https://ojshostng.com/index.php/ajbr

Afr. J. Biomed. Res. Vol. 27 (January 2024); 121- 128

Research Article

Biochemical evaluation of *In-vitro* Toxicity of Aqueous and Methanol Extracts of *Asparagus laricinus* in Sprague Dawley Rats

Mokgawa S.D. and *Makhoahle P.M

Faculty of Health and environmental Sciences, Central University of Technology-Free State, South Africa

ABSTRACT

Traditional medicine has been a fertile source of revealing lead novel molecules which are then subjected to investigations using the techniques of the modern drug discovery. The objective of the study was to evaluate variations in serum biochemical parameters of aqueous and ethanolic extracts of *Asparagus laricinus* roots. The powdered plant roots material was dissolved in distilled water to prepare 2%, 10% and 20% concentration. For the second extraction. the material was also dissolved in ethanol and the increasing concentrations were controlled by varying the volumes of the solution administered. 54 Sprague Dawley rats (180g - 250g), 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 ad libitum for eight weeks to 0%, 2%, 10% and 20% extracts. The ethanolic group was divided into five subgroups of 6 rats which were exposed for two weeks (via gavage) to increasing doses of 50, 100, 200 and 400mg/kg/day extracts and a control (unexposed) group. 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 from the rats for biochemical testing. Biochemical tests were done for CHOL, BUN, CREA, ALP, AST and ALT as indicators of any possible 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 dosages as well as weight at 400g/kg/day, even though AST and ALT have high SEM values. The median change in weight remained slightly below 50g over the entire two-week period of experimentation. Biochemical results could not show any patterns in abnormalities although we observed statistically significant results on few parameters. In conclusion, the toxicological evaluation of *Asparagus laricinus* extracts may be considered relatively safe when given orally, as it did not produce any remarkable biochemical adverse effects in both the male and female Sprague Dawley rats.

Keywords: *Sprague Dawley rats, Asparagus laricinus , biochemical tests, ethanol extract, biochemical parameters*

**Author for correspondence: Email: pmakhoahle@cut.ac.za; Tel:+27515074120*

Received: July 2023; Accepted: November 2023

DOI: https://doi.org/10.4314/ajbr.v27i1.16

© 2024 The Author(s).
This article has been published under the terms of Creative Commons Attribution-Noncommercial 4.0 International License (CC BY-NC 4.0), which permits noncommercial unrestricted use, distribution, and reproduction in any medium, provided that the following statement is provided. "This article has been published in the African Journal of Biomedical Research"

INTRODUCTION

Traditional medicine is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not (World Health Organization 2001; 2002). It is used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (World Health Organization 2001; 2002). Traditional systems in general have had to meet the needs of the local communities for many centuries. China and India, for example, have developed very sophisticated systems such as acupuncture and ayurvedic medicine. Traditional medicine is generally available, affordable, and commonly used in large parts of Africa, Asia, and Latin America.

Frequently used plants in traditional medicine are assumed safe, due to their long- term use (Elgorashi *et al*., 2002), and are considered to have no side effects because they are 'natural' (Popat *et al*., 2001). However, recent scientific reports have shown that many plants used as food, or in traditional medicine, are potentially toxic, mutagenic and carcinogenic (Schimmer *et al*., 1988; Higashimoto *et al*., 1993; Schimmer *et al*., 1994; Kassie *et al*., 1996; De Sã Ferrira & Ferrão Vargas, 1999; Elgorashi *et al*., 2003; Marques *et al*.,

2003). The danger is that continuous consumption may result in several side effects and complications that may affect different tissues and organs.

The genus Asparagus consist of almost 100 species which comprises of herbs, shrubs, and vines. This plant is a monocot, belonging to the family of Asparagaceae, under the order Asparagales, and possesses great variety throughout Africa, especially in South Africa (Brummitt., 1992). With South African as part of the world using relying on medicinal plants for quick and cheap remedies, *Asparagus laricinus* is found to be among the plants entrusted for the treatment of several ailments. Historically the roots have being used for the treatment of tuberculosis, and its use as a diuretic in the Khoisan and Cape Dutch ethnobotany (van Wyk et al., 2001). Among the Batswana mostly occupying the North West province in South Africa, the roots are used for treatment of sores, redwater, urinary infections, umblical cord inflammation and general ailments (van der Merwe., 2001). While the mix tribes in the most populated Gauteng province in the country use the leaves and stem for the treatment of different illness (Dzerefos et al., 2001).

In previous studies, the safety of *Asparagus laricinus* extracts was investigated using *in vitro* anticancer activity against three human cell lines, namely, breast MCF7, renal TK10 and melanoma UACC62 (Fouche *et al*., 2006; Mashele & Kolesnikova, 2010). In the present study, the toxicity of *Asparagus laricinus* extracts is further investigated *in vivo* using Sprague-Dawley rats because other studies concentrated on the different plants such as in in vivo antifungal study done in South Africa (Seepe *et al*., 2020). There is no research to date that reported on in vivo testing about *Asparagus laricinus* which gave this study an opportunity to close this existing scientific gap.

MATERIALS AND METHODS

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.

Animals: 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 rats 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 8-week period, while the other six controls were used for two weeks of exposure to ethanol and dichloromethane extracts.

Ethical consideration: Written approval for the final version of the protocol was obtained from the Interfaculty Animal Ethics Committee (Ethics number 16/2012) of the Faculty of Health Sciences at the University of the Free State, before the extracts could be administered to the rats. The study was conducted at the animal facility by the researcher, under the guidance of personnel who are skilled and trained in the handling of experimental animals, and the ethical guidelines were followed at all times.

At the end of the study the rats were administered with Halothane (anaesthetic) and blood samples collected. They were finally sacrificed by administration of higher doses of halothane for further testing in hematology and histology.

Plant authentication (validation): 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 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 was discontinue.

Group 1 (Water extract): It consisted of 24 rats which were further subgrouped into 4 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. Water extracts were diluted to prepare 2%, 10% and 20% concentrations. The different concentrations were administered to three of the four groups ad libitum. The last group served as controls, where tap water was administered instead of the extract. The extracts were administered over a period of eight weeks, using a 200ml feeding bottle per cage. Bottles were cleaned and replaced with a fresh extract after every two days.

Group 2: Ethanol extract: It consisted of 30 rats which were further subgrouped into 5 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 varying at 50mg/kg/day, 100mg/kg/day,

200mg/kg/day, and 400mg/kg/day. Although the controls had water ad libitum, water was administered by gavage every time the exposed groups were administered with the extracts.

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 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 scientists employed by the Animal Research Unit at the University of the Free State.

Laboratory investigations: Substances ingested by animals including food, medication and fluids (extracts in our case) are absorbed in the gastrointestinal tract after digestion. 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.

Selected biochemical tests include enzymes such as aspartate aminotransferase (AST); alanine aminotransferase (ALT); alkaline phosphatase (ALP) which assesses the extent of the damage to hepatic tissues. Blood urea nitrogen and creatinine are specifically selected for the assessment of kidney function, and would therefore reflect damage to the nephrons and other tissues of the kidney. Blood urea nitrogen (BUN) and creatinine clearance are well established biomarkers of renal function that can be measured cheaply and easily using an enzyme/oxidation reaction assay and high performance liquid chromatography (HPLC), respectively (Mouton & Holder, 2006). Lastly, cholesterol is necessary for the assessment of damage to the heart and vascular system.
Blood samples for biochemical analysis were allowed to clot, followed by centrifugation at 3000rpm for 10 minutes. Serum was collected in 2ml tubes and analysed within five hours, and some aliquots stored in a refrigerator set at minus 4°C.The samples were kept for one week in order to allow repeat analysis in case of unreliable, doubtful or inaccurate results.

Biochemical parameters
Quantitative analysis of Aspartate aminotransferase (AST); Alanine aminotransferase (ALT); Alkaline phosphatase (ALP); Cholesterol (CHOL); blood urea nitrogen (BUN) and Creatinine were performed using Dimension XpandPlusTM auto analyzer (supplied by Siemens).

Principle for the determination of Aspartate aminotransferase: Aspartate aminotransferase (AST) catalyzes the transamination from L-aspartate to α-ketogluterate, forming L-glutamate and oxaloacetate. The oxaloacetate formed is reduced to malate by malate dehydrogenase (MDH), with simultaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH). The change in absorbance with time due to the conversion of NADH to NAD is directly proportional to the AST activity and is measured using a bichromatic (340nm, 700nm) rate technique (Bergmeyer *et al*., 1978).

Principle for the determination of Alanine aminotransferase: Alanine aminotransferase catalyzes the transamination of L-alanine to α-ketogluterate, forming L-glutamate and pyruvate. The pyruvate formed is reduced to lactate by actate dehydrogenase (LDH) with simultaneous oxidation of reduced NADH. The change in absorbance is directly proportional to the alanine aminotransferase activity and is measured using a bichromatic (340nm, 700nm) rate technique (Bergmeyer *et al*., 1978).

Principle for the determination of Alkaline phosphatase: Alkaline phosphatase catalyzes the transphosphorylation of p-nitrophenylphosphate (p-NPP) in the presence of the transphosphorylating buffer, 2-amino-2-methyl-1- propanol (AMP). The reaction is enhanced through the use of magnesium and zinc ions. The change in absorbance at 405 nm due to the formation of p-nitrophenol (p- NP) is directly proportional to the ALP activity, since the reactants are present in non- rate-limiting quantities and is measured using a bichromatic (405 nm, 510 nm) rate technique (Bowers & McComb,1966).

Principle for the determination of Cholesterol: Cholesterol esterase (CE) catalyzes the hydrolysis of cholesterol esters to produce free cholesterol, which, along with pre-existing free cholesterol, is oxidized in a reaction catalyzed by cholesterol oxidase (CO) to form cholest-4-ene-3-one and hydrogen peroxide. In the presence of horseradish peroxidase (HPO), the hydrogen peroxide thus formed is used to oxidize N,Ndiethylaniline-HCl/ 4-aminoantipyrine (DEA-HCl/AAP) to produce a chromophore that absorbs at 540 nm. The absorbance due to oxidized DEA-HCl/AAP is directly proportional to the total cholesterol concentration, and is measured using a polychromatic (452, 540, 700 nm) endpoint technique (Rautela & Liedtke, 1978).

Principle for the determination of Blood Urea Nitrogen (BUN): Urease specifically hydrolyzes urea to form ammonia and carbon dioxide. The ammonia is used by the enzyme glutamate dehydrogenase (GLDH) to reductively aminate α-ketoglutarate, with simultaneous oxidation of reduced NADH. The change in absorbance at 340 nm due to the disappearance of NADH is directly proportional to the BUN concentration in the sample and is measured using a bichromatic (340 nm, 383 nm) rate technique (Burtis & Ashwood, 2001).

Principle for the determination of Creatinine: In the presence of a strong base such as NaOH, picrate reacts with creatinine to form a red chromophore. The rate of increasing absorbance at 510 nm, due to the formation of this chromophore is directly proportional to the creatinine concentration in the sample and is measured using a biochromatic (510, 600 nm) rate technique. Bilirubin is

oxidized by potassium ferricyanide to prevent interference (Knapp & Mayne, 1987).

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 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-Wallis 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

Comparison of results obtained for the control group and the experimental population: The results of the combined control and experimental groups are presented in this section, starting with weight followed by clinical chemistry parameters investigations.

Weight profile: The median weight of all controls (n=12) and the experimental group combined (n=42) were statistically compared using Kruskal-Wallis test. No significant differences ($p>0.05$) were observed between the median weights of unexposed and exposed rats at the start and termination of the experiment was observed, as illustrated by Table 1.

Biochemical parameters: Liver enzymes, cholesterol, urea and creatinine results for the combined groups of ethanol and aqueous extract controls (n=12); in comparison with combined different treatment groups (n=42) were statistically analysed using a Kruskal-Wallis test . Again, no significant statistical differences ($p>0.05$) across the different extracts and levels of concentrations were observed (Table 2).

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. With a 95% confidence level, 95% of all sample means will be expected to lie within a confidence interval of ± varying standard errors of the sample median per biochemical test performed as indicated in table 2 above. Nonetheless the p values are >0.05 indicating to statistical difference overall to the biochemical tests.

Table 3 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 weight of rats exposed to 200mg/kg/day (p=0.0100), and also for the termination weight of rats exposed to 400mg/kg/day (p=0.0303). There was no difference in the change of weight for the rest of the dosages administered.

Biochemical parameters: Median results for biochemical tests were compared between the controls (n=6) and experimental group (n=6) using a Kruskal-Wallis test.There was no statistical difference between the controls and increasing doses of ethanol extracts in all biochemical results, as illustrated by Table 4.

Table 1:
Median change in weight of the controls (unexposed) and experimental (exposed) rats.

		Median	IQR	Min – Max	p-value
Start weight (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 weight (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 Weight	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)

Table 2:
Comparison of biochemical parameters between combined controls and experimental groups.

		CHOL	BUN	CREA	ALP	AST	ALT
Median ±SEM	Control	2.00±0.06	6.25±0.30	47.00±2.55	107.5±6.11	125.00±21.66	58.50±9.47
	Exposed	2.00±0.00	6.00±0.25	29.00±1.33	131.0±2.90	123.50±14.62	53.00±9.59
IQR	Control	2.00-2.00	5.85-6.85	14.50- 74.50	92.00- 141.50	101.50- 185.50	51.00- 86.00
	Exposed	2.00-2.00	5.40-6.50	12.00- 71.00	91.00- 151.00	102.00- 164.00	45.00- 66.00
p-value		0.061	0.215	0.876	0.365	0.9254	0.219

*(P<0.05) statistically significant**; CHOL** (Cholesterol)**. BUN** (Blood Urea Nitrogen)**, CREAT** (Creatinine), **ALP** (Alkaline Phosphatase), **AST** (Aspartate Aminotransferase), **ALT** (Alanine Aminotransferase).*

Table 3:
Median weight of controls (unexposed) and experimental (exposed) rats at different levels of ethanol extract

		Control	dose of ethanol extract (mg/kg/day)			
			50	**100**	**200**	**400**
Start weight	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 weight	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 weight	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 4:
Comparison of biochemical parameters between controls and rats exposed to ethanol across all concentrations.

		CHOL	BUN	CREA	ALP	AST	ALT
Median	Control	2.00±0.00	6.55±0.44	14.50±3.26	141.50±6.26	149.00±30.48	69.50±13.82
	Exposed 50mg/kg	2.00±0.00	6.15±0.37	8.50±3.92	167.00±6.82	165.50±126	72.50±72.62
	p-value	1.000	0.688	0.514	0.0542	0.936	0.873
	Exposed 100mg/kg	2.00±0.00	6.90±0.39	24.00±4.20	146.50±8.40	119.50±19.38	47.50±4.47
	p-value	1.00	0.749	0.199	0.630	0.229	0.078
	Exposed 200mg/kg	2.00±0.00	6.25±0.46	14.00±0.47	132.00±10.21	164.00±35.79	65.00±21.63
	p-value	1.000	0.422	0.867	0.7479	0.7483	0.747
	Exposed 400mg/kg	2.00±0.00	6.10±0.21	23.00±4.26	145.50±6.33	121.50±21.86	54.00±3.47
p-value		1.000	0.127	0.295	0.261	0.229	0.192

Comparative study of the control group versus various aqueous extracts group : The median change in weight for both treated (experimental) and untreated (control) groups over the experimental period (8 weeks), were recorded. Results for controls and experimental groups were compared using a Kruskal-Wallis test. There was no significant difference (p>0.05) in the median weight between the controls (n=6) and all experimental groups (n=18), as illustrated in Table 5. Furthermore, the change in median weights throughout the entire experimental period was approximately 50g, as illustrated by Figure 5 below. The change in weight was similar in all groups, with 2% aqueous extract having a slightly higher weight, which was not significant.

Biochemical parameters: Several figures were created for the comparison of the median results of controls (n=6), and experimental results (n=6) for the increasing doses of aqueous extracts. Table 6 summarizes the medians and p-values across increasing aqueous extracts. No significant differences were observed throughout increasing doses of aqueous extracts in biochemical parameters, except for the blood urea nitrogen (p=0.0081) for animals exposed to 20% aqueous extract. Figures 6, 7 and 8 illustrates a summary of comparisons of median results between the controls (n=6) and experimental groups (n=6), at increasing doses of aqueous extracts for biochemical parameters.

Table 5:
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 weight	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 weight	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 weight	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 6:
Comparison of biochemical parameters between controls and exposed rats across all aqueous extract.

		CHOL	BUN	CREA	ALP	AST	ALT
Median±SME	Control	2.00±0.11	6.15±0.17	74.5±1.84	92.00±5.95	101.5±12.24	56.5±5.11
	Exposed 2%	2.00±0.00	5.85±0.17	80.00±1.02	89.50±2.16	100.50±3.29	47.50±1.7
p-value		0.3173	0.4217	0.1488	0.7471	0.5745	0.5738
	Exposed 10%	2.00±0.00	5.95±0.11	69.00±1.84	88.00±2.33	136.00±8.41	53.50±1.88
p-value		0.3137	0.9356	0.1978	0.8726	0.4233	0.3350
	Exposed 20%	2.00±0.00	5.05±0.11	73.00±2.84	98.00±6.60	111.00±20.00	53.00±9.06
p-value		0.3173	0.0081*	0.7483	0.8726	0.5189	0.9361

(P<0.05) statistically significant **; CHOL (Cholesterol), **BUN** (Blood Urea Nitrogen), **CREAT** (Creatinine), **ALP** (Alkaline Phosphatase), **AST** (Aspartate Amino Transferase), **ALT** (Alanine Aminotransferase).*

Table 6 above indicates a 95% confidence level, 95% of all sample means will be expected to lie within a confidence interval of ± varying standard errors of the sample medians, AST and ALT appeared to have very high SEM meaning the median cannot be a true reflection of the sample population.

DISCUSSION

Biochemical tests were selected as indicators of the damage to the tissue of organs, including the liver, kidney and spleen. For better analysis extremely high or low results would then estimate the severity of the damage to such tissues.

Further 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. The median change in weight remained slightly below 50g over the entire two-week period of experimentation. There were no statistical differences between the treatment groups and controls with regard to the selected biochemical tests. The 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 BUN results with the 20% water extract. There were no statistical differences between the treatment and control groups regarding the rest of the selected biochemical tests. It is worth noting that some of the standard errors of the sample median were too high indicating the median cannot be a true representative of the study population.

With the variety of plant species been scientifically and traditionally identified for their medicinal properties and values respectively. Accessibility and affordability have always made traditional medicine convenient for healthcare and nutritional use in Africa (World Health Organization., 2002; Tchacondo et al., 2012). The species Asparagus laricinus has been extensively studied for its anticarcinogenic properties (Mashele & Konesnikova, 2010), and the active ingredients identified (Fuku et al., 2013 Although medicinal plants may have biological activities that are beneficial to humans, the potential toxicity of these bioactive substances has not been well established (Rosidah et al., 2009; Idoh et al., 2016). Moreover, despite the widespread use, few scientific studies have been undertaken to ascertain the safety and efficacy of traditional remedies in vivo (Graça et al., 2007).

Dose-response assessment of the effect of the extract was done by analysis of the blood samples collected at the end of the research for haematology analysis and tissues to be used for histology.

This study contributes to the body of knowledge because the literature does not reveal any *in vivo* 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 (Danmalam et al., 2009); Moringa oleifera Lam. (Moringaceae) (leaves) (Isitua & Ibe, 2003; Sreelata et al., 2011; Ugwu et al., 2013; Berkovich et al., 2013); Newbbouldia laevis (Bignoniaceae) (stem leaves and roots) (Azuine et al., 1995) and Nigella sativa (Ranunculaceae) (seeds) (Ali & Blunden, 2003; Ekanen & Yusuf, 2008;) to cite but a few. Generally, biochemical results could not conclusively indicate the presence of toxicity, as the majority of the results were not significantly different in both the treated and untreated groups of rats. This study will expand further by doing in-vivo hematological and histological investigation on the same extracts on Sprague Dawley rats.

In conclusion the study was able to show that that there is no toxic or adverse effects on the tissue and organs of animal models exposed to extracts of Asparagus laricinus (Sprague Dawley rats). This was done by administering both aqueous and ethanol extracts of Asparagus laricinus to groups of animals, and also varying the concentrations of these extracts.

Acknowledgments

- *Animal Research Unit of the University of the Free State, Bloemfontein, South Africa*
- *Funding: Central University of Technology, Postgraduate Staff funding from the research office.*

REFERENCES

Ali, B.H. and Blunden G.(2003). Pharmacological and toxicological properties of Nigella sativa. *Phytotherapy Research,* 17(4):299-305.

Angelo, A.I. and Edzard, E.(2009). Interactions between herbal medicines and prescribed drugs: an updated systematic review. *Drugs*, 69: 1777-1798.

Azuine, M.A., Ibrahim, K., Enweren, M., Gamaniel, K., and Wabembe, C.(1995). Cytotoxic and anticancer activity

of Newbbouldia laevis in mice. *West African Journal of Pharmacology and Drug Research*, 11:72-9.

Balunas, M. J. and Kinghorn, A. D.(2005) Drug discovery from medicinal plants. *Life Sciences*, 78: 431–441.

Bergmeyer, H.U., Scheibe, P., and Whahlefeld, A.W (1978). Optimization of methods for aspartate aminotransferase and alanine aminotransferase. *Clinical Chemistry*, 24(1): 58-73.

Berkovich, L., Earon, G., Ron, I., Rimmon, A., Vexler, A., and Levi-Ari, S.(2013). 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*,13:212.

Bowers, G.N. and McComb, R.B.(1966) A continuous spectrophotometric method for measuring the activity of serum alkaline phosphatise. *Clinical Chemistry*, 12:70.

Brandt, H.D., Osuch, E., Mathibe, L., and Tsipa, P.(1995). Plants associated with accidental poisoned patients presenting at Ga-Rankuwa Hospital, Pretoria. Suid- Afrikaanse Tydskrif vir Wetenskap, 91: 57–59.

Burtis, C., Ashwood, E., eds. (2001): Tietz Fundamentals of Clinical Chemistry, 5th ed. Philadelphia: WB Saunders,415.

Cragg, G.M. and Newman D.J.(2005). Plants as a source of anti-cancer agents. Perspective paper. *Journal of Ethnopharmacology*, 100: 72-79.

Cunningham, A.B. (1982) Imithi isiZulu: the Traditional Medicine Trade in KwaZulu- Natal. MSc thesis, University of Natal, Pietermaritzburg.

Danmalam, U.H., Abdullahi, LM., Ugunu, A., Musa, K.Y. (2009) Acute toxicity studies and hypoglycemic activity of of the methanol extract of the leaves of Hyptis suaveolens Poit. (Lamiaceae). *Nigerian Journal of Pharmaceutical Sciences*, 8(2):87-92.

De Sã Ferrira, I.C.F and Ferrão Vargas, V.M. (1999). Mutagenicity of medicinal plant extracts in Salmonella/microsome assay. *Phytotherapy Research*, 13: 397–400.

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

Ekanen, J.T. and Yusuf, O.K. (2008): Some biochemical and haematological effects of black seed (Nigella sativa) oil on T. brucei-infected rats. *African Journal of Biomedical Research*,11:79-85.

Elgorashi, E.E., Taylor, J.L.S., Maes, A., De Kimpe, N., van Staden, J., and Verschaeve, L. (2002). The use of plants in traditional medicine: potential genotoxic risks. South *African Journal of Botany*, 68: 408– 410.

Elgorashi, E.E., Taylor, J.L.S., Maes, A., De Kimpe, N., van Staden, J., and Verschaeve, L.(2003). Screening of medicinal plants used in South African traditional medicine for genotoxic effects. *Toxicology Letters*, 143(2): 195-207

Fabricant, T.S. and Farnsworth, N.R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*, 109: 69-75.

Fennell, C.W., Light, M.E., Sparg, S.G., Stafford, G.I., and van Staden, J. (2004). Assessing South African medicinal plants for efficacy and safety: agricultural and storage practices. *Journal of Ethnopharmacology*, Dec;95(2-3):113-21

Flint, A. and Payne, J.(2013). Reconciling the irreconciliable? HIV/AIDS and the potential for middle ground between the traditional and biomedical healthcare sectors in South Africa. *Forum for Developmental Studies*, 40(1):47-68.

Fouche, G., Khorombi, E., Kolesnikova, N., Maharaj, V.J., Nthambeleni, R., and van der Merwe, M.(2006). Investigation of South African plants for anticancer properties. *Pharmacologyonline*, 3: 494–500.

Fuku, S., Al-Azzawi, A.M., Madamombe-Manduna, I.T., and Mashele,S.(2013). Phytochemistry and Free Radical Scavenging Activity of *Asparagus laricinus* . International *Journal of Pharamacology*, 9(5): 312-317.

Gelfand, M., Mavi, S., and Drummond, R.B.(1993). The Traditional Medical Practitioner in Zimbabwe: His Principles of Practice and Pharmacopoeia. Mambo Press, Gweru, Zimbabwe.

Graça, C., Freitas, CS., Baggio, CH., Dalsenter, PR., and Marques, MC.(2007). Mikania laevigata syrup does not induce side effects on reproductive system of male Wistar rats. *Journal of Ethnopharmacology*, Apr 20; 111(1):29-32.

Gurib-Fakim, A.(2006) Medicinal plants: Traditions of yesterday and drugs tomorrow, Molecular Aspects of Medicine, 27: 1–93.

Hamburger, M., and Hostettmann, K.(1991). Bioactivity in plants: the link between phytochemistry and medicine. *Phytochemistry*, 30(12): 3864-3874.

Heibatullah, Kalantari., Mohsen, Rezaei., Masoud, Mahdavinia., Rashi, Jahangirnejad., Golnaz, and Varnaseri., (2012). Determination of the Mutagenicity Potential of Sankol Herbal Medicine by Single Cell Gel Electrophoresis in Rat Hepatocytes in Comparison With H2O2. Jundishapur *Journal of Natural Pharmaceutical Products*, summer; 7(3): 123–126.

Higashimoto, M., Purintrapiban, J., Kataoka, K., Kinouchi, T., Vinitketkumnuen, U., Akimoto, S., Matsumoto, H., and Ohnishi, Y.(1993). Mutagenicity and antimutagenicity of extracts of three species and a medicinal plant in Thailand. *Mutatation Research*, 303: 135-142.

Idoh, K., Agbonon, A., Potchoo, Y., and Gbeassor, M.(2016). Toxicological assessment of the hydroethanolic leaf extract of clrodenrum capitatum in Wistar rats. *The Pan African Medical Journal*, 24:66.

Isitua, C.C and Ibeh, I.N.(2003) Toxicological assessment of aqueous extract of Moringa oleifera and Caulis bambusae in rabbits. *Journal of Clinical Toxicology*, S12:003.

Kassie, F., Parzefall, W., Musk, S., Johnson, I., Lamprecht, G.,Sontag, G., and Knasmöller, S.(1996). 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*, 102 (1): 1-16.

Kavitha, C. V., Basappa, S., Nanjunda, S., Mantelingu, K., Doreswamy, S., Sridhar, M.A., Prasad, J.S., and Rangappa, K.S.(2006). Synthesis of new bioactive venlafaxine analogs: Novel thiazolidin-4-ones as antimicrobials. *Bioorganic and Medicinal Chemistry*, (14): 2290–2299.

Kucuk Guzel, G., Kocatepe, A., De Clercq, E., Sahin, F., and Gulluce, M. (2006). Synthesis and biological activity of 4-thiazolidinones, thiosemicarbazides derived from diflunisal hydrazide. *European Journal of Medical Chemistry*, (41): 353–359.

Mander, M. and Le Breton, G. (2005). Plants for therapeutic use. In: Mander, M., Mckenzie, M. (Eds.), Southern African trade directory of indigenous natural products. Commercial Products from the Wild Group. Stellenbosch University, pp. 3-8.

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

Marques, R.C.P., de Medeiros, S.R.B., da Silva Dias, C., Barbosa-Filho, J.M., and Agnez-Lima, L.F. (2003). Evaluation of the mutagenic potential of yangambin and the hydroalcoholic extract of Ocotea duckei by the Ames test. *Mutation Research*, 536:117–120.

Marshall N. (1998) Searching for a cure: conservation of medicinal wildlife resources in east and southern Africa. TRAFFIC International.

Mashele, S.S. and Kolesnikova, N. (2010). In vitro anticancer screening of *Asparagus laricinus* extracts. *Pharmacologyonline*, (2): 246-52.

McGaw, L.J., Jäger, A.X., and van Staden, J. (2000). Antibacterial, anthelminthic and anti-amoebic activity in South African medicinal plants. *Journal of. Ethnopharmacology*, 73: 247- 263.

Mouton, R. and Holder, K. (2006). Laboratory tests of renal function. Anaesthesia & Intensive Care Medicine, Volume 7, Issue 7, July 2006, pp 240-243.

Newman, D.J., Cragg, G.M., and Snader, K.M. (2003). Natural products as sources of new drugs over the period 1981–2002. *Journal of Natural Products*, 66 (7): 1022–1037.

Ojewole, J. A., Kamadyaapa, D. R., Gondwe, M.M., Moodley, K., and Musabayane, C.T. (2007) "Cardiovascular effects of Persea americana Mill (Lauraceae) (avocado) aqueous leaf extract in experimental animals," *Cardiovascular Journal of South Africa*,18(2): 69–76.

Popat, A., Shear, N.H., Malkiewicz, I., Stewart, M.J., Steenkamp, V., Thomson, S., and Neuman, M.G. (2001). The toxicity of Callilepis laureola, a South African traditional herbal medicine. *Clinical Biochemistry*, 34: 229–236.

Rautela, G.S. and Liedtke, R.J. (1978) Automated enzymic measurement of total cholesterol in serum. *Clinical Chemistry*, 1978:24.

Rosidah, Y.M.F., Sadikun, A., Ahmad, M., Akowuah, G.A., and Asmawi, M.Z. (2009). Toxicology evaluation of standardized methanol extract of Gynura procumbens. *Journal of Ethnopharmacology*, Jun 22;123(2):244-249.

Schimmer, O., Haefele, F., and Kruger, A. (1988). The mutagenic potencies of plant extracts containing quercetin in Salmonella typhimurium TA98 and TA100. *Mutation Research*, 206: 201–208.

Schimmer, O., Kruger, A., Paulini, H., and Haefele, F.(1994). An evaluation of 55 commercial plant extracts in the Ames mutagenicity test. *Pharmazie*, 49: 448–451.

Shale, T.L., Stirk, W.A., van Staden, J. (1999). Screening of medicinal plants used in Lesotho for anti-bacterial and anti-inflammatory activity. *Journal of Ethnopharmacology*, 67: 347–354.

Springfield, E.P., Eagles, P.K.F., and Scott, G.(2005). Quality assessment of South African herbal medicines by means of HPLC fingerprinting. *Journal of Ethnopharmacology* 101, 75–83.

Sreelata S, Jeyachitra A, Padma PR (2011). Antiproliferation and induction of Moringa oleifera leaf extract on human cancer cells. *Food and Chemical Toxicology*, 49:1270-5.

Ugwu, O.P.C., Nwondo, O.F.C., Joshua, P.E., Bawa, A., Ossai, E.C., and Odo C.E. (2013). Phytochemical and acute toxicity studies of Moringa oleifera ethanol leaf extract. International *Journal of Life Science, Biotechnology and Pharma Research*, 2(2): 66-71.

van Wyk, B.E., van Oudtshoorn, B., and Gericke, N. (1997). Medicinal plants of South Africa. Pretoria: Briza Publications. ISBN 1 875093 09 5

Vigorita, M.G., Ottana, R., Monforte, F., Maccari, R., Trovato, A., Monforte M.T., and Taviano M. F. (2001). Synthesis and antiinflamatory, analgesic activity of 3,3‟-(1,2 ethandiyl)-bis[2-aryl-4- thiazolidinone] chiral compounds. *Bioorganic and Medicinal Chemistry Letters*, 11(21): 2791–2794.

Williams, VL., Balkwill, K., and Witkowski, E.T.F. (1997). Muthi traders on the Witwatersrand, South Africa –an urban mosaic. *South African Journal of Botany* 63: 378–381.

World Health Organization (2001). 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).

World Health Organization. (2002) WHO traditional medicine strategy 2002-2005. Geneva: WorldHealth Organization: http://apps.who.int/medicinedocs/en/d/Js2297e/

World Health Organisation (2005). "Cancer", Factsheet number 297 February. http://www.who.int/mediacentre/factsheets/fs297/en/index.html

World Health Organization (2008). "Traditional medicine," Factsheet number 134 December.http://www.who.int/mediacentre/factsheets/2003/fs134/en/print.html.