992. [Cited 2023 August 21]; Available from: https://link.springer.com/article/10.1023/A:1013310809275

8. van Wyk BE, van Oudtshoorn B, and Gericke N. Medicinal plants of South Africa. Pretoria: Briza Publications. 1997. [cited 2023 August 21]. Available from: https://books.google.co.za/books/about/Medicinal_Plants_of_South_Africa.html?id=2jHbAAAAMAAJ&redir_esc=y

9. Brandt HD, Osuch E, Mathibe L, and Tsipa P. Plants associated with accidental poisoned patients presenting at Ga-Rankuwa Hospital, Pretoria. SA J of Science. 1995; 91: 57–59. [cited 2023 August 24] Available https://www.scopus.com/record/display.uri?eid=2-s2.0-0029104755&origin=inward&txGid=013d6f8e1c218dec5aa39c2cc66a737f

10. Flint A. and Payne J. Reconciling the irreconciliable? HIV/AIDS and the potential for middle ground between the traditional and biomedical healthcare sectors in South Africa. Forum for Devel Studies .2012 June 12 online; 40(1):47-68.

11. Cunningham AB. Imithi isiZulu: the Traditional Medicine Trade in KwaZulu-Natal. MSc thesis, University of Natal, Pietermaritzburg. 1992. [cited 2023 August 21]. Available from: https://www.scirp.org/reference/referencespapers?referenceid=143608

12. Mander M, and Le Breton G. Plants for therapeutic use. In: Mander, M., Mckenzie, M. (Eds.), Southern African trade directory of indigenous natural products. Comm Prod from the Wild Group. Stellenbosch University. 2005; 3-8. [cited 2023 August 21]. Available from: https://books.google.co.za/books/about/Southern_African_Trade_Directory_of_Indi.html?id=xTPiAAAACAAJ&redir_esc=y

13. Marshall N. Searching for a cure: conservation of medicinal wildlife resources in east and southern Africa. TRAFFIC International.1998. [cited 2023 August 22]. Available from: https://www.traffic.org/site/assets/files/9719/conservation-of-medicinal-wildlife-resources-in-east-and-southern-africa.pdf

14. Williams VL, Balkwill K, and Witkowski ETF. Muthi traders on the Witwatersrand, South Africa –an urban mosaic. SA J. of Botany. 1997 December; 63: 378–381.

15. Gelfand M, Mavi S, and Drummond RB. The Traditional Medical Practitioner in Zimbabwe: His Principles of Practice and Pharmacopoeia. Mambo Press, Gweru, Zimbabwe. 1993. [cited 2023 August 22]. Available from: https://books.google.co.za/books/about/The_Traditional_Medical_Practitioner_in.html?id=mHEKAQAAIAAJ&redir_esc=y

16. Elgorashi EE, Taylor JLS, Maes A, et al. The use of plants in traditional medicine: potential genotoxic risks. SA J. of Botany. 2002 September 1; 68: 408– 410.

17. Popat A, Shear NH, Malkiewicz I, et al. The toxicity of Callilepis laureola, a South African traditional herbal medicine. Clin Biochem. 2001 MAy; 34: 229–236.

18. Schimmer O, Haefele F, and Kruger A. The mutagenic potencies of plant extracts containing quercetin in Salmonella typhimurium TA98 and TA100. Mut Res. 1988 October; 206: 201–208.

19. Higashimoto, M., Purintrapiban NJ, Kataoka K, Kinouchi T, et al. Mutagenicity and antimutagenicity of extracts of three species and a medicinal plant in Thailand. Mut Res.1993 November; 303: 135-142.

20. Schimmer O, Krüger A, Paulini H, Haefele F. An evaluation of 55 commercial plant extracts in the Ames mutagenicity test. Pharmazie. 1994 June;49(6):448-451.

21. Kassie F, Parzefall W, Musk S, et al. Genotoxic effects of crude juices from Brassica vegetables and juices and extracts from phytopharmaceutical preparations and spices of cruciferous plants origin in bacterial and mammalian cells. Chemicho-Biological Interactions. 1996 September 27; 102 (1): 1-16.

22. De Sã Ferrira ICF, and Ferrão Vargas VM. Mutagenicity of medicinal plant extracts in Salmonella/microsome assay. Phytotherapy Res. 1999 August, 13: 397–400.

23. Elgorashi EE, Taylor J.L.S, Maes A, et al. Screening of medicinal plants used in South African traditional medicine for genotoxic effects. Toxicology Letters. 2003 July 20; 143(2): 195-207.

24. Marques RCP, de Medeiros SRB, da Silva Dias, C., Barbosa-Filho, J.M., and Agnez-Lima, L.F.. Evaluation of the mutagenic potential of yangambin and the hydroalcoholic extract of Ocotea duckei by the Ames test. Mut Res. 2003 April 20; 536:117–120. https://www.academia.edu/20448316/Evaluation_of_the_mutagenic_potential_of_yangambin_and_of_the_hydroalcoholic_extract_of_Ocotea_duckei_by_the_Ames_test

25. Brummitt RK. Vascular plant families and genera. Royal Botanical Gardens, Kew. 1992; 804. [cited 2023 August 22]. Available from: https://search.worldcat.org/title/36164919

26. van Wyk BE. A review of Khoisan and cape Dutch medical ethnobotany. J of Ethnopharmac. 2008 October 28; 119: 331-341.

27. van der Merwe D, Swan GC, and Botha CJ. Use of ethoveterinary medicinal plants in cattle by Setswana speaking people in Madikwe area of the Northwest province of South Africa. J of SA Veterinary Association. 2001 July 09; 72: 189-196.

28. Dzerefos CM, and Witkowski ETF. Density and potential utilization of medicinal grassland plants from Abe Bailey Nature Reserve, South Africa. Biodiversity and Conservation. 2001; 10: 1875 – 1896. [cited 2023 August 22]. Available from: https://link.springer.com/article/10.1023/A:1013177628331#preview

29. Mashele SS, and Fuku S. Evaluation of the antimutagenic and mutagenic properties of Asparagus laricinus. Med Tech SA. 2011 December; 2: 33-36.

30. Fouche G, Khorombi E, Kolesnikova N, Maharaj VJ, Nthambeleni R, and van der Merwe M. Investigation of South African plants for anticancer properties. Pharmacologyonline. 2006 3: 494–500. [cited 2023 August 23]. Available from: https://pharmacologyonline.silae.it/files/archives/2006/vol3/048.Fouche.pdf

31. Mashele SS, and Kolesnikova N. In vitro anticancer screening of Asparagus laricinus extracts. Pharmacologyonline. 2010; (2): 246-52. [cited 2023 August 22]. Available from: https://pharmacologyonline.silae.it/files/archives/2010/vol2/023.Mashele.pdf

32. Isitua CC, and Ibeh IN. Toxicological assessment of aqueous extract of Moringa oleifera and Caulis bambusae in rabbits. Journal of Clinical Toxicology. 2013 January 01; S12:003.

33. Awodele O, Oreagba IA, Odoma S, da Silva JA, and Osunkalo VO. Toxicological evaluation of the aqueous leaf extract of Moringa oleifera Lam. (Moringaceae). J of Ethnopharmachology. 2012 January 31; 139(2):330-6.

34. Ugwu OPC, Nwondo OFC, Joshua PE, Bawa A, et al. Phytochemical and acute toxicity studies of Moringa oleifera ethanol leaf extract. Inter J of Life Science, Biotech and Pharma Research. 2013; 2(2): 66-71. [cited 2023 August 26]. Available from:file:///C:/Users/pmakhoahle/Downloads/ijlbpr_51835952bf12e%20(5).pdf

35. Pawar B, Gupta t, Vasdev N, Tekade M, Arafat B, Tekade RK. (2023). Chapter 1 - Understanding pharmacotoxicology, In Advances in Pharmaceutical Product Development and Research, Essentials of Pharmatoxicology in Drug Research, Academic Press. 2023;1:1-28. [cited 2023 August 27]. Available from: https://doi.org/10.1016/B978-0-443-15840-7.00025-7

36. ABX user manual. 2005;2-13; [cited 2023 August 22]. Available from the machine packages

37. Mapanga, R.F. and Musabayane, C.T. The renal effects of blood glucose-lowering plant-derived extracts in diabetes mellitus – an overview. Renal Failure. 2010 February 01. 32:132–138.

38. Leong AS. Microwave fixation and rapid processing in a large throughput histopathology laboratory. Pathology, 1991; 23:271–273. [cited 2023 August 27]. Available from https://doi.org/10.3109/00313029109063583

39. Danmalam UH, Abdullahi LM, Ugunu A, Musa KY. Acute toxicity studies and hypoglycemic activity of of the methanol extract of the leaves of Hyptis suaveolens Poit. (Lamiaceae). Nigerian J of Pharma Sci. 2009 January; 8(2):87-92.

40. Sreelata S, Jeyachitra A, Padma PR. Antiproliferation and induction of Moringa oleifera leaf extract on human cancer cells. Food and Chem Toxicology. 2011 June; 49:1270-5.

41. Berkovich L, Earon G, Ron I, Rimmon A, Vexler A, and Levi-Ari S. Moringa oleifera aqueous leaf extract down-regulates nuclear factor-Kappa B and increases cytotoxic effect of chemotherapy in pancreatic cancer cells. *BMC Complementary and Alternative Medicine*. 2013 August 19; 13:212.

42. Azuine MA, Ibrahim K, Enweren, M, Gamaniel K, and Wabembe C. Cytotoxic and anticancer activity of *Newbbouldia laevis* in mice. W*est African Journal of Pharma and Drug Res*. 1995; 11:72-9. doi: [cited 2023 August 29]. Available from 10.4314/wajpdr.v11i1.53382

43. Ali,BH, and Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. *Phytotherapy Research*. 2003 April 15;17(4):299-305.

44. Ekanen JT, and Yusuf OK. Some biochemical and haematological effects of black seed (*Nigella sativa)* oil on T. brucei-infected rats. *African J of Biom Res*. 2008; 11:79-85. [cited 2023 August 29]. Available from https://doi.org/10.5897/AJB08.130

**Cite this article:** Mokgawa SD, Makhoahle PM, Mashele S. The In-Vivo Assessment of the Effect of Traditionally Used *Asparagus laricinus* Extracts for Anticancer on the Kidney, Liver, and Spleen of Rats. Pharmacogn J. 2024;16(1): 76-87.