MANAGEMENT OF CCl_4 INDUCED HEPATOTOXICITY ON WISTAR RATS USING METHANOL EXTRACT OF *Pleurotus ostreatus* CULTIVATED WITH EXTRACT OF *Allium cepa*.

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ABSTRACT

This study was designed to assess the ameliorating properties of methanol extract of *Pleurotus ostreatus* cultivated with extract of *Allium cepa* on liver function test of CCl_4 induced hepatotoxity in wistar albino rats. Seventy wistar albino rats were used for the study and were grouped into 7 of ten rats each. Groups 2-7 received a dose of 0.3ml/kg body weight (b.w) of CCl_4 in 1:1 olive oil intraperitoneally twice a week. Groups 3, 4, 5 and 7 orally received a dose of 100mg/kg b.w, 200mg/kg b.w, 300mg/kg b.w and 200mg/kg b.w of methanol extract respectively. Groups 6 and 7 orally received a dose of 5.2mg/kg b.w of livolin and group 7 orally received a dose of 50mg/kg b.w of vitamin C. These drugs were administered for 30 days and the following biochemical markers; Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), Direct and Total Bilirubin (DBIL and TBIL), Total Protein (TPRO) and Albumin (ALB) were estimated on day 10, 20 and 30 after treatment. The values of ALT, AST, ALP, DBIL and TBIL were significantly increase at $p<0.05$ on day 10, 20 and 30 in group 2 compared to group 1 and the values of TPRO and ALB significantly decreased at $p<0.05$ on day 10, 20 and 30 in group 2 compared to group 1. The values of ALT, AST, ALP, DBIL and TBIL significantly decreased at $p<0.05$ on day 10, 20 and 30 in groups 3,4 5,6 and 7 compared to group 2 and the values of TPRO and ALB significantly increased at $p<0.05$ on day 10, 20 and 30 in groups 3, 4 5 ,6 and 7 also compared to group 2. The histopathology findings showed that there were marked distortion of hepatocytes in group 2 on day 30 and there were regeneration of hepatocytes treatment groups especially group 5 on day 10, 20 and 30. These findings suggest that methanol fruiting body extract of *Pleurotus ostreatus* cultivated with extract of *Allium cepa* possesses hepatoprotective properties and can be used to manage cases of liver damage.

Keywords: *Pleurotus ostreatus*, *Allium cepa*, Liver Function markers.

No: of Tables: 6 **No:of Figures: 9** **No:of References: 28**

INTRODUCTION

The liver is large in size and well organized to carry out a major role in protein, fat and carbohydrate metabolism. It is the place where metabolism waste products like ammonia are converted to harmless substances. In connection with the spleen, it serves in damage of remaining of the erythrocytes cells and with their parts recycling. It also serves a role of producing plasma proteins including clotting factors and lipoprotein by chemical process and maintains stable blood glucose concentration (glyconeogenesis), decomposing it to glucose when required (glycogenolysis) and producing glucose from non carbohydrate like ammonia (gluconeogenesis) (Ward and Daly, 1999; Kmiec, 2001, Pocock and Gillian, 2006; andKrishnendu, 2012).

Liver diseases have become a major problem in all over the world and are connected with high rate of diseases that result to death (Baranisrinivasan *et al*., 2009). In developed countries, the major causes are excessive alcohol consumption and viral induced chronic liver diseases while in developing countries hepatitis B and C viruses, parasitic disease, hepatotoxic drugs, high doses of paracetamol and environmental toxins are the common frequent causes.

Herbal medicine is a part of science that plant-based preparations are used to alleviate disease. Over the past thirty years, the use of herbal drugs have increased as it is known that if used properly, herbs can help treat various conditions and have fewer side effects compared to conventional medicine (Kala *et al.,* 2006). Plants can synthesize different chemical compounds and some of these compounds exhibit pharmacological activity, this is the basis of herbal drug. These plants contain bioactive compounds that can affect one or more identified biological process such as improving homeostatic, free radicals scavenging ability, cholesterol lowering capability, anti-inflammatory, antimicrobial, antiviral, anticancer and antiparasitic activity (Manjulika *et al.,* 2004).

MATERIALS AND METHODS

Experimental Plants: Red *Allium cepa* (Onion) bulbs and *Pleurotus ostreatus* fruiting bodies were bought from Choba Market, Choba, Port Harcourt, Rivers State. Dr. Nwosu of plant science department, University of Port Harcourt, Rivers State identifies both plants.

Experimental Animals: Seventy *Wistar Albino* rats weighing between 100-200g of three months old breed were procured from department of biochemistry, University of Port Harcourt animal house. The animals were randomly selected, weighed and distributed into seven groups on weight bases. These animals were put in plastic cages and left under suitable laboratory conditions for two weeks for them to adapt to the new environment before beginning of the experiment. The cages were cleaned daily. The animals were fed with commercial growers mash product of Top Feeds Ltd., Sapele, Nigeria

and water given *ad libitum*. The animals' body weight were recorded.

METHODS

Preparation of *Allium cepa* extract by sohxlet method.

Fresh, healthy Red bulbs of *Allium cepa* were washed, sliced into small pieces and blended in a warring blender. Four hundred grams of the sample was placed in a Sohxlet extractor that was inserted on a filter paper. The extractor was connected to a pre-weighed dried distillation flask before acetone was poured into the distillation flask through the condenser, joined to the Soxhlet extractor. This set up was tied using retort stand clamp. Cooled water from the jet was permitted to move continuously into the condenser, and the heated solvent refluxed as a result. Onion sample in the solvent that was poured into the distillation flask was extracted in the process of refluxing continuously. When onion extract was observably extracted completely from the sample under test, the condenser and the extractor were disconnected, and the solvent was heated to concentrate the onion extract.

Air oven was used to dry the flask to constant weight and re-weighed to get the weight crude onion extract (Sheema *et al.,* 2015).

Method of cultivation of *Pleurotus ostreatus* fruiting body with extract of *Allium cepa*bulb.

This involves 5 stages:

(i) Preparation of tissue culture of *Pleurotus ostreatus* using Potato Dextrose Agar (PDA) medium.

Potato Dextrose Agar (PDA) medium was prepared by pealing 200g irish potatoes and boiled in water for some minutes. This was filtered and the irish potato's water was measured to 1000ml. Twenty grams of glucose and 20 grams of powdered Agar were added to the irish potato's water and this was stirred properly and shared into two conical flasks of 500ml which were covered with cotton wool and foil paper held with rubber band. The two conical flasks were placed inside the pressure pot and sterilized for 15-20 minutes under pressure. Twenty grams of *Allium cepa* was mixed with the mixture in one of the conical flasks allowed to cool and were poured into petri-dishes. Inner tissues of the grown *Pleurotus ostreatus* (that was purchased from Choba market) were removed from the stem of the fruiting bodies and were dropped into the petri-dishes. These were covered with foil paper held with rubber band and kept in dark cupboard for seven days to form mycelium (Vasil and Thorpe, 1998).

(ii) Grains sterilization: Two kilograms of guinea corn grains were washed in water to remove the bad ones. The grains were poured into a pressure pot and per boiled in water for some minutes to make it soft. The water was sieve out and the grains were sprained on the ground for some hours for it to dry properly before they were bottled in spawn bottles.

(iii) Transfer of the mycelium into the sterilized guinea corn grains in the spawn bottles.

The mycelium that were formed in stage 1 were cut into parts and were added to the grains mixed properly in the spawns bottles and were kept in the innoculation room for two weeks to form spawns.

(iv) Mushroom substrate preparation: The substrate used were 93kg of saw dust, 7kg of wheat brown, 400g of calcium carbonate and 60 to 65 % of water. These were mixed properly after which they were bagged and sterilized for 4 hours before the spawns were transferred into them and were kept in the incubation room for one month for ramification to occur.

(v) Development of the full grown fruiting bodies of *Pleurotus ostreatus*: After ramification had taken place, the bags were cut open and were watered for duration of two weeks before the fruiting bodies started emerging and were harvested after maturation (Vasil and Thorpe, 1998).

Methanol extraction of *Pleurotus ostreatus* cultivated with *Allium cepa* extract

Dried *Pleurotus ostreatus*were blended in a warring blender, three hundred grams of *Pleurotus ostreatus* sample was macerated in 300ml of methanol for three days in a macerating jar. Then the sample was filtered using a Whatman No.1 filter paper. The filtrate was concentrated with a rotary evaporator at 65° C and was finally dried in a thermostat water bath at 60° C to become an extract (Chaturvedi, 2011).

EVALUATION OF BIOCHEMICAL PARAMETERS

The animals to be sacrificed were first anaesthetized with chloroform (inhalational anesthesia) followed by cervical dislocation. Each animal was then placed on a dissecting slab and then cut along the thorax down the abdominal region; blood was collected via cardiac puncture and dispensed into the Heparin bottle for biochemical assays (ALT, AST and ALP). ALT, AST and ALP were analyzed by kinetic methods using kits from Randox (United Kingdom) using a double-beam spectrophotometer. All other reagents were of analytical grade. Other biochemical markers; TBIL, DBIL, T PROTEIN and ALBUMIN were analysed using RANDOX test kits following the standard operating procedures enumerated on the test manual.

HISTOPATHOLOGICAL METHOD

The liver tissues of the sacrificed animals were collected, excised and rinsed in ice cold 0.9% normal saline solution. They were blotted dry and fixed in 100% formalin for 48 hours and were subjected to dehydration with acetone of strength 70, 80 and 100% respectively each for 1 hour and were cleared in xylene and embedded in paraffin wax. Serial sections of 70 μl thickness were cut from each paraffin blocks (from control and treated groups) using microtone. They were stained with herematoxylin eosin for photomicroscopic observation (Bora, 1995).

STATISTICAL ANALYSIS

All data are presented as means ±SD, and were analyzed using the One Way Analysis Of Variance (ANOVA). The results were considered significant when P values are less than 0.05 (P<0.05) and non-significant when p values are greater than 0.05 (P>0.05).

RESULTS

The result of the effect of methanol extract of *Pleurotus ostreatus* cultivated with extract of *Allium cepa* on the liver function parameters and histopathology of the liver on day 10, 20 and 30 are as follows:

Table 1.0: Result of the Effect of Methanol extract of *Pleurotus ostreatus* on Aspartate Transaminase (AST) activities (μ/l).

Groups	Treatment	Day 10	Day 20	Day 30
1	NormalControl.	36.33±0.33^{ab}	36.00±1.00^{abc}	36.33±0.33^{abc}
2	CCl_4 treated only.	61.67±9.67^{abc}	50.67±1.33^{abc}	50.00±1.00^{abc}
3	CCl_4 + 100mg/kg extract.	27.33±4.33^{b}	23.00±0.00^{ab}	28.33±2.67^{b}
4	CCl_4 + 200mg/kg extract.	23.00±4.00^{b}	19.00±0.00^{ab}	23.00±0.00^{ab}
5	CCl_4 + 300mg/kg extract.	21.00±5.00^{b}	17.00±1.00^{ab}	31.00±0.00^{b}
6	CCl_4+ 5.2mg/kg Livolin.	17.67±4.67^{bc}	12.33±5.33^{abc}	27.00±4.00^{abc}
7	CCl_4 + 200mg/kg extract+5.2mg/kg Livolin+ 50mg/kg Vitamin C.	19.00±0.00^{b}	8.67±1.67^{ab}	21.67±1.33^{ab}

Values are represented as Mean ± Standard error of mean; n =3 per group.
Values in the same column with common superscript letter (a, b, c) are significantly different at P < 0.05.
Superscript A (^{a}) represents significant difference when group 1 is compared to other groups at P < 0.05.
Superscript B (^{b}) represents significant difference when group 2 is compared to other groups at P < 0.05.
Superscript C (^{C}) represents significant difference when group 6 is compared to other groups at P < 0.05.

Values without superscript shown no significant difference when group 1, 2 and 6 are compared to other groups at $P < 0.05$.

Table 1.1: Result of the Effect of Methanol Extract 0f *Pleurotus ostreatus* on Alanine amino Transaminase (ALT) activities (μ/l).

Groups	Treatment	Day 10	Day 20	Day 30
1	Normal Control (NC).	20.67 ± 0.33^{ac}	21.33 ± 0.67^{ac}	22.00 ± 1.00^{ac}
2	CCl_4 treated only.	23.33 ± 0.17^{bc}	25.17 ± 0.60^{bc}	25.00 ± 1.16^{bc}
3	CCl_4 + 100mg/kg extract.	12.00 ± 0.00^{ab}	20.23 ± 3.23^{c}	15.33 ± 1.67^{b}
4	CCl_4 + 200mg/kg extract.	9.33 ± 1.33^{ab}	12.00 ± 0.00^{ab}	13.67 ± 1.67^{ab}
5	CCl_4 + 300mg/kg extract.	8.00 ± 2.00^{ab}	6.67 ± 1.33^{ab}	11.33 ± 0.67^{ab}
6	CCl_4 + 5.2mg/kg Livolin.	9.33 ± 1.33^{abc}	8.00 ± 2.00^{abc}	10.00 ± 0.00^{abc}
7	CCl_4 + 200mg/kg extract+5.2mg/kg Livolin+ 50mg/kg Vitamin C.	8.00 ± 0.00^{ab}	8.00 ± 2.00^{ab}	9.33 ± 2.67^{ab}

Values are represented as Mean $\pm$ Standard error of mean; n =3 per group.
Values in the same column with common superscript letter (a, b, c) are significantly different at $P < 0.05$.
Superscript A (^{a}) represents significant difference when group 1 is compared to other groups at $P < 0.05$.
Superscript B (^{b}) represents significant difference when group 2 is compared to other groups at $P < 0.05$.
Superscript C (^{C}) represents significant difference when group 6 is compared to other groups at $P < 0.05$.
Values without superscript shown no significant difference when group 1, 2 and 6 are compared to other groups at $P < 0.05$.

Table 1.2: Result of the Effect of Methanol Extract 0f *Pleurotus ostreatus* on Alkaline Phosphatase (ALP) activities (μ/l).

Groups	Treatment	Day 10	Day 20	Day 30
1	Normal Control (NC).	469.33±0.67^{ab}	471.67±3.33^{abc}	475.00±14.3^{ac}
	CCl_4 treated only.			
2		761.33±101.67^{abc}	673.67±74.33^{abc}	641.67±58.33^{bc}
	CCl_4 + 100mg/kg			
3	extract.	396.00±78.00^{b}	381.33±9.33^{b}	405.33±16.67^{b}
	CCl_4 + 200mg/kg			
4	extract.	361.33±1.33^{b}	305.33±47.33^{b}	326.33±33.33^{b}
	CCl_4 + 300mg/kg			
5	extract.	391.33±34.38^{b}	245.00±3.00^{ab}	203.33±27.33^{ab}
	CCl_4 + 5.2mg/kg			
6	Livolin.	413.33±23.33^{bc}	229.33±6.33^{abc}	282.00±59.00^{abc}
	CCl_4 + 200mg/kg			
7	extract+5.2mg/kg			
	Livolin+ 50mg/kg Vit.C.	200.00±49.00^{ab}	203.33±27.33^{ab}	214.00±14.00^{ab}

Values are represented as Mean ± Standard error of mean; n =3 per group.
Values in the same column with common superscript letter (a, b, c) are significantly different at $P < 0.05$.
Superscript A (^{a}) represents significant difference when group 1 is compared to other groups at $P < 0.05$.
Superscript B (^{b}) represents significant difference when group 2 is compared to other groups at $P < 0.05$.
Superscript C (^{C}) represents significant difference when group 6 is compared to other groups at $P < 0.05$.
Values without superscript shown no significant difference when group 1, 2 and 6 are compared to other groups at $P < 0.05$.

Table 1.3: Result of the Effect of Methanol Extract 0f *Pleurotus ostreatus* on Albumin (Alb) levels (g/dl).

Groups	Treatment	Day 10	Day 20	Day 30
1	Normal Control (NC).	4.10 ± 0.10^{ab}	4.13 ± 0.03^{abc}	4.00 ± 0.12^{ab}
2	CCl_4 treated only.	2.70 ± 0.10^{abc}	2.77 ± 0.03^{abc}	2.80 ± 0.15^{abc}
3	CCl_4 + 100mg/kg extract.	3.73 ± 0.03^{ab}	3.53 ± 0.03^{abc}	3.17 ± 0.07^{ac}
4	CCl_4 + 200mg/kg extract.	3.87 ± 0.03^{b}	3.67 ± 0.03^{ab}	3.60 ± 0.10^{b}
5	CCl_4 + 300mg/kg extract.	3.97 ± 0.09^{b}	3.80 ± 0.00^{ab}	3.70 ± 0.00^{b}
6	CCl_4 + 5.2mg/kg Livolin.	3.83 ± 0.09^{bc}	3.77 ± 0.07^{abc}	3.70 ± 0.06^{bc}
7	CCl_4 + 200mg/kg extract+5.2mg/kg Livolin+ 50mg/kg Vitamin C.	3.97 ± 0.03^{b}	3.93 ± 0.03^{ab}	3.90 ± 0.10^{b}

Values are represented as Mean ± Standard error of mean; n =3 per group.
Values in the same column with common superscript letter (a, b, c) are significantly different at $P < 0.05$.
Superscript A (^{a}) represents significant difference when group 1 is compared to other groups at $P < 0.05$.
Superscript B (^{b}) represents significant difference when group 2 is compared to other groups at $P < 0.05$.
Superscript C (^{C}) represents significant difference when group 6 is compared to other groups at $P < 0.05$.
Values without superscript shown no significant difference when group 1, 2 and 6 are compared to other groups at $P < 0.05$.

Table 1.4: Result of the Effect of Methanol Extract 0f *Pleurotus ostreatus* on Total Protein (TPRO) levels (g/dl).

Groups	Treatment	Day 10	Day 20	Day 30
1	Normal Control (NC).	7.17 ± 0.17^{abc}	7.23 ± 0.19^{abc}	7.17 ± 0.12^{ab}
2	CCl_4 treated only.	5.07 ± 0.07^{abc}	5.00 ± 0.00^{abc}	5.07 ± 0.13^{abc}
3	CCl_4 + 100mg/kg extract.	5.47 ± 0.03^{ac}	5.67 ± 0.07^{abc}	5.60 ± 0.10^{abc}
4	CCl_4 + 200mg/kg extract.	6.27 ± 0.07^{ab}	6.37 ± 0.03^{ab}	6.53 ± 0.03^{ab}
5	CCl_4 + 300mg/kg extract.	6.50 ± 0.10^{ab}	6.63 ± 0.17^{ab}	6.70 ± 0.00^{ab}
6	CCl_4 + 5.2mg/kg Livolin.	6.43 ± 0.18^{abc}	6.73 ± 0.03^{abc}	6.77 ± 0.13^{bc}
7	CCl_4 + 200mg/kg extract+5.2mg/kg Livolin+ 50mg/kg Vitamin C.	6.90 ± 0.00^{b}	6.93 ± 0.07^{b}	7.00 ± 0.06^{b}

Values are represented as Mean ± Standard error of mean; n =3 per group.
Values in the same column with common superscript letter (a, b, c) are significantly different at $P < 0.05$.
Superscript A (^{a}) represents significant difference when group 1 is compared to other groups at $P < 0.05$.
Superscript B (^{b}) represents significant difference when group 2 is compared to other groups at $P < 0.05$.
Superscript C (^{C}) represents significant difference when group 6 is compared to other groups at $P < 0.05$.
Values without superscript shown no significant difference when group 1, 2 and 6 are compared to other groups at $P < 0.05$.

Table 1.5: Result of the Effect of Methanol Extract 0f *Pleurotus ostreatus* on Direct Bilirubin (DBIL) levels ($\mu mol/l$).

Groups	Treatment	Day10	Day20	Day30
1	Normal Control (NC).	$6.90\pm0.50_{ab}$	7.67 ± 0.33^{abc}	7.67 ± 0.17^{abc}
2	CCl_4 treated only.	8.83 ± 0.17^{abc}	8.70 ± 0.10^{abc}	8.93 ± 0.09^{abc}
3	CCl_4 + 100mg/kg extract.	6.20 ± 0.30^{b}	4.87 ± 0.12^{abc}	1.80 ± 0.30^{ab}
4	CCl_4 + 200mg/kg extract.	5.13 ± 0.03^{ab}	4.30 ± 0.20^{ab}	2.67 ± 0.07^{ab}
5	CCl_4 + 300mg/kg extract.	3.63 ± 0.23^{abc}	3.83 ± 0.03^{ab}	2.23 ± 0.27^{ab}
6	CCl_4 + 5.2mg/kg Livolin.	6.40 ± 0.30^{bc}	4.00 ± 0.06^{abc}	2.33 ± 0.12^{abc}
7	CCl_4 + 200mg/kg extract+5.2mg/kg Livolin+ 50mg/kg Vitamin C.	4.37 ± 0.32^{abc}	3.87 ± 0.07^{ab}	2.30 ± 0.00^{ab}

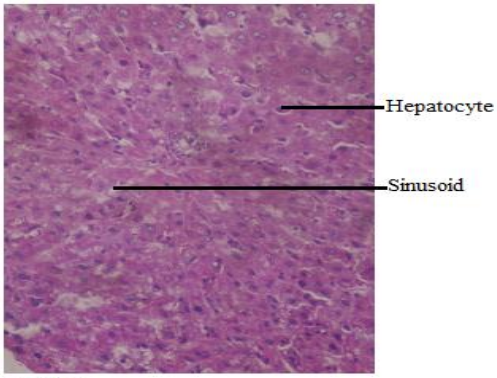
Values are represented as Mean ± Standard error of mean; n =3 per group.
Values in the same column with common superscript letter (a, b, c) are significantly different at $P < 0.05$.
Superscript A (^{a}) represents significant difference when group 1 is compared to other groups at $P < 0.05$.
Superscript B (^{b}) represents significant difference when group 2 is compared to other groups at $P < 0.05$.
Superscript C (^{C}) represents significant difference when group 6 is compared to other groups at $P < 0.05$.
Values without superscript shown no significant difference when group 1, 2 and 6 are compared to other groups at $P < 0.05$.

Table 1.6: Result of the Effect of Methanol Extract 0f *Pleurotus ostreatus* on Total Bilirubin (TBil)levels ($\mu mol/l$).

Groups	Treatment	Day10	Day20	Day30
1	Normal Control (NC).	14.50 ± 0.00^{abc}	15.00 ± 0.00^{abc}	14.00 ± 0.58^{abc}
2	CCl_4 treated only.	16.33 ± 0.33^{abc}	16.47 ± 0.17^{abc}	15.90 ± 0.21^{abc}
3	CCl_4 + 100mg/kg extract.	12.97 ± 0.37^{abc}	12.20 ± 0.00^{abc}	9.00 ± 0.40^{abc}
4	CCl_4 + 200mg/kg extract.	10.83 ± 0.23^{ab}	12.03 ± 0.03^{abc}	7.90 ± 0.30^{ab}
5	CCl_4 + 300mg/kg extract.	10.63 ± 0.23^{ab}	11.23 ± 0.13^{abc}	7.90 ± 0.50^{ab}
6	CCl_4 + 5.2mg/kg Livolin.	11.00 ± 0.60^{abc}	9.93 ± 0.13^{abc}	7.03 ± 0.27^{abc}
7	CCl_4 + 200mg/kg extract+5.2mg/kg Livolin+ 50mg/kg Vitamin C.	11.17 ± 0.27^{ab}	9.07 ± 0.37^{abc}	7.70 ± 0.30^{ab}

Values are represented as Mean $\pm$ Standard error of mean; n =3 per group.
Values in the same column with common superscript letter (a, b, c) are significantly different at $P < 0.05$.
Superscript A (^{a}) represents significant difference when group 1 is compared to other groups at $P < 0.05$.
Superscript B (^{b}) represents significant difference when group 2 is compared to other groups at $P < 0.05$.
Superscript C (^{C}) represents significant difference when group 6 is compared to other groups at $P < 0.05$.
Values without superscript shown no significant difference when group 1, 2 and 6 are compared to other groups at $P < 0.05$.

LIVER HISTOPATHOLOGY

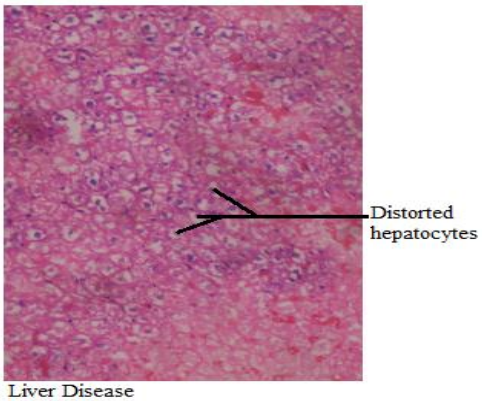


Control

(H×E 400)

RESULT OF THE EFFECT OF METHANOL EXTRACT OF *Pleurotus osreatus* ON HEPATIC HISTOPATHOLOGY ON DAY 10 FOR GROUP 1(CONTROL).

The section of the liver shows normal hepatocytes and sinusoids.

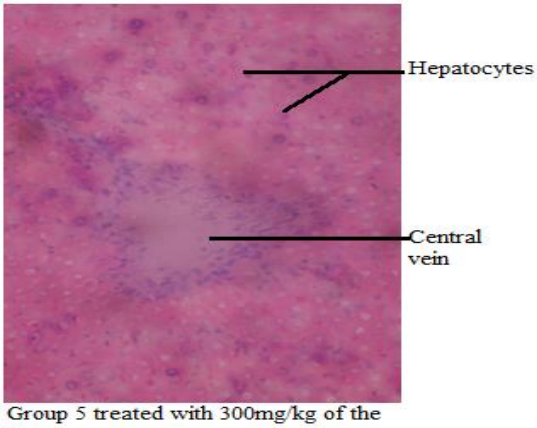


Liver Disease

(H×E 400)

RESULT OF THE EFFECT OF METHANOL EXTRACT OF *Pleurotus osreatus* ON HEPATIC HISTOPATHOLOGY ON DAY 10 FOR GROUP 2 (LIVER DISEASE).

The section of the liver shows distorted hepatocytes after the administration of CCl_4 to the experimental rats.

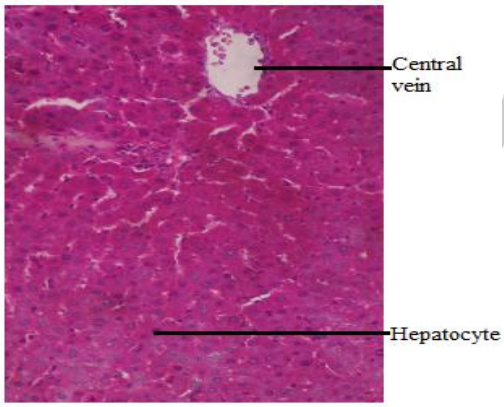


Group 5 treated with 300mg/kg of the Extract

(H×E 400)

RESULT OF THE EFFECT OF METHANOL EXTRACT OF *Pleurotus osreatus* ON HEPATIC HISTOPATHOLOGY ON DAY 10 FOR GROUP 5.

The section of the liver shows regeneration of hepatocytes.

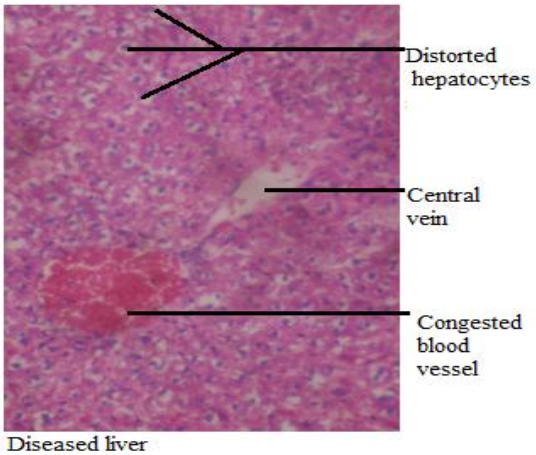


Control

(H×E 400)

RESULT OF THE EFFECT OF METHANOL EXTRACT OF *Pleurotus osreatus* ON HEPATIC HISTOPATHOLOGY ON DAY 20 FOR GROUP 1 (CONTROL).

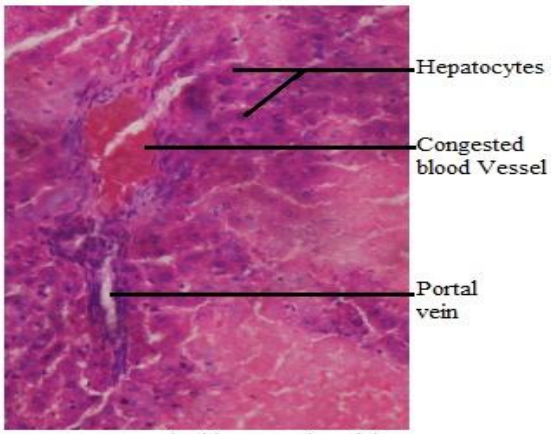
The section of the liver shows normal hepatocytes and central vein.



Diseased liver

(H×E 400)

RESULT OF THE EFFECT OF METHANOL EXTRACT OF *Pleurotus osreatus* ON HEPATIC HISTOPATHOLOGY ON DAY 20 FOR GROUP 2 (LIVER DISEASE).

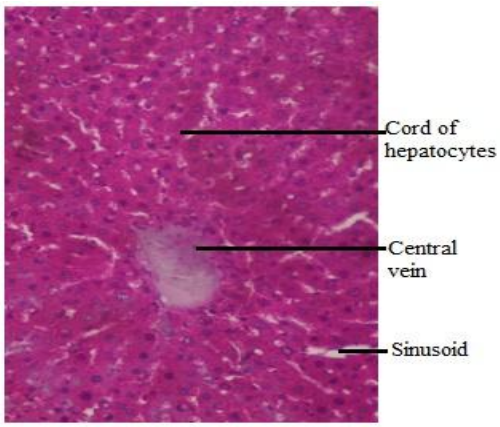


Group 5 Treated with 300mg/kg of the Extract

(H×E 400)

RESULT OF THE EFFECT OF METHANOL EXTRACT OF *Pleurotus osreatus* ON HEPATIC HISTOPATHOLOGY ON DAY 20 FOR GROUP 5.

The section of the liver shows regeneration of hepatocytes.

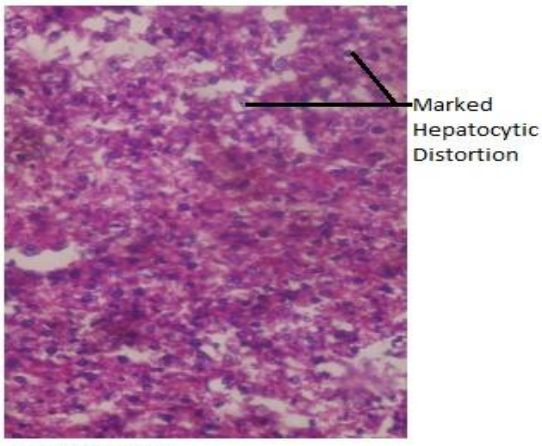


Control

(H×E 400)

RESULT OFTHE EFFECT OF METHANOL EXTRACT OF *Pleurotus osreatus* ON HEPATIC HISTOPATHOLOGY ON DAY 30 FOR GROUP 1 (CONTROL).

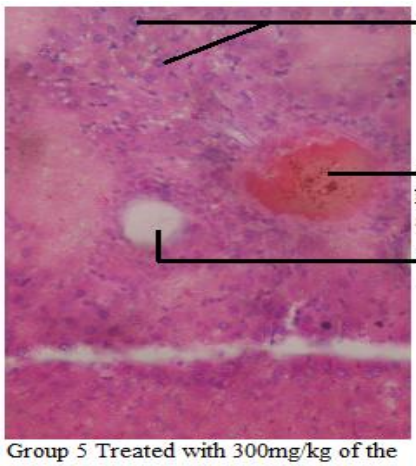
The section of the liver shows normal hepatocytes and central vein.



Diseased Liver

(H×E 400)

RESULT OF THE EFFECT OF METHANOL EXTRACT OF *Pleurotus osreatus* ON HEPATIC HISTOPATHOLOGY ON DAY 30 FOR GROUP 2 (LIVER DISEASE).



Group 5 Treated with 300mg/kg of the Extract

(H×E 400)

RESULT OF THE EFFECT OF METHANOL EXTRACT OF *Pleurotus osreatus* ON HEPATIC HISTOPATHOLOGY ON DAY 30 FOR GROUP 5.

The section of the liver shows regeneration of hepatocytes.

DISCUSSION

The effect of methanol fruiting body extract of *Pleurotus ostreatus* cultivated with crude extract of red bulb *Allium cepa on* liver function test of carbon tetrachloride induced hepatotoxicity in wistar rats were investigated.

Onions are common kitchen spices which posses so many health benefits; this vegetable increases the health values of many foods when added to them (Khiari *et al.,* 2009).The cultivation of *Pleurotus ostreatus with Allium cepa* extract shows a fast growth of the mycelium when compared to the growth of the mycelium without *Allium cepa* extract.

Oxidative stress has been shown to be involved as primary factor in progression of many degenerative ailments like cancer, diabetes type 2, atherosclerosis, cataracts, liver disease, neurodegenerative disorders, etc. (Jayakumar *et al.,* 2006) and it occurs when there is an over production of reactive species. These reactive species if not deactivated, their chemical reaction can cause injury to macromolecules in cells like proteins, carbohydrates, lipids, and nucleic acids. Too many quantities of ROS are dangerous for they can cause oxidation of biomolecular substances that can result to cell damage and death, or form oxidative damage that result in various ailments and disorders (Halliwell and Gutteride, 2000).

Carbon Tetrachloride has been established to cause hepatotoxicity in both man and experimental subjects (Adewale *et al.,* 2013). Administration of CCl_4 to experimental rats induced chronic liver injury that results to fibrosis, scar production and damage of normal tissue architecture (Chaudhary *et al.,* 2010).

Medicinal plants owe their therapeutic features as a result of the presence of various phytochemical substances in their leaves, stems, barks, roots, and fruits (Sofowora, 2008). Fruiting body extract of *Pleurotus ostreatus* has been recognised to be possible source of antioxidants and have the ability to highly stop lipid peroxidation (Chaudhary *et al.,* 2010). The occurrence of compounds like phenolic compounds, and flavonoid are responsible for the fruiting bodies of *Pleurotus ostreatus* protection due to their antioxidant features (Liu, 2004; Gupta *et al.,* 2011) that scavenge reactive oxygen species.

The result of Table 1.0, 1.1 and 1.2 on day 10 shows that there was a high increase in the activities of AST, ALT and ALP in group 2 compared to group 1. Groups treated with the dosage of methanol extract of *Pleurotus ostreatus* showed a significant reduction at P<0.05 in the activities of AST, ALT and ALP when compared to group 2. This explanation was also applicable to the activities of AST, ALT and ALP on day 20 and 30.

The result of Table 1.3 and 1.4 on day 10 shows that there was a significant reduction in the levels of ALB and TPRO in group 2 compared to group 1. Groups treated with the dosage of methanol extract of *Pleurotus ostreatus* showed a significant increase at P<0.05 in the levels of ALB and TPRO when compared to group 2. This explanation was also applicable to the levels of ALB and TPRO on day 20 and 30.

The result of Table 1.3 1.5, and 1.6 on day 10 shows a significant increase at P<0.05 in the levels of DBIL, TBIL and TBARS in group 2 when compared to group 1. Groups treated with the dosage of methanol extract of *Pleurotus ostreatus* showed a significant decrease at P<0.05 in the levels of DBIL, TBIL and ALB when compared to group 2. This explanation was also applicable to the levels of DBIL, TBIL and ALB on day 20 and 30.

This improvement to near normal level was an indication of stabilization of plasma membrane as well as repair of hepatic parenchyma. The histopathology study provides direct evidence of the possibility of the extract to be able to reduce disruption of the hepatocytes structure and accelerates hepatic regeneration.

CONCLUSION

From this research, it has been established that methanol fruiting body extract of *Pleurotus ostreatus* cultivated with red bulb *Allium cepa* showed the effectiveness in the treatment of Liver disease due to its protective effect against CCl_4 induced hepatotoxicity.

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