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Synergistic Effect of Ethanol Extracts of *Moringa oleifera* and *Pleurotus ostreatus* on Liver Enzymes and Some Renal Functions of Alloxan-induced Diabetic Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TAN and CCM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author TAN managed the analyses of the study. Authors TAN, CCM and LCC managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Objective: To evaluate the effect of the combined ethanol extracts of *Moringa oleifera* and *Pleurotus ostreatus* on the liver and some renal function of alloxan-induced diabetic rats by checking the concentrations of the liver enzymes (AST, ALT & ALP), total bilirubin, total protein, albumin, creatinine, urea & uric acid**.**
Study Design: Animal experimental study.
Place of Study: Department of Biochemistry, Faculty of Science University of Port Harcourt P.M.B 5323 Port Harcourt Nigeria.
Year of Analysis: 2015.
Methods: The animals were induced diabetes mellitus type 1 with alloxan at 120 mg/kg body weight

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intraperitoneally. The combined extract was administered to the diabetic wistar albino rats at a different combination percentage of 60% (1,800 mg/kg) *Moringa oleifera* and 40% (600 mg/kg) *Pleurotus ostreatus* and 40% (1,200 mg/kg) *Moringa oleifera* and 60% (900 mg/kg) *Pleurotus ostreatus* respectively. The rest of the diabetic rats were treated with 100% (3,000 mg/kg) *Moringa oleifera,* 100% (1,500 mg/kg) *Pleurotus ostreatus* while 7.1mg/kg of metformin was used as the standard drug. The effect of the combined treatment of the extracts of *Moringa oleifera* and *Pleurotus ostreatus* on the liver and renal function of the diabetic animals were monitored by measuring the concentrations of the liver enzymes (alkaline phosphatase, alanine transaminase and aspartate transaminase), total bilirubin, total protein, albumin, creatinine, urea and uric acid.

Results: The combined ethanol leaf extracts of *Moringa oleifera* and *Pleurotus ostreatus* significantly (p<0.05) lowered the ALT, AST, ALP, total bilirubin and uric acid and significantly increased the total protein concentration in all the diabetic rats been treated when compared with the diabetic (disease) control rats that were not treated. It was also observed that there was no significant (p<0.05) difference in the concentration of albumin, creatinine and urea during the period of treatment.

Conclusion: This study concluded that the combined ethanol extracts of *Moringa oleifera* and *Pleurotus ostreatus* produced a significant reduction of elevated liver enzymes and are capable of controlling/regulating impaired kidney functions.

Keywords: Alloxan; intraperitoneally; Moringa oleifera; transaminase; Pleurotus ostreatus.

1. INTRODUCTION

The liver and kidney are organs found in vertebrates and some other animals that play a major role in metabolism with numerous functions in the human body. The liver's function includes regulation of glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification and produces bile (an alkaline compound that aids in digestion via the emulsification of lipids). The liver, a highly specialized tissue consisting of mostly hepatocytes regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions [1,2]. The liver plays an enormous role in glucose regulation and thus is affected in diabetic conditions as fat accumulation in the liver may be linked to excess glycogen, which is common among diabetics. Fatty deposits may be due to the increased transport of fat to the liver from the intestines or to decreased removal of fat from the liver. Liver cells may become inflamed as a result of fatty deposition (diabetic or nonalcoholic steatohepatitis). Also, enlarged liver and enzyme abnormalities are characteristic of fatty liver. This condition may also cause abdominal pain, nausea and vomiting, or rarely fluid accumulation around the liver. Symptoms are likely to improve with better blood sugar control. Thus, the concentration of liver enzymes and other liver damage biomarkers are used to estimate the level of liver damage. Occasionally, oral medications used in the treatment of diabetes have unwanted effects on the liver. The bean-shaped organs (kidneys) also play several vital regulatory roles in vertebrates which includes the removal of excess organic molecules from the blood, regulation of electrolytes, maintenance of acid–base balance, and regulation of blood pressure (via maintaining salt and water balance). They also serve the body as a natural filter of the blood, and remove water soluble wastes, which are diverted to the bladder. The kidneys excrete wastes such as urea and ammonium, and they are responsible for the reabsorption of water, glucose, and amino acids. It also produces hormones including calcitriol, erythropoietin, and the enzyme renin. There are numerous diseases of the kidney, but individuals with kidney disease frequently display characteristic clinical features. Common clinical conditions involving the kidney include the nephritic (inflammatory) and nephrotic (non-inflammatory) syndromes, renal cysts, acute kidney injury, chronic kidney disease, urinary tract infection, nephrolithiasis, and urinary tract obstruction [3]. Diabetic kidney disease (diabetic nephropathy) which is one of the major chronic kidney diseases is a complication that occurs in some people with diabetes. It can progress to kidney failure in some cases. Early treatment aims to prevent or delay the progression of the disease and to reduce the risk of developing cardiovascular diseases such as heart attack and stroke which are much more common than average in people with this disease. To diagnose

for kidney diseases, some markers are been used to ascertain when the kidneys are not functioning appropriately. The markers of renal function test such as creatinine, urea, uric acid and other electrolytes are used to assess the normal functioning of kidneys [4]. They indicate the glomerular filtration rate, concentrating and diluting capacity of kidneys (tubular function). If there is an increase or decrease in the values of these markers it indicates dysfunction of kidney. As many herbal medicines have potential and opportunity for the development of newer therapeutics for anti-hyperglycemic and anti-hyperlipidemic agents from natural resources [5, 6], this led to the use of *Moringa oleifera* and *Pleurotus ostreatus* (Mushroom) for the treatment of diabetes and other diseases. *Moringa oleifera* on its own have shown various beneficial pharmacological effects in the prevention and treatment of a variety of diseases such as diabetes mellitus [7], microbial infections [8] and inflammatory conditions [9]. *Moringa oleifera* is a remarkably nutritious vegetable with several antioxidant properties. It is exceptionally rich in vitamins A, C & E and key elements including selenium, and also contains other nutrients required for a healthy lifestyle. *Moringa* leaves are a rich source of beta carotene, protein, amino acids, vitamin C, calcium and potassium [10]. This makes *Moringa* leaves an ideal, safe and natural treatment with wide medicinal uses such as, an anti-inflammatory, hepatoprotective, antihyperytensive and anti-tumor agent [11], which for centuries has been advocated for traditional and industrial uses. *Pleurotus ostreatus* (mushroom) is a fungi, well-known for its nutritional and medicinal value due to its composition of a variety of bioactive substances with pharmacological properties [12]. Edible mushrooms have higher protein and minerals contents and contain less fat but are rich in B vitamins, vitamin D, vitamin K and sometimes vitamins A and C [13-17]. Mushrooms are not only sources of nutrients but also have been reported as therapeutic foods, useful in preventing diseases such as hypertension, diabetes, hypercholesterolemia and cancer [18-20].

2. MATERIALS AND METHODS

2.1 Collection of Plant Sample and Preparation of Leaves Extracts

The leaves of *Moringa oleifera* and *Pleurotus ostreatus* (Mushroom) plant used for extractions were obtained from Dilomat Farms, Rivers State University of Science and Technology, Rivers State [21]. The leaves of *Moringa oleifera* and the *Pleurotus ostreatus* plant were cleaned and shade dried at room temperature, after which they were pulverized to yield a powder. Powdered Moringa leaves weighing 1,000 g and powdered mushroom weighing 1,500 g were soaked in 3,000 ml and 4,500 ml of 95% ethanol respectively for 12-48 hours, after which they were sieved using a muslin cloth and afterwards filtered through a Whatmann filter paper No. 1. The filtrates were concentrated using a rotary evaporator at 45℃. The weight of the concentrates were taken and the percentage yield calculated and kept at 4^{o} C until usage. The extracts were diluted with distilled water at different concentrations for oral administration.

2.2 Reagents

The reagents were sourced commercially and they include: Metformin (Merck Serono Ltd. U.K), chloroform (BDH chemicals Ltd.), alloxan monohydrate (Qualkems Lab. Reagents), ethanol 95% (SIGMA chemicals) and Biochemical reagent kits (MINDRAY).

2.3 Experimental Animals

A total of sixty (60) albino rats of both sexes weighing 160-250 g were used and were marked for easy identification. They were housed in the Pharmacology Department animal house at Ofrima, Abuja park of the University of Port Harcourt, Choba, Rivers State. They were left for 1 week to acclimatize to laboratory conditions during which they had free access to normal feed (Top feeds- grower's mash) and clean water. In the experiment, a total of fifty-seven (57) rats were used, the other three (3) were used for pilot studies to ascertain that the rats could be made diabetic by alloxan treatment at the dose level of 120mg/kg body weight [22]. The rats were grouped in the following pattern below. Mode of administration of *Moringa oleifera* and *Pleurotus ostreatus* were adopted from Bakre et al. [23] and Imoh and Okon [24] respectively.

Group 1: Non-diabetic animals. They were given distilled water and normal feed throughout the course of this study (normal control).

Group 2: Diabetic animals but were not treated with any drug or extract (disease control).

Group 3: Diabetic animals treated with 7.1 mg/kg metformin

Group 4: Diabetic animals treated with mushroom extract (1,500 mg/kg)
Group 5: Diabetic animals treated with *Moringa oleifera* extract (3,000 mg/kg)
Group 6: Diabetic animals treated with 60% *Moringa oleifera* extract (1,800 mg/kg) and 40% mushroom extract (600 mg/kg)
Group 7: Diabetic animals treated with 40% *Moringa oleifera* extract (1,200 mg/kg) and 60% mushroom extract (900 mg/kg).

2.4 Acute Toxicity Study

Wistar albino mice of both sexes were used in determining the LD50 of the ethanolic leaf extracts of *Moringa oleifera* and *Pleurotus ostreatus*. The safety of the extracts was carried out according to the method of Lorke [25] with slight modification. In the first stage, a total of eighteen (18) rats (i.e. nine rats for each extract) were grouped into six (6) groups of three (3) rats each, which were given 10, 100, and 1000 mg extract/kg body weight of the extracts (*M. oleifera* and *P. ostreatus*) respectively. The rats were kept under the same conditions and observed for signs of toxicity and mortality for the first 6 hours and thereafter daily for 14 days. In the second stage, the following doses- 1600, 1900 and 5000 mg extract/kg body weight were administered to another set of six (6) groups of three (3) rats for both extracts separately. These rats were carefully monitored for the first 6 hours after treatment and afterwards daily for 14 days for signs of toxicity and/or mortality.

2.5 Administration of Alloxan

Alloxan weighing 1.0 g was dissolved in 20 ml of distilled water from which a single dose (120 mg/kg body weight) was slowly administered intraperitoneally to the rats within few minutes of its (alloxan) preparation. Diabetes was confirmed using a drop of blood from the tail artery on a blood glucometer 2-3 days following alloxan injection and was found to have increased by two to three (2-3) times the normal value.

2.6 Investigation of the Effect of the Combined Extracts on Liver and Renal Function of Diabetic Rats

A total number of fifty-seven (57) rats were used for the experiment proper. The plasma activity of alanine transaminase (ALT) and aspartate transaminase (AST) were determined using Reitman and Frankel method [26] while that of alkaline phosphatase (ALP) was determined using colorimetric method of Deutsche Gesellschaft für Klinische Chemie [26], Bromcresol green（BCG）method was used to determine the level of Plasma Albumin in the samples according to the method of Doumas and Watson [27], Biuret method was used to determine the level of total protein in the samples according to the method of Flack and Woollen [28] and Jendrassik-Grof method [29] was used to determine the level of total bilirubin in the samples. Modified Jaffé method according to Bartels and Bohmer [30] was used to determine the level of creatinine in the samples, Urease-Berthelot's method [31,32] was used to determine the level of urea in the samples, Uricase-peroxidase (Enzymatic colorimetric method) of Fossati et al. [32,33], was used to determine the level of uric acid The animals were grouped into 7 groups of 9 animals each except for the normal control group with 3 animals.

2.7 Statistical Analysis

All data were subjected to statistical analysis. Values were reported as mean ± standard deviation (X±S.D). One-way ANOVA test was used to compare means in the different groups. The results were considered significant at p-values of less than 0.05, that is at 95% confidence level ($p<0.05$).

3. RESULTS AND DISCUSSION

The ethanolic extracts of *Moringa oleifera* and *Pleurotus ostreatus* showed no sign of toxicity and/or mortality during the acute monitoring and 14 days monitoring in both stages of the experiment. The oral LD50 value of both plant extracts was estimated to be greater than 5,000 mg/kg body weight in rats. The preliminary qualitative analysis of *Moringa oleifera* and *Pleurotus ostreatus* were conducted and the result revealed the presence of saponin, tannin, phenol, flavonoid, cardiac glycoside, terpenoid and steroid. The result of this analysis is shown in Table 1. Also, the quantitative phytochemical constituents of the leaves of *Moringa oleifera* and *Pleurotus ostreatus* (mushroom) plant were determined using gas chromatography (GC). From the result as shown in Tables 2 and 3 respectively, the quantitative analysis of *Moringa oleifera* revealed that it contains high level of catechins, phenols and sapogenins. It also contains low levels of sparteins, oxalates and kaempferol. Other phytochemicals such as

anthocyanins, epicatechin and rutin were also detected. The quantitative analysis of *Pleurotus ostreatus* shows the presence of saponins, phytates, oxalates, anthocyanins, tannins, phenols, lunamarine and other phytochemicals like ribalinidine, quercetin and rutin in trace amounts as shown below. The effects of the combined ethanol extract of *Moringa oleifera* and *Pleurotus ostreatus* on AST, ALT, ALP, total bilirubin, total protein, albumin, creatinine, urea and uric acid are shown in Tables 4-12.

Table 1. Phytochemical content of the leaves of *Moringa oleifera* and *Pleurotus ostreatus*

Phytochemical	*M. oleifera*	*P. ostreatus*
Saponin	+	+
Tannin	++	+
Phenol	+	++
Flavonoid	+	+
Alkaloid	-	++
Cardiac glycoside	++	++
Terpenoid	++	++
Protein	-	++
Steroid	++	++

Table 2. Quantitative phytochemical constituents of *Moringa oleifera* leaf extract

Component	Concentration (ug/ml)
Spartein	0.2137
Oxalate	4.2931
Anthocyanin	37.2228
Phenol	140.0281
Sapogenin	80.1033
Catechin	147.3057
Epicatechin	48.8936
Rutin	50.8902
Kaempferol	5.2335

Table 3. Quantitative phytochemical constituents of *Pleurotus ostreatus* extract

Component	Concentration (ug/ml)
Phytate	0.2195
Oxalate	0.2022
Anthocyanin	0.1981
Tannin	0.1871
Phenol	0.1756
Lunamarine	0.1702
Saponin	1.1855
Ribalinidine	0.1414
Quercetin	0.0509
Rutin	0.0354

Table 4. Effect of combined treatment of *Moringa oleifera* and *Pleurotus ostreatus* (mushroom) on AST activity of diabetic rats

Groups	AST (U/L)		
	Week 1	Week 3	Week 5
Normal control	43.67± 33.67	37.44± 21.29[b]	41.72± 15.31[b]
Disease control	252.33± 23.11	262.17± 56.86[ag]	240.17± 37.89[acdefg]
Treated with metformin	134.23± 47.90	123.30± 38.79	55.27± 25.71[b]
Treated with 100% mushroom	117.93± 15.56	91.57± 98.73	49.30± 55.37[b]
Treated with 100% *Moringa oleifera*	114.17± 44.24	75.57± 95.83	37.73± 36.91[b]
Treated with 60% *Moringa &* 40% Mushroom	122.70± 174.09	96.07± 109.93	45.10± 25.65[b]
Treated with 40% *Moringa &* 60% Mushroom	98.30± 141.81	36.33± 9.30[b]	43.50± 20.08[b]

Values are presented as mean ± standard deviation.

** Superscript "a,b,c,d,e,f and g" shows significant difference, (p<0.05) when groups "1,2,3,4,5,6 and 7" are compared with other groups respectively*

Table 5. Effect of combined treatment of *Moringa oleifera* and *Pleurotus ostreatus* (mushroom) on ALT activity of diabetic rats

Groups	ALT (U/L)		
	Week 1	Week 3	Week 5
Normal control	43.37± 1.70	41.21± 2.18	38.43± 12.20[b]
Disease control	74.07± 5.05	75.80± 7.17	86.63± 4.70[a]
Treated with metformin	56.50± 4.81	63.10± 18.46	52.67± 15.17
Treated with 100% mushroom	49.20± 1.90	89.47± 75.68	58.73± 16.50
Treated with 100% *Moringa oleifera*	150.80± 167.99	82.23± 20.58	70.50± 16.50
Treated with 60% *Moringa &* 40% Mushroom	72.00± 10.25	58.50± 9.02	63.50± 14.03
Treated with 40% *Moringa &* 60% Mushroom	77.00± 17.92	71.71± 26.96	59.00± 18.24

Values are presented as mean ± standard deviation.

** Superscript "a and b" shows significant difference, (p<0.05) when groups "1 and 2" are compared with each other*

Table 6. Effect of combined treatment of *Moringa oleifera* and *Pleurotus ostreatus* (mushroom) on ALP activity of diabetic rats

Groups	ALP (U/L)		
	Week 1	Week 3	Week 5
Normal control	45.93± 16.28	51.38± 36.47	43.31± 12.11[b]
Disease control	154.60± 90.24	226.67± 96.81	280.47± 49.33[acdefg]
Treated with metformin	104.20± 12.47	116.07± 77.76	97.67± 13.19[b]
Treated with 100% mushroom	220.43± 223.71	190.00± 98.96	90.30± 24.97[b]
Treated with 100% *Moringa oleifera*	274.57± 302.80	169.40± 146.19	63.20± 13.20[b]
Treated with 60% *Moringa &* 40% Mushroom	97.43± 47.86	108.17± 118.88	102.97± 64.41[b]
Treated with 40% *Moringa &* 60% Mushroom	200.57± 152.35	116.57± 83.33	107.70± 18.32[b]

Values are presented as mean ± standard deviation.

** Superscript "a,b,c,d,e,f and g" shows significant difference, (p<0.05) when groups "1,2,3,4,5,6 and 7" are compared with other groups respectively*

Table 7. Effect of combined treatment of *Moringa oleifera* and *Pleurotus ostreatus* (mushroom) on total bilirubin concentration of diabetic rats

Groups	Total bilirubin (mg/dL)		
	Week 1	Week 3	Week 5
Normal control	7.41± 4.30[b]	7.13± 3.80[b]	6.86± 3.39[b]
Disease control	29.64± 23.11[acef]	35.34± 10.45[acd]	37.62± 18.67[ac]
Treated with metformin	7.41± 4.30[b]	6.88± 3.91[b]	7.03± 3.27[b]
Treated with 100% mushroom	35.34± 10.45	9.57± 2.31[b]	14.25± 11.64
Treated with 100% *Moringa oleifera*	9.69± 2.61[b]	14.25± 11.64	13.11± 8.08
Treated with 60% *Moringa &* 40% Mushroom	14.25± 11.64[b]	13.11± 8.08	13.68± 5.92
Treated with 40% *Moringa &* 60% Mushroom	13.11± 8.08	13.68± 5.92	12.41± 7.86

Values are presented as mean ± standard deviation.

** Superscript "a,b,c,d,e and f" shows significant difference, (p<0.05) when groups "1,2,3,4,5 and 6" are compared with other groups respectively*

Table 8. Effect of combined treatment of *Moringa oleifera* and *Pleurotus ostreatus* (mushroom) on total protein concentration of diabetic rats

Groups	Total protein (g/L)		
	Week 1	Week 3	Week 5
Normal control	68.97± 2.50	62.13± 2.31	66.72± 2.50
Disease control	65.40± 3.22	68.40± 5.86	63.23± 4.33
Treated with metformin	64.47± 0.97	82.40± 3.70	73.73± 4.35
Treated with 100% mushroom	71.27± 9.82	73.17± 6.10	68.67± 2.57
Treated with 100% *Moringa oleifera*	66.30± 4.20	75.90± 7.69	68.37± 4.85
Treated with 60% *Moringa &* 40% Mushroom	71.60± 3.40	71.97± 6.65	73.13± 2.90
Treated with 40% *Moringa &* 60% Mushroom	66.57± 5.70	74.47± 6.21	71.30± 3.75

Values are presented as mean ± standard deviation.

Table 9. Effect of combined treatment of *Moringa oleifera* and *Pleurotus ostreatus* (mushroom) on albumin concentration of diabetic rats

Groups	Albumin (g/L)		
	Week 1	Week 3	Week 5
Normal control	29.77± 2.64	28.32± 2.58	31.11± 3.83
Disease control	38.10± 15.54	41.60± 22.04	34.53± 3.49
Treated with metformin	29.03± 8.59	34.17± 11.43	42.20± 7.86
Treated with 100% mushroom	30.10± 11.63	37.33± 10.58	33.00± 7.79
Treated with 100% *Moringa oleifera*	30.93± 11.12	39.03± 8.83	43.97± 11.07
Treated with 60% *Moringa &* 40% Mushroom	37.87± 6.33	32.80± 10.57	39.37± 11.25
Treated with 40% *Moringa &* 60% Mushroom	43.90± 17.15	36.00± 1.80	38.30± 11.89

Values are presented as mean ± standard deviation.

Table 10. Effect of combined treatment of *Moringa oleifera* and *Pleurotus ostreatus* (mushroom) on creatinine concentration of diabetic rats

Groups	Creatinine (µmol/L)		
	Week 1	Week 3	Week 5
Normal control	89.73± 0.42	86.02± 0.31	73.65± 4.31
Disease control	90.03± 9.08	88.60± 15.70	92.03± 5.24
Treated with metformin	62.30± 0.95	66.90± 5.80	65.40± 2.96
Treated with 100% mushroom	95.93± 3.12	72.57± 5.46	64.87± 3.82
Treated with 100% *Moringa oleifera*	105.47± 69.72	83.37± 16.30	87.33± 11.00
Treated with 60% *Moringa &* 40% Mushroom	83.00± 11.48	73.90± 9.79	88.83± 13.77
Treated with 40% *Moringa &* 60% Mushroom	79.97± 10.71	71.50± 16.87	62.80± 7.60

Values are presented as mean ± standard deviation

Table 11. Effect of combined treatment of *Moringa oleifera* and *Pleurotus ostreatus* (mushroom) on urea concentration of diabetic rats

Groups	Urea (mmol/L)		
	Week 1	Week 3	Week 5
Normal control	7.91± 7.44	6.92± 1.24	6.97± 5.83
Disease control	9.43± 3.33	8.70± 4.20	9.84± 6.22
Treated with metformin	6.55± 1.79	5.19± 3.88	4.87± 2.02
Treated with 100% mushroom	7.60± 8.79	5.45± 2.30	5.84± 4.73
Treated with 100% *Moringa oleifera*	9.64± 11.35	4.74± 2.73	3.53± 0.81
Treated with 60% *Moringa &* 40% Mushroom	3.96± 0.55	6.93± 1.56	4.20± 1.23
Treated with 40% *Moringa &* 60% Mushroom	5.63± 3.78	5.97± 3.98	4.60± 1.90

Values are presented as mean ± standard deviation

Table 12. Effect of combined treatment of *Moringa oleifera* and *Pleurotus ostreatus* (mushroom) on uric acid concentration of diabetic rats

Groups	Uric acid (µmol/L)		
	Week 1	Week 3	Week 5
Normal control	208.53± 121.54	121.32± 9.73	114.32± 8.92
Disease control	395.70± 285.98	366.40± 192.30	457.33± 241.85[fg]
Treated with metformin	138.23± 32.89	194± 10.44	195.50± 72.95
Treated with 100% mushroom	191.83± 17.38	227.23± 27.84	189.63± 16.44
Treated with 100% *Moringa oleifera*	130.90± 45.85	158.97± 46.23	171.63± 10.99
Treated with 60% *Moringa &* 40% Mushroom	152.13± 43.08	141.53± 28.60	126.63± 25.57[b]
Treated with 40% *Moringa &* 60% Mushroom	133.80± 8.54	148.23± 44.59	113.20± 36.20[b]

Values are presented as mean ± standard deviation.

** Superscript "b,f and g" shows significant difference, (p<0.05) when groups "2,6 and 7" are compared with other groups respectively.*

***ALT – Alanine aminotransferase; AST - Aspartate aminotransferase; ALP – Alkaline phosphatase*

3.1 Discussion

Moringa oleifera and *Pleurotus ostreatus* individually have been proven to be an effective hypoglycemic and hypolipidemic agent in diabetic animals. This work was carried out to assess the effects of the combined treatment of *Moringa oleifera* and *Pleurotus ostreatus* on liver and renal functions in diabetic rats. The acute toxicity study of the extracts revealed that the oral LD50 value of ethanolic extract of *Moringa oleifera* was estimated to be greater than 5,000 mg/kg body weight in rats. This is in agreement with the study of Bakre et al. [23] in which it was reported that the oral LD50 of the ethanol extract of *M. oleifera* leaves was greater than 6400 mg/kg in mice [23]. The oral LD50 value of ethanolic extract of *Pleurotus ostreatus* was also estimated to be greater than 5,000 mg/kg body weight. This is in agreement with the study of Imoh and Okon [24] that no mortality and significant changes in general behavior of rats were observed to the maximum dose level of 5,000 mg/kg b.wt of orally administered

P. ostreatus for 72 h treatment. According to Bruce [34], any substance with LD50 estimated to be greater than 2000-5000 mg/kg body weight given orally could be considered of low toxicity and being safe. This implies that the extracts can be consumed at higher doses without fear of toxicity. From the results showed in Tables 4-6, it was seen that there was a significant increase (*p<0.05*) in the activities of all liver enzymes (ALT, AST and ALP) of the diabetic group (disease control animals) when compared with that of other groups (treated animals) which showed a significant (*p<0.05*) decrease in the activities of liver enzyme. After the first week of treatment, there was no significant difference among the groups in all the liver enzymes. But as treatment progressed to week 3, significant increase (*P<0.05*) was observed in AST activity when untreated animals (disease control animals) were compared with the animals treated with the standard drug (Metformin), combined extracts of 60% mushroom and 40% *Moringa* extract (MSMO). After week 5 treatment, there was significant increase in the AST and ALP activities of the untreated animals. This shows that in diabetic condition, the liver enzymes are elevated, thus the liver is damaged but the *Moringa*, mushroom, metformin and the combined treatment extracts of *Moringa* and mushroom were able to control the activities of liver enzymes in the various treatment groups. Increase in liver enzymes activity as observed in this study may reflect damage of liver cells. Serum ALT level is known to increase in liver disease and it has been used as a tool for measuring hepatic necrosis [35]. High rise in serum ALP activity as shown by the untreated (disease control) animals may be considered as a sensitive indicator of cholestasis in early stages or mild circumstances preceding other indicators such as hyperbilirubinemia [35]. ALT or AST is a liver specific enzyme and its elevated concentrations are usually linked to lot of health problems. Such suspected health issues include viral hepatitis, diabetes, congestive heart failure, liver damage, problems associated with the bile ducts like biliary tract obstruction [36]. This is an indication of damage to the liver since there are significant differences between the concentration of ALT, AST and ALP when the diabetic control animals were compared with other experimental groups and normal control animals. However, there was no significant difference in the concentrations of ALT, AST and ALP of the treated animals respectively when compared to the normal animals (control group). *Moringa oleifera* leaf has repairing effect on the liver due to its nutritional properties such as the presence of essential amino acid like methionine and cysteine [37,38] and thus boosting the total proteins and albumin level [39]. Concentration of total bilirubin was measured in order to ascertain synthetic functionality of the liver. The biochemical examination of the serum total bilirubin showed a significant (*p<0.05*) decrease in the treated animals when compared with the diabetic (disease) control animals. From the result shown in Table 7, after the first week of treatment, there was a significant difference (*p<0.05*) in total bilirubin concentration between diabetic (disease) control animals and the normal control animals (non diabetic animals) and the animals treated with metformin, *Moringa* and 60% *Moringa* and 40% mushroom combination extracts respectively. This shows the efficacy of *Moringa* & metformin in reducing and controlling the level of bilirubin. As treatment commenced, significant difference was seen only when diabetic control animals were compared with normal control animals and animals treated with metformin i.e. after the 3rd and 5th week of treatment. This reveals that metformin has a high affinity for regulating bilirubin level in the blood. There was also a significant increase in the total protein level of all the treated animal groups as treatment progressed but this is not exactly the same with the untreated animals which showed a decreased level of total protein as ailment progresses. Serum albumin is also used as an indicator of liver impairment, reduced absorption or protein loss [40]. Albumin apart from being a useful indicator of the integrity of glomerular membrane is also important in determining the severity of disease [41]. Decreased albumin may be due primarily to reduction in synthesis by the liver and secondarily to reduced protein intake which further confirms hepatic damage [42,43]. From the result as shown in Table 9, it showed that there was no significant difference in albumin concentration when diabetic group (untreated animals) were compared with other experimental groups (treated animals). Some renal functions were assayed and the result showed an observable significant increase ($p<0.05$) in the urea and creatinine level in the diabetic control group (untreated animals) when compared with the other groups (treated animals) which showed normal or significant decrease. Though there was no significant difference ($p<0.05$) in the concentration of urea when the diabetic group was compared with all other experimental groups, but there was an increase in urea concentration in the diabetic group relative to the normal animals and other treated

animals (see Tables 10-12). Urea and creatinine measurements are mostly used in ascertaining the functionality of the kidney but are also used to check the synthetic function of the liver since both molecules and/or their precursors can be synthesized in the liver. Urea is one of a number of non-protein nitrogenous substances that accumulate in the plasma when renal excretion is reduced. Causes of increased blood urea levels include: high protein diet, intestinal haemorrhage, dehydration, severe haemorrhage, shock, etc. Urea level could be decreased due to the following: liver failure, low protein diet, anabolic steroids, diabetes insipidus, etc [35]. The uric acid result showed a significant increase ($p<0.05$) in week 5 when the diabetic control group was compared with the group treated with metformin and the two different concentrations of combined ethanol extract of *Moringa oleifera* and *Pleurotus ostreatus*. Lowering the uric acid levels reduces the severity of metabolic syndrome, which includes combination of high triglycerides, abdominal obesity, insulin resistance, low HDL and hypertension [44]. Reduction of uric acid concentration by eliminating fructose or with xanthine oxidase (XOD) enzyme inhibitors reduces the severity of the metabolic syndrome [45]. Fructose increases blood uric acid levels (glucose does not). High blood uric acid reduces the availability of nitric oxide (NO) in the body needed for normal circulation. Insulin requires nitric oxide for glucose absorption. It is demonstrated that fructose is a likely cause of the metabolic syndrome by causing hyperuricemia. The ability of these combined extracts in reducing uric acid level is due to its flavonoids content. In a study carried out by Mo et al. [46] to determine the ability of flavonoids in lowering uric acid level in mice; quercitin, morin (3,5,7,2′,4′-Pentahydroxyflavone), kaempferol, apigenin and puerarin were all found to lower uric acid levels of the blood even at the low 50 mg/kg dose. Quercitin, morin, kaempferol, myricetin and puerarin all lowered uric acid levels in the liver. Flavonoids, figs, black tea, astragulus, cocoa polyphenols and white mulberry twigs have been shown to have xanthine oxidase inhibitory activity, reducing uric acid to prevent gout [46].

4. CONCLUSION

This study concluded that metformin and the combined ethanolic extracts of *Pleurotus ostreatus* and *Moringa oleifera* produced a significant reduction of liver enzymes, total bilirubin concentration and increased total protein concentration in alloxan-induced diabetic rats. The extracts and metformin also produced a significant reduction and regulation of creatinine, urea and uric acid concentration. Thus, the combined ethanol extracts of *Pleurotus ostreatus* and *Moringa oleifera* has a repairing effect on the liver and kidneys.

ETHICAL APPROVAL

The ethical approval code for this study as granted by the Institution is: UPH/BCH/REC/015/028

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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