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Histopathological Effects in Albino Rats Fed with Pleurotus ostreatus Cultivated on Composted Wood Dust Substrates

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Abstract
.This study evaluated the histopathological effects of feeding albino rats with Pleurotus ostreatus cultivated on composted wood dust of Pycnanthus angolensis and Spondias mombin mixed with various animal dungs. Experimental rats were fed with mushroom-supplemented diets for twenty-one days, after which histopathological analyses were conducted on their liver, kidney, and intestine. The results revealed that most organs displayed normal architecture, with no signs of necrosis, haemorrhage, or inflammation. Mild histological alterations such as inflammatory cell infiltration and hepatocellular karyolysis were observed in some treatments, particularly those involving poultry droppings and sheep dung substrates. Rats fed with mushrooms from composted substrates showed significantly higher weight gain compared to controls, with the highest gain (13.67 g) recorded in the group fed with S. mombin and cow dung substrate. These findings indicate that P. ostreatus cultivated on composted wood dust substrates is non-toxic and nutritionally beneficial to experimental animals, suggesting its safety for human consumption.

Keywords: Albino rats, Biosafety assessment, Composted substrates, Histopathology, Pleurotus ostreatus

Introduction
Edible mushrooms are widely recognized for their nutritional and medicinal value, containing high-quality proteins, vitamins, minerals, and bioactive compounds (Chang & Wasser, 2017). Among these, Pleurotus ostreatus (oyster mushroom) is particularly notable for its adaptability to various lignocellulosic substrates and its potential role in bioconversion of agricultural wastes (Adebayo et al., 2014). However, substrate composition can influence both nutritional quality and possible bioaccumulation of toxic elements (Jonathan et al., 2012). The safety assessment of edible mushrooms cultivated on unconventional substrates such as composted wood dust and animal dungs is crucial to ascertain their biosafety for consumption. Histopathological examination of experimental animals provides a reliable method for evaluating potential toxicological effects of such dietary components (Sasidharan et al., 2008). This study, therefore, investigates the histopathological effects of feeding albino rats with P. ostreatus grown on composted wood dust of Pycnanthus angolensis and Spondias mombin supplemented with different animal dungs. The aim was to determine whether these substrates affect organ morphology or general health, thereby assessing the biosafety of mushrooms cultivated on such substrates.

Methods and Materials
The body weights of the experimental rats were determined following the procedure outlined by Buchweit, Frølund, and Pedersen (2012). Prior to the feeding trials, all animals were allowed to acclimatize to their new environment for one week under standard laboratory conditions. Each rat was weighed individually at the beginning of the experiment to obtain its initial weight. Subsequent measurements were taken daily throughout the feeding period using a precision digital electronic balance (Gilbertini, Italy). The daily weight gain or loss for each animal was determined by subtracting the initial body weight from the corresponding final body weight recorded on each day. This approach provided an accurate measure of the growth performance and physiological response of the rats to the various mushroom-supplemented diets.

Histopathology results of biosafety
The result shown in Plates 1-6 was the histopathology of rats fed with cultivated *Pleurotus ostreatus.* The results of most of the rats showed no form of haemorrhage, necrosis, infiltration or inflammation of the organs examined both of the ones fed with mushroom and the control. However, various pathological changes were observed in some that ranged from the washed submucosa of the intestinal villi to inflammatory cell infiltration of kidney

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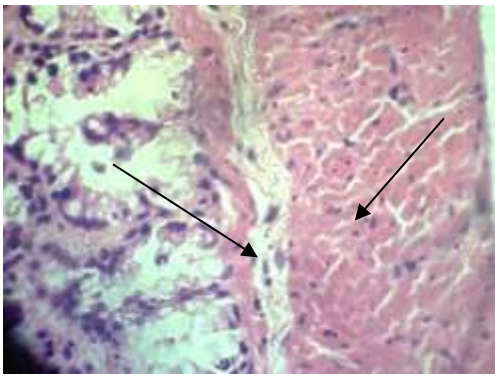
nephrones as well as liver hepatocytes with aggregation of lymphatic cells and keryolysis. These histopathologically analysed organs from the biosafety analyses are shown in Plates 1-6. The intestines of albino rats fed with mushroom were normal. Rats fed with mushroom cultivated on composted wood dust of *Pycnanthus angolensis* and cow dung with intestine histology showing normal wall, but had inflammatory cell infiltration. There was no necrosis. Rats fed with mushroom cultivated on composted wood dust of *Spondias mombin* and cow dung showed normal intestine with proper blood channel/vesels having infiltration of cells. Intestine showing diffused goblet cells of the intestinal villi. No necrosis, haemorrhagen or inflammation.

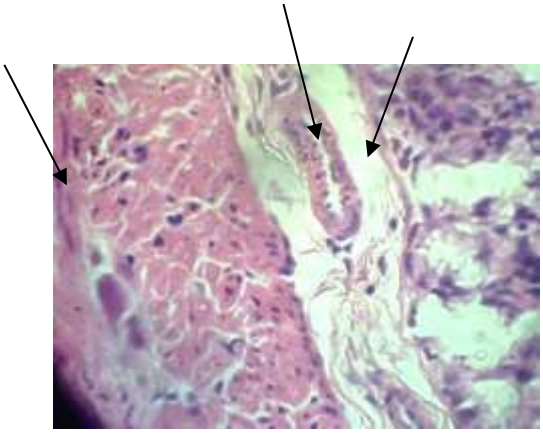
The kidneys generally were well-formed and showed no presence of or visible decrimination of toxic protein and amino acids. Kidney nephrons with distinct Bowman's capsules with central infiltration of cells. However, kidneys of rats fed with mushroom cultivated on composted wood dust of *Spondias mombin* and sheep dung was poorly formed; kidney nephrons were without distinct Bowman's capsules and presence of inflammatory cell infiltrations. There was thickening of glomerular basement. The visible signs shown in these kidneys nephrones are pointer to nephritis/cellulitis, which may be traceable to the diets of the animals. The liver hepatocytes were generally well-formed in albino rats that were fed with mushroom cultivated on varying substrates. However, washed liver hepatocytes with keryolysis which is a sign of aplasia usually due to blood surrounding the organ was observed in rats fed with mushroom cultivated on composted wood dust of *Pycnanthus angolensis* and poultry droppings.

Results

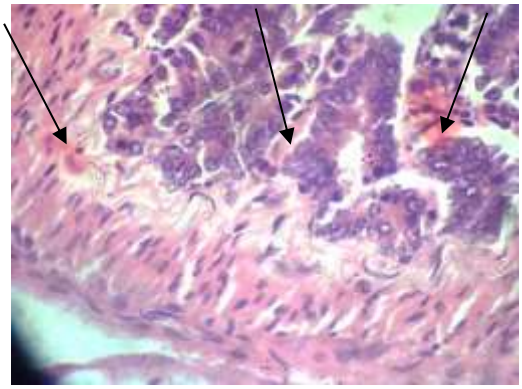
The results shown in Tables 1 are the weights of rats fed with *Pleurotus ostreatus*. The results of the weight analyses showed that mushroom from composting increased the weight of the rats as feeding progressed. Rat fed with mushroom supplemented feed from compost substrate gained more weight than the control rats. Rats fed with mushroom supplemented feed from composted *S. mombin* and cow dung substrate had a highest weight gain of 13.67g in 21days.



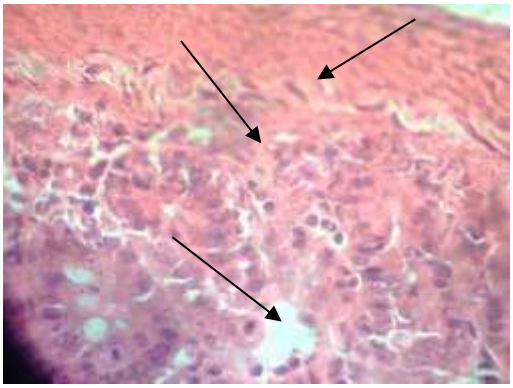
a. normal wall, but has inflammatory cell infiltration. No necrosis



b. inflammatory cell infiltration, without prominent finger-like structure of the villi



c. dot necrosis, profuse inflammatory cell infiltration and haemorrhage



d. washed submucosa, no distinct intestinal villi with cellular infiltration of lymphocyte tissues

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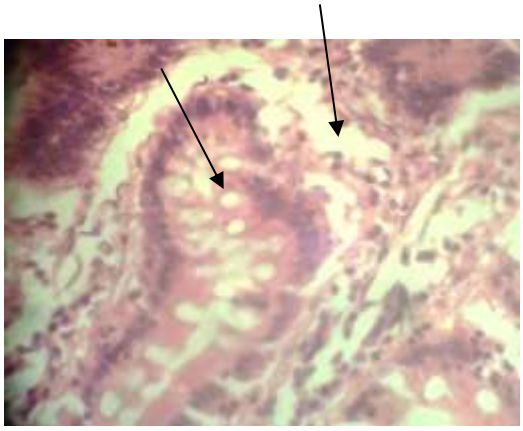
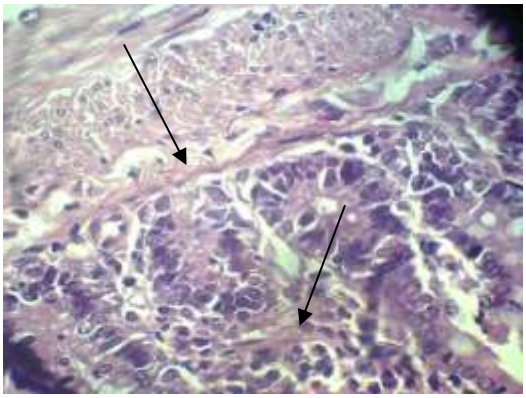


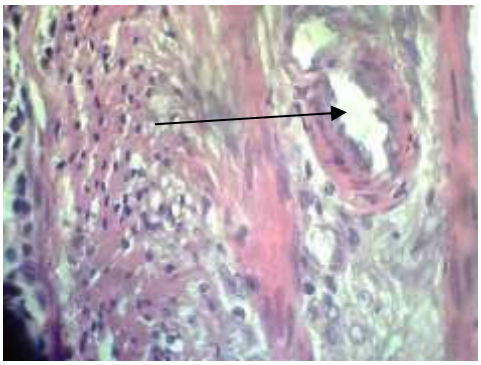
e. Normal intestine but with inflammatory cell infiltrations surrounding goblet cells.



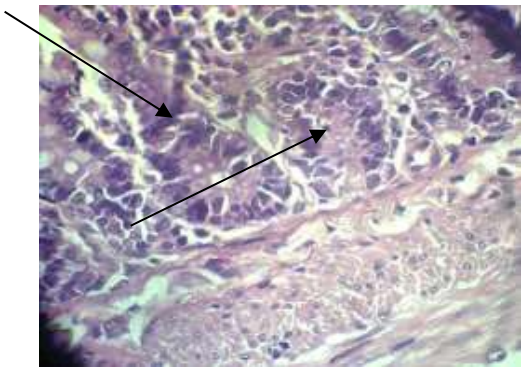
f. Normal intestine villi with distinct submucosa but having infiltration of cells.

Plates 1a-f: Histopathology of the intestine of albino rats fed with *P. ostreatus.* from Composted wood dust of *P.angolensis*

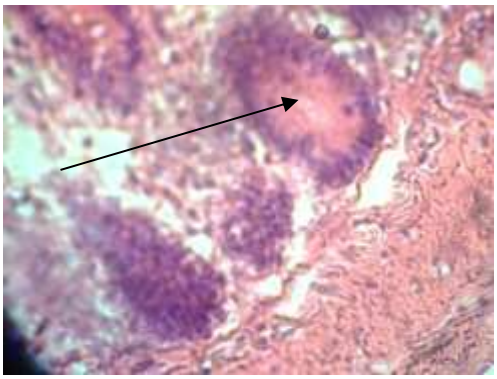
Key: a- cow dung, b- goat dung, c- poultry dropping, d- sheep dung, e-wood dust 100% f-supplemented diet(control)



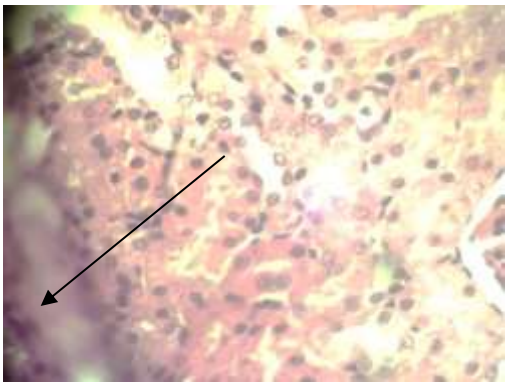
a. Normal intestine with proper blood channel/vesels having infiltration of cells.



b. Normal intestine villi with distinct submucosa but having infiltration of cells.



c. Diffused goblet cells of the intestinal villi. No necrosis, haemorrhage or inflammatory wall.



d. Intestine eroded infiltration of cells without partial destruction of the villi.

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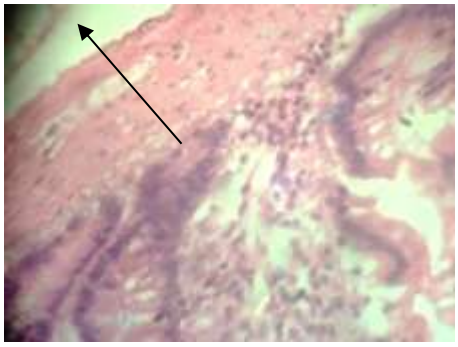
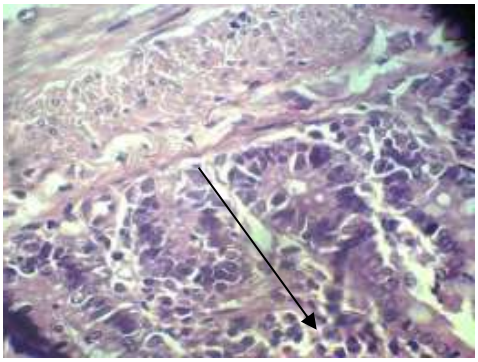


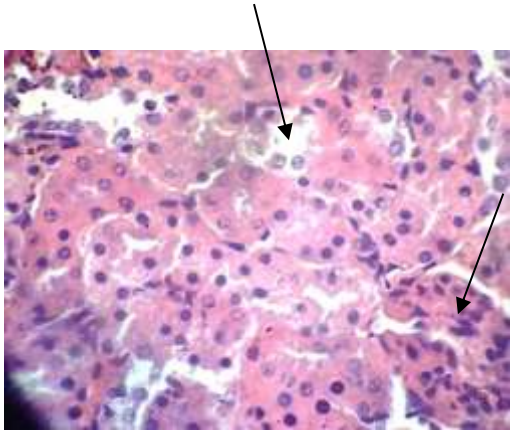
e. Normal intestine with washed surface and infiltrations of cells.



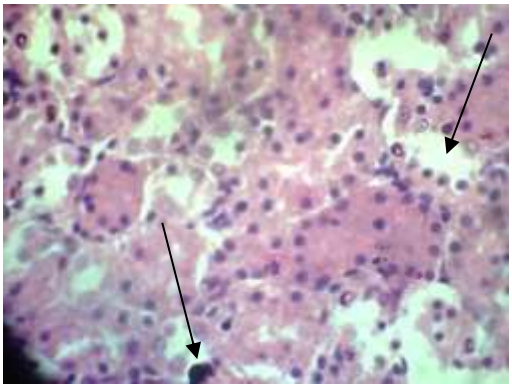
f. Normal intestine villi with distinct submucosa but having infiltration of cells.

Plates 2a-f: Histopathology of the intestine of albino rats fed with *P. ostreatus* from composted wood dust of *Spondias mombin*

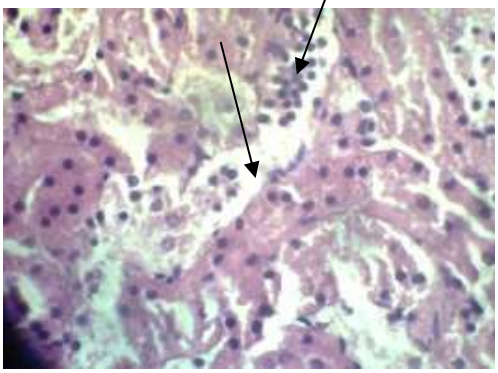
Key: - , Key: a- cow dung, b- goat dung, c- poultry dropping, d- sheep dung, e-wood dust 100% f-supplemented diet(control)



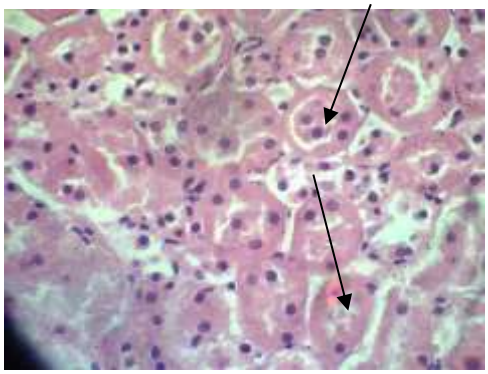
a. Normal kidney with distinct Bowman's capsules with inflammatory cell infiltration.



b. Normal kidney nephrons/Bowman's capsule with infiltration of cells.



c. Well-formed kidney nephrons with distinct Bowman's capsules with central infiltration of cells.



d. Well-formed Bowman's capsules with moderate mononuclear cell infiltrations.

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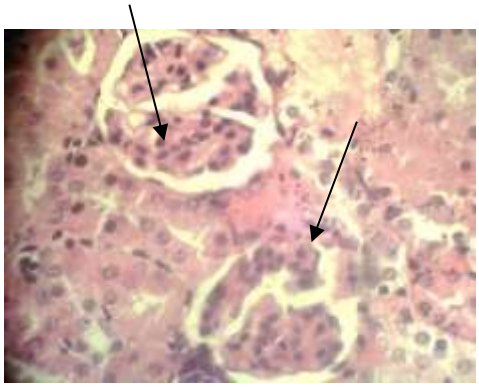
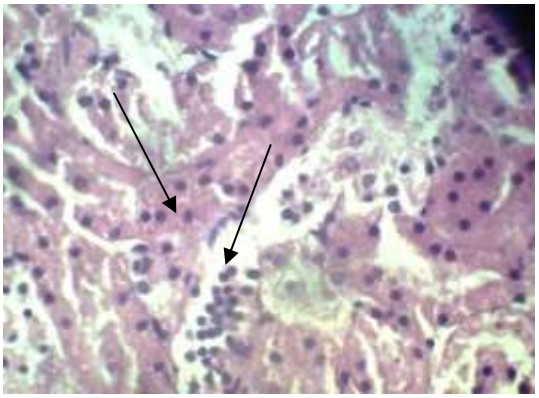


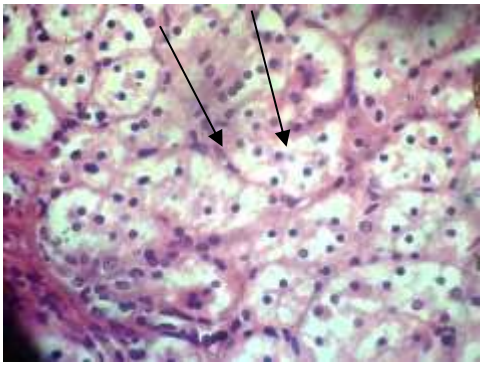
e. Normal kidney with intact Bowman's capsules having infiltration of cells.



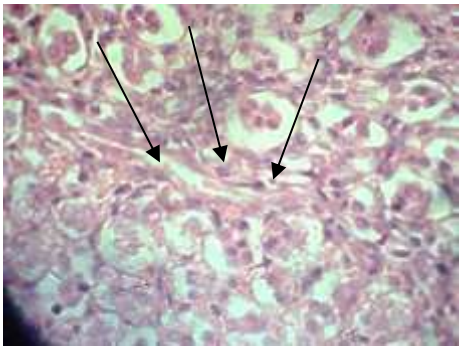
f. Well-formed kidney nephrons with distinct Bowman's capsules with central infiltration of cells.

Plates 3a-f: Histopathology of the kidney of albino rats fed with *P. ostreatus* from Composted wood dust of *P. angolensis*

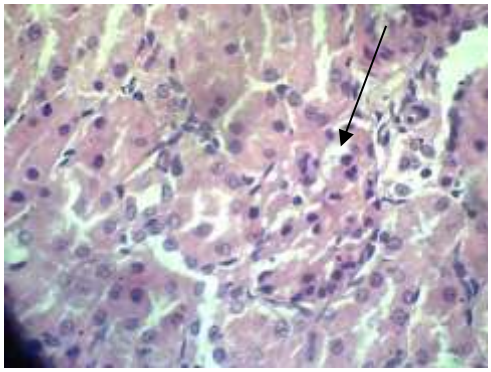
Key: a- cow dung, b- goat dung, c- poultry dropping, d- sheep dung, e-wood dust 100% f-supplemented diet(control,



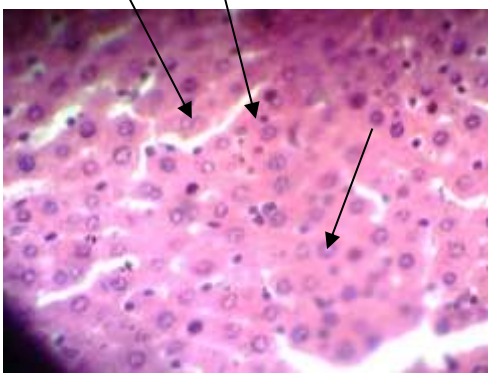
a. Well-formed Bowman's capsules with high mononuclear cell infiltrations.



b. Kidney Bowman's capsules re washed and have mass deposit of cast cells surrounding them.



c. Well-formed Bowman's capsules of kidney nephrons but with inflammatory cell infiltrations.



d. Poorly formed kidney nephrons without distinct Bowman's capsules and presence of inflammatory cell infiltrations. There is thickening of glomerular basement.

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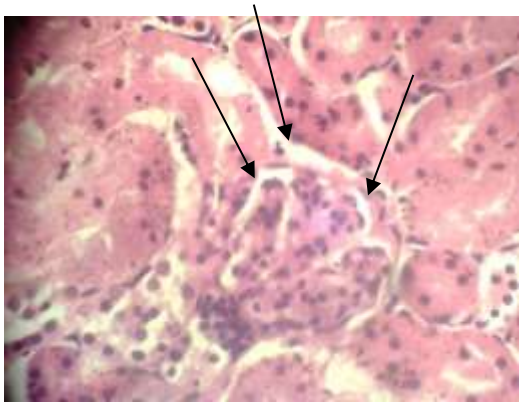
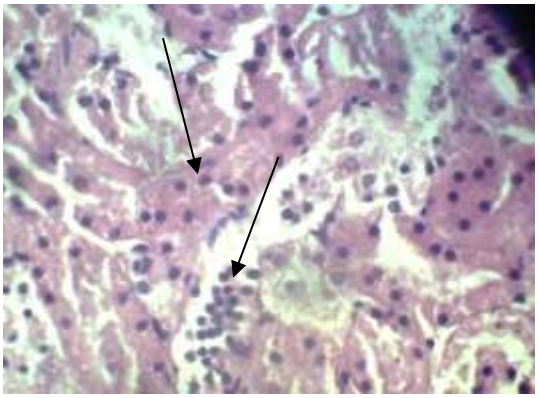


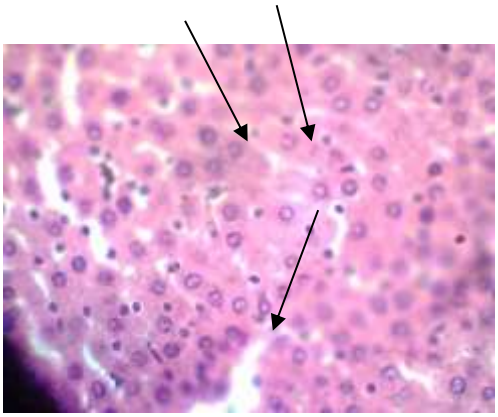
e. Normal kidney with intact Bowman's capsules, infiltration of cells without haemorrhage.



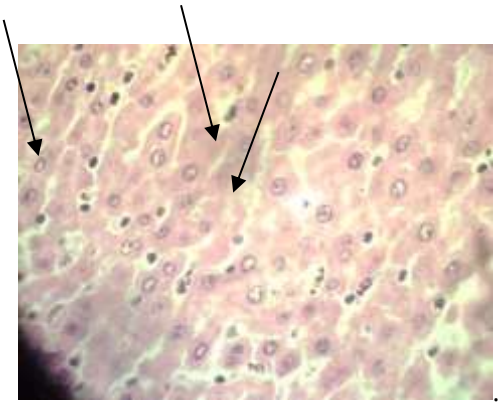
f. Well-formed kidney nephrons with distinct Bowman's capsules with central infiltration of cells.

Plates 4a-f: Histopathology of the kidney of albino rats fed with *P. ostreatus* from composted wood dust of *Spondias mombin*

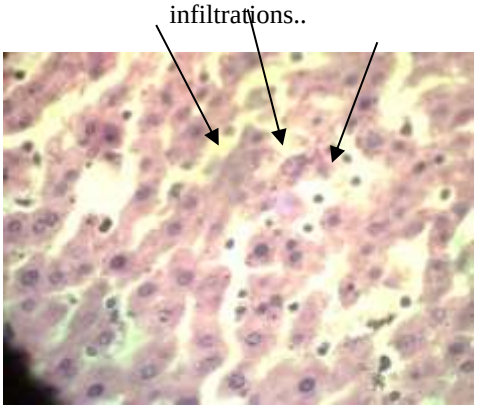
Key: a- cow dung, b- goat dung, c- poultry dropping, d- sheep dung, e-wood dust 100% f-supplemented diet(control)



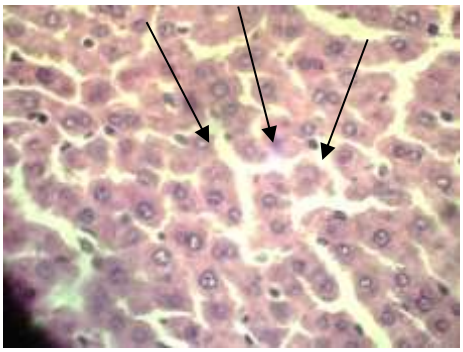
a. Normal liver hepatocytes but with hepatocellular keryolysis at extreme edge and inflammatory cell infiltrations..



b. Normal liver with intact hepatocyte and sinusoids.



c. Washed liver hepatocytes with keryolysis and inflammatory cell infiltrations.



d. Liver hepatocytes are intact, but have keryolysis and disappearing sinusoids. There are inflammatory cells infiltrations.

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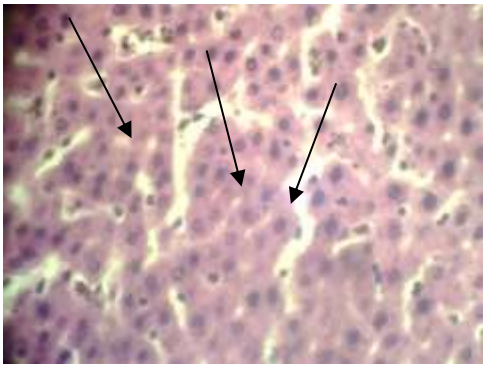
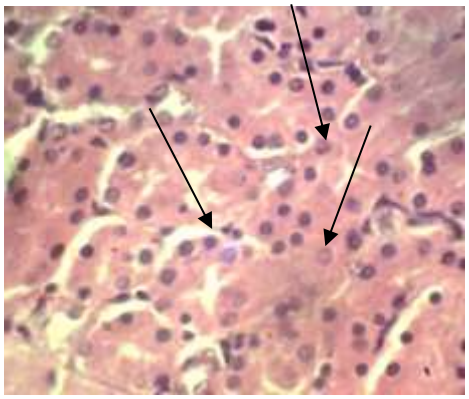


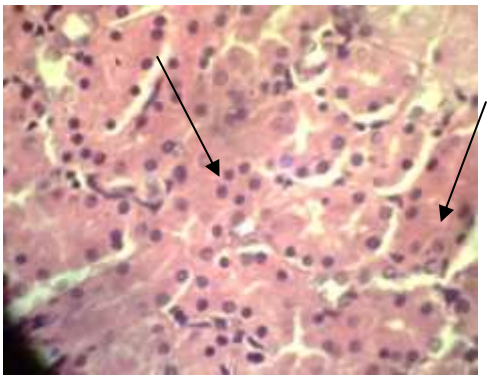
e. Well-formed liver hepatocytes but has cell infiltration and keryolsis.



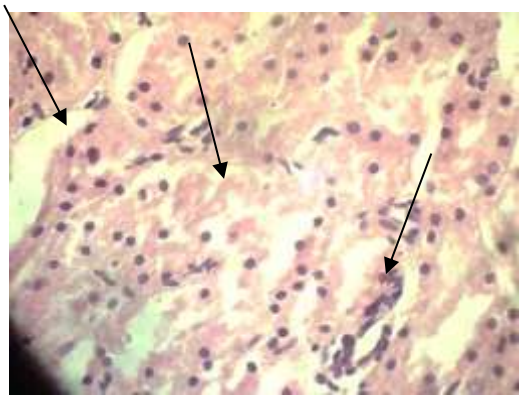
f. Liver hepatocytes are formed but washed. There is no keryolysis, while there are infiltrations of cell.

Plates 5a-f: Histopathology of the liver of albino rats fed with *P. ostreatus* from Composted wood dust of *P. angolensis*

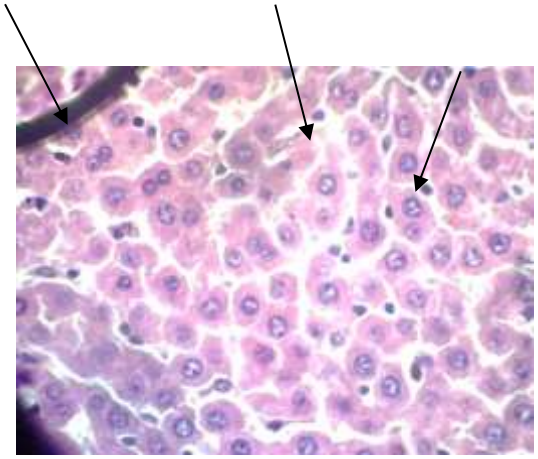
Key: a- cow dung, b- goat dung, c- poultry dropping, d- sheep dung, e-wood dust 100% f-supplemented diet(control)



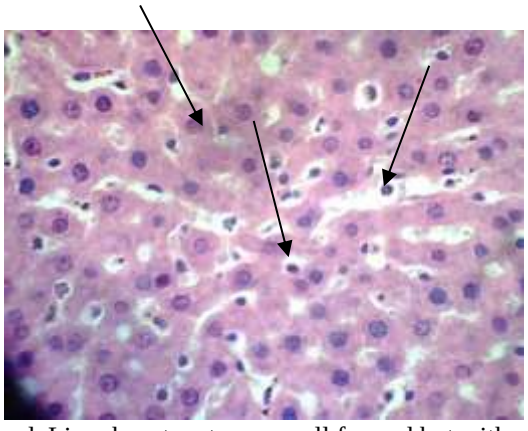
a. Liver hepatocytes are formed but washed. There is no keryolysis, while there are infiltrations of cell.



b. Normal liver with cell infiltrations and slight karyolsis.



c. Well-formed liver hepatocytes but with disappearing sinusoids. There is little keryolysis between hepatocytes which are however not prominent.



d. Liver hepatocytes are well formed but with disappearing sinusoids and presence of inflammatory cell infiltrations.

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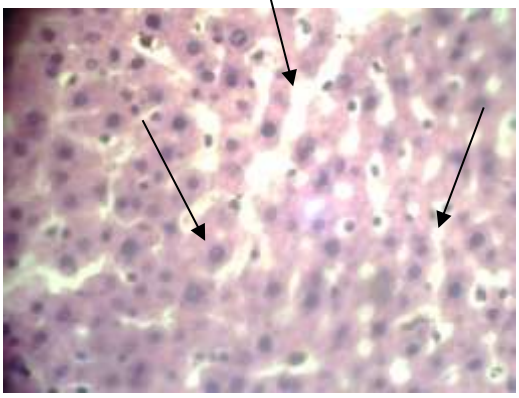
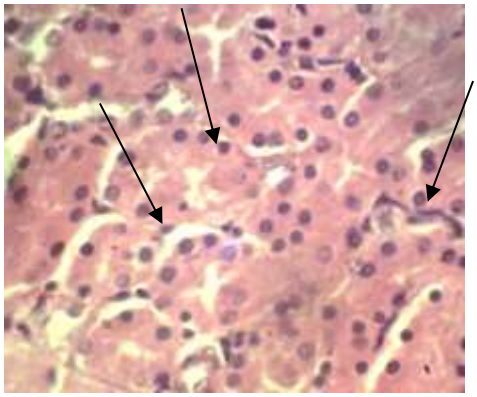


e. Liver hepatocytes are well formed, has cell infiltration with little central hepatocellular drainage.



f. Liver hepatocytes are formed but washed. There is no keryolysis, while there are infiltrations of cell.

Plates 6a-f: Histopathology of the liver of albino rats fed with *P. ostreatus* from composted wood dust of *Spondias mombin*

Key: a- cow dung, b- goat dung, c- poultry dropping, d- sheep dung, e-wood dust 100% f-supplemented diet(control)

Table 41: Weight of Albino Rats fed with *Pleurotus ostreatus* from composting for biosafety analysis
Values followed by the same letter in a row are not significantly different at P≤0.05.

Substrates	Days 0	Days 3	Days 6	Days 9	Days 12	Days 15	Days 18	Day 21
P. angolensis and Cow dung	51.33±0.58^{a}	51.00±0.00^{a}	53.67±0.58^{a}	54.33±0.58^{a}	56.67±0.58^{a}	58.33±1.15^{a}	60.33±1.15^{a}	62.00±0.00^{a}
P. angolensis and Goat dung	45.67±0.58^{b}	44.67±0.58^{e}	45.33±0.58^{b}	47.00±0.00^{b}	49.67±1.15^{b}	51.67±1.15^{b}	53.33±0.58^{b}	55.00±0.00^{b}
P. angolensis and Sheep dung	60.67±1.15^{c}	59.67±1.15^{b}	61.33±0.58^{b}	63.00±1.00^{c}	64.33±0.58^{b}	66.00±0.00^{b}	68.67±0.58^{c}	70.33±1.15^{bc}
P. angolensis and poultry droppings	49.00±0.00^{cd}	48.67±0.58^{d}	49.00±0.00^{c}	51.00±0.00^{d}	53.33±0.58^{b}	55.33±0.58^{c}	57.67±0.58^{de}	59.33±0.58^{cd}
S. mombin and Cow dung	56.33±1.15^{c}	56.00±1.00^{cd}	58.00±0.00^{d}	60.33±1.15^{e}	63.00±0.00^{cd}	65.67±1.15^{c}	68.67±1.15^{f}	70.67±0.58^{e}
S. mombin and Goat dung	52.00±0.00^{f}	52.33±1.00^{e}	54.33±1.00^{e}	55.67±1.00^{f}	57.33±0.58^{e}	59.00±0.00^{d}	61.33±0.58^{g}	63.67±0.58^{f}
S.mombin and Sheep dung	60.00±0.00^{fg}	59.33±0.58^{f}	61.33±0.58^{fg}	63.67±0.58^{g}	64.67±0.15^{f}	66.00±0.00^{e}	68.00±1.00^{g}	69.33±0.15^{f}
S. mombin and poultry droppings	51.33±1.15^{f}	52.00±0.00^{g}	54.00±0.00^{g}	55.33±1.15^{h}	57.00±0.00^{f}	58.33±1.15^{fg}	60.00±0.00^{h}	61.67±1.15^{g}
(100% *P. angolensis)*	48.67±0.58^{h}	47.33±0.58^{h}	48.67±1.15^{f}	50.00±0.00^{h}	51.67±1.15^{g}	53.33±1.15^{g}	55.00±1.00^{i}	56.33±0.58^{h}
(100% *S. mombin)*	49.00±0.00^{i}	48.33±0.58^{f}	49.00±0.00^{h}	50.33±0.58^{i}	52.00±0.00^{gh}	53.67±0.58^{h}	55.33±0.58^{i}	56.67±1.15^{h}
Control	53.33±1.15^{e}	53.00±1.00^{d}	55.33±1.15^{e}	56.00±0.00^{e}	57.67±1.15^{e}	58.33±0.58^{e}	59.00±1.00^{e}	60.33±0.58^{d}

Discussion

The architectural preservation of the intestinal sections across the majority of the experimental groups suggests that *Pleurotus ostreatus* cultivated on composted lignocellulosic waste does not possess acute enterotoxic properties. The structural continuity of the mucosal layer and the retention of villi integrity indicate that the fungal metabolites present in the diet did not trigger cytotoxic effects on the enterocytes.

The focal inflammatory cell infiltration observed in groups supplemented with poultry and sheep dung substrates requires a nuanced immunological interpretation. Rather than representing pathological injury, such mononuclear infiltration often reflects an adaptive immune response or "immunological surveillance" in the gut-associated lymphoid tissue (GALT). Mushrooms such as *P. ostreatus* are rich in β-glucans and other immunomodulatory polysaccharides that serve as ligands for pattern recognition receptors on immune cells, potentially stimulating mucosal immunity (Schwartz, 2017; Effiong et al., 2024). Furthermore, the presence of diffused goblet cells in

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certain samples points toward an enhanced protective mechanism. Goblet cells are responsible for secreting mucins that form a physical barrier against pathogens; their proliferation is a known physiological response to the high dietary fiber and polysaccharide content characteristic of the *Pleurotus* genus (Ghosh et al., 2021; Hou et al., 2023). The observed integrity of the Bowman's capsules and the clear definition of the nephron units in most groups support the safety profile of the mushroom-based diets regarding renal filtration. However, the thickening of the glomerular basement membrane and signs of nephritis in specific groups notably those involving *S. mombin* and sheep dung suggest that the chemical composition of the growth substrate significantly influences the toxicological profile of the fruiting body. Mushrooms are efficient bio-accumulators of minerals and heavy metals from their growth media. Substrates enriched with animal manure may contain higher concentrations of nitrogenous compounds or trace metals which, if hyper-accumulated by the mycelium, could exert secondary osmotic stress on the renal parenchyma (Ab Rhaman et al., 2021; Malinowski et al., 2021). These minor histological shifts likely represent a compensatory metabolic response to the increased solute load processed by the kidneys during the 21-day trial.

The liver sections generally maintained a healthy coronal arrangement of hepatocytes, which is a hallmark of systemic biosafety. The localized hepatocellular karyolysis and "washed-out" appearance noted in the *P. angolensis* and poultry dropping group likely reflect a state of high metabolic demand rather than irreversible necrosis. In food toxicology, such changes can occur when the liver is actively processing diverse bioactive compounds or xenobiotics. Interestingly, while certain substrates induced mild stress, P. ostreatus is widely documented for its hepatoprotective qualities, often attributed to its ability to upregulate endogenous antioxidant enzymes like superoxide dismutase (SOD) (Ogbomida et al., 2018; Zapaśnik et al., 2025). The absence of widespread hemorrhage or necrotic lesions across all treatments confirms that the mushrooms produced from these composted wood dust substrates are largely biosafe and do not induce systemic hepatotoxicity.

The superior weight gain observed in rats fed mushroom-supplemented diets, particularly the 13.67 g gain in the *S. mombin* and cow dung group, underscores the high biological value of the bioconverted substrate. *P. ostreatus* effectively upgrades the nutritional profile of lignocellulosic waste by increasing the crude protein content and liberating essential amino acids (Effiong et al., 2024; Olagunju et al., 2023). The significant growth compared to the control group indicates that the mushrooms provided a highly digestible source of protein and micronutrients, facilitating efficient nutrient Partitioning and metabolic growth in the experimental models (Deboleto et al., 2024).

Conclusion

The findings of this study confirm that *P. ostreatus* successfully bioconverts lignocellulosic wood waste, particularly when enriched with animal dungs, into a nutritionally dense food source. The significant weight gain observed in the experimental rats validates the high biological value and digestibility of the fungal proteins and polysaccharides produced through this composting process. From a microbiological and toxicological standpoint, the general preservation of the cellular architecture in the liver, kidneys, and intestines across most treatment groups demonstrates that these mushrooms do not harbor acute systemic toxins.

While minor histological shifts such as focal mononuclear infiltration in the gut and mild renal glomerular thickening were noted in specific substrate combinations (notably those involving poultry and sheep dungs), these do not represent clinical pathology. Instead, they likely reflect a localized immunomodulatory response to the mushroom's bioactive β-glucans or a metabolic adaptation to the specific mineral profile of the growth medium. In conclusion, the cultivation of *P. ostreatus* on composted *P. angolensis* and *S. mombin* wood dust is a viable and safe biotechnological approach to food production. This method not only offers a solution for agricultural waste management but also provides a biosafe, growth-promoting protein source suitable for animal and potentially human consumption. Future research should focus on the long-term (chronic) exposure effects and the specific heavy metal translocation patterns from different animal dungs to ensure absolute safety standards in commercial mushroom farming.

Recommendations

1. Future studies should include biochemical analyses to complement histopathological results and confirm the absence of toxic metabolites.
2. Nutritional profiling of mushrooms from various compost substrates should be conducted to identify optimal substrate combinations.
3. Scaling up production using composted wood dust and animal dung should be encouraged for sustainable mushroom farming.
4. Human consumption studies should be pursued to validate these findings in broader dietary contexts.

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