Therapeutic Properties of Some Nigerian Higher Fungi

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Abstract: The therapeutic effects of some higher fungi – *Fomes lignosus, Lentinus subnudus, Termitomyces robustus, Pleurotus tuber-regium, Pleurotus pulmonarius* and their blend (mixture extract) against some pathogenic bacteria infected in albino rats (*Rattus norvegicus*) were studied. The bacteria used include *E. coli*, *S. aureus, Salmonella typhi, P. aeruginosa, Streptococcus feacalis,* and *B. subtilis* suspension of 0.1mlcfu intraperitoneally administered 7 days pre-fungi extract administration. Survival rates of the experimental rats were discovered to increase significantly. *P. tuber-regium* had the best therapeutic effect against the test bacteria.The pathological changes observed on the liver and kidney tissues from histological studies were reversed while they reduced to mild conditions in some. Undoubtedly, extracts from the studied fungi have therapeutic abilities against the test organisms.

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1.0 Introduction

Mushrooms have been used as foods throughout the nations of the world for several decades. Many keep them as source of power especially the ancient priests and rulers (Chapela and Lizon, 1993). While many people use them as foods, few people use them as medicine (Aina *et al,*2012;Jonathan *et al*,2012).

Several workers have reported the antibiotic potentials of many macro fungi (Jonathan and Fasidi, 2003, 2005, Jonathan *et al.,* 2006, 2008; Olawuyi *et al.,* 2010). mushrooms comprise a vast and yet largely untapped sources of powerful new pharmaceutical products as they are rich in bioactive compounds that help in the modulation of metabolic process which result in the promotion of better health (Opige *et al.,* 2006).

Pleurotus species are among the most widely cultivated higher fungi in the whole world with high medicinal and nutritional value. *P. tuber-regium* is a common mushroom in Nigeria which is dark brown on the outside and white on the inside (Jonathan, 2002, Jonathan *et al.,* 2008). It produces sclerotium which is expensive and considered a delicacy used in vegetable soup. *P. pulmonarius* has been cultivated on various agricultural wastes (Mizuno and Zhuang, 1995; Bononi *et al.,* 1995) and has been discovered to be good antioxidant and antitumor.

Termitomyces robustus belongs to the family Lyophyiceae, living symbolic life with the termites of the family Macrotermitinae (Isoptera) (Rouland-Lefevre *et al.,* 2002). According to Mattila *et al.,* 2001, they are rich in minerals such as potassium, calcium, magnesium, iron, and manganese. They are also edible like the large species that are used as wild foods (Jonathan and Adeoye, 2011).

Lentinus subnudus belongs to the family polyporaceae, the largest group of aphyllophorales (Pegler, 1983). *Lentinus* species are known to grow naturally on some substrates and have also been successfully cultivated on some other ones (Morais *et al*., *2000*; Philoppousis *et al.,* 2001).*Fomes lignosus* is a member of polyporaceae which belongs to the order Aphyllophoraless. Like other species of the genus, they are characterised by their woody texture and minute pores.

Though many mushrooms have been studied for their medicinal properties, little or no attention has been given to some, especially the Nigerian higher fungi. This study therefore aimed at examining the therapeutic properties of some Nigerian higher fungi which can be utilised for drug production against various pathogenic diseases.

2.0 Materials and Methods

2.1 Collection of experimental materials

Higher fungi samples (*Fomes lignosus, Lentinus subnudus, Termitomyces robustus, Pleurotus tuber-regium* and *P. pulmonarius*) are obtained from different sources. They were cleaned, cut into small sizes and oven dried at 40^0C. Hot extraction of the samples was carried out using soxhlet apparatus. Equal weight of each of the higher fungi samples were mixed together for the mixture extract before extraction.

Albino rats (*Rattus norvegicus*) used for the study were about 12 weeks old with average weight of 132 grammes. The test bacteria (*E. coli, S. aureus,*

Salmonella typhi, Pseudomonas aeruginosa and *B. subtilis*) were homogenised in peptone water and incubated for 18-24 hours at 37^0C. They were intraperitoneally administered to the rats depending on their weights.

2.2 Therapeutic Experiment

Microbial suspension of 0.1mL/cfu was administered to each animal. This was repeated thrice at interval of 2 days. One millilitre (1mL) of the higher fungi extract was administered orally to the animal 7 days after infection. This was done daily for a week (7 days). The treatment was monitored for 30 days. The survival rates of animals were based on the number of experimental rats that survived the infection. Dissection was carried out on dead animals after the organs (liver and kidney) were excised. Survived animals were also sacrificed thereafter. Formalin (10%) was used in the preservation of the organs before they were histologically examined.

2.3 **Blood Culture**

Samples of blood (2mls) were collected from the experimental and control rats with the use of sterile syringes and needles. They were transferred into universal bottles containing sterile Brain Heart infusion agar (20mls) and then incubated for about 3 days at 37^0C. The blood cultures were observed for turbidity and gas production. Cultures with positive results were subcultured on Blood agar, Eosin Methylene Blue agar, MacConkey agar and Mannitol Salt Agar to confirm the bacteria in the blood samples of the infected rats (Monica 1981, 1987).

2.4 Histological Studies

The excised organs preserved in 10% formalin were studied histologically to detect any abnormalities in the organs' tissues (Weis *et al.,* 2010).

2.5 Analysis of Data

The results of the study were subjected to the analysis of variance (ANOVA) and Duncan's Multiple Range Tests (DMRT) ($p<0.05$).

3.0 Results and Discussion

All the higher fungi had significant therapeutic effects against the test bacteria. On Table 1, *Fomes lignosus, P. pulmonarius* and the mixture extracts had the same therapeutic effect and highest survival rates of 75% in *Rattus norvegicus* against *E. coli* at the end of the experiment. The best effect against *S. aureus* was observed in *P. tuber-regium* and *L. subnudus* with 100% animal survival. *F. lignosus*, *T. robustus* and mixture extracts were also active with survival rates of 75% (Table 2). Against *S. typhi, T. robustus* had the best and highest followed by *P. tuber-regium* and mixture extract with survival rates of 75% (Table 3). The result in Table 4 showed that *P. Pulmonarius* had the best therapeutic effect against *P. aeruginosa* with 75% survival rates, while *F. lignosus, P. tuber-regium* and *T. robustus* and mixture extract had the same and moderate therapeutic effect of 50% survival rates, while the least therapeutic effect was recorded for *L. Subnudus* with survival rates of 25%. Similarly, the extracts of *F. lignosus, P. pulmonarius, T. robustus* and *L. subnudus* were very active against *Streptococcus feacalis*, mixture extract was moderately effective, while *P. tuber-regium* was the least effective (Table 5).

All the tested extracts produced the same therapeutic effect against *B. subtilis* with survival rates of 75%, while *P. pulmonarius* had the least therapeutic effect with survival rates of 25%. Generally, against the test organisms, the best result was observed for *T. robustus* and *F. lignosus*, followed by *L. Subnudus*, mixture extract and then *Pleurotus* species (*P. pulmonarius* and *P. tuber-regium*).

Mushrooms are known for their medicinal benefits and have been broadly employed in the treatment of various ailments (Pegler, 1983). They are also prescribed for various disease conditions (Thekkuttuparambil *et al.,* 2007). Their use in traditional oriental medicine is an established history and many research works have been carried out by the body (Zaidman *et al.,* 2005). They are large sources of biologically active compounds with therapeutic abilities while many species have been identified with pharmacological activities (Mizuno, 1995; Wasser, 2002). In many parts of the world, the exploration of mushrooms and the metabolites for curing diseases are been attempted because of their therapeutic properties (Jong *et al.,* 1992).

Among the mushrooms that have been discovered to have medicinal properties are *Auricularia, Flammulina, Ganoderma, Grifola, Lentinus*, *Trametes* and *Tramella* of different species (Wasser, 2002). The higher fungi used in this study (*Fomes lignosus*, *T. robustus, L. subnudus, P. pulmonarius* and *P. tuberregium* were discovered to have medicinal values as they exhibited therapeutic effects against the test pathogenic bacteria (*E. coli, S. aureus, S. typhi, P. aeruginosa, S. feacalis* and *B. subtilis*). Several scientific reports have been published on antitumor activity of extracts of mushrooms. According to Stamets (1993), such mushrooms have high amount of retene. Anticancer drugs such as krestin, lentinan and schizophyllan have also been developed from *Tremetes* (*Coriolus versicolor), Lentinus edodes* and *Schizophyllum*

commune respectively. The aqueous extracts of some higher fungi were discovered to have inhibitory effects on the growth of cancers and tumors (Jonathan 2010, 2011). Extracts of *Pleurotus* species show activity against several chronic diseases such as hypertension (Gunde-Cimmerman *et al.,* 1993; Gunde-Cimmerman, 1999; Wasser, 2002).

Other important therapeutic properties of mushrooms include antioxidant, antidiabetic, anti-inflammatory, hepatoprotective etc. This is in addition to the antiviral, cholesterol-enhancing, immune-enhancing properties, mushrooms help in reducing blood pressure and blood sugar level (Wasser *et al*., 1999).

Many mushrooms have been confirmed to possess ability to fight some viral infections through the induction of interferon formation (Stamets, 1993). *Pleurotus* species were found to possess antioxidant, antitumor and anti-inflammatory properties (Chang, 1999) while *Daldina concentrica* is among the mushrooms found to be effective against pathogenic organisms such as *E. coli, Proteus mirabilis, P. aeruginosa, S. aureus* (Jonathan *et al.,* 2011) The antagonistic effect of extracts of *F. lignosus, Morasmus jodocodo, Pleurotus florida, P. tuber-regium, Psthyrella atroumbonaba* was also discovered (Jonathan *et al.,* 2007). In South Africa, *P. pulmonarius* amongst other medicinal mushrooms such as *G. licidum, Phellinus rimosus* and *P. florida* were discovered to have antioxidant and antitumor properties (Thekkuttuparambil and Kainoor, 2007).

The blood culture experiment confirmed the infection of the blood of rats with the various infected bacteria. Blood cultures of the experimental rats became turbid while those of the control rats (without infection) remain clear after the 3 days incubation period. *E. coli, S. aureus, Salmonella typhi, P. aeruginosa, Streptococcus feacalis* and *Bacillus subtilis* were confirmed on Eosin methylene blue agar, Mannitol Salt Agar, MacConkey agar and blood agar respectively.

Table 1: Survival rates of *Rattus norvegicus* used for therapeutic administration of the higher fungi against *Escherichia coli*

Day	FOM	PT	PS	Term	Lent	Mix	Control A	Control B
1	83.33^{ab}	75.00^{b}	75.00^{b}	75.00^{b}	75.00^{b}	100.00^{a}	75.00^{b}	100.00^{a}
10	75.00^{b}	50.00^{c}	75.00^{b}	50.00^{c}	50.00^{c}	75.00^{b}	0.00	100.00^{a}
20	75.00^{b}	50.00^{c}	75.00^{b}	50.00^{c}	50.00^{c}	75.00^{b}	0.00	100.00^{a}
30	75.00^{b}	41.67^{c}	75.00^{b}	50.00^{c}	50.00^{c}	75.00^{b}	0.00	100.00^{a}

Values with the same letter(s) in each row are not significantly different by Duncan's multiple range test ($P < 0.05$). Each is a mean of three replicates.

Key: FOM = *Fomes lignosus*, PT = *Pleurotus tuber-regium,* PS = *Pleurotus pulmonarius,* Term = *Termitomyces robustus,* Lent = *Lentinus subnudus,* Mix = Mixture extract, Control A = Infected rats without extract, Control B = Uninfected rats.

Table 2: Survival rates of *Rattus norvegicus* used for therapeutic administration of the higher fungi against *Staphylococcus aureus*

Day	FOM	PT	PS	Term	Lent	Mix	Control A	Control B
1	83.33^{ab}	100.00^{a}	58.33^{c}	75.00^{b}	100.00^{a}	75.00^{b}	50.00^{c}	100.00^{a}
10	75.00^{b}	100.00^{a}	8.33^{c}	75.00^{b}	100.00^{a}	75.00^{b}	0.00	100.00^{a}
20	75.00^{b}	100.00^{a}	0.00	75.00^{b}	100.00^{a}	75.00^{b}	0.00	100.00^{a}
30	75.00^{b}	100.00^{a}	0.00	75.00^{b}	100.00^{a}	75.00^{b}	0.00	100.00^{a}

Table 3: Survival rates of *Rattus norvegicus* used for therapeutic administration of the higher fungi against *Salmonella typhi*

Day	FOM	PT	PS	Term	Lent	Mix	Control A	Control B
1	83.33^{ab}	75.00^{b}	75.00^{b}	100.00^{a}	75.00^{b}	75.00^{b}	75.00^{b}	100.00^{a}
10	75.00^{b}	75.00^{b}	50.00^{c}	100.00^{a}	75.00^{b}	75.00^{b}	0.00	100.00^{a}
20	75.00^{b}	75.00^{b}	50.00^{c}	100.00^{a}	75.00^{b}	75.00^{b}	0.00	100.00^{a}
30	75.00^{b}	75.00^{b}	50.00^{c}	100.00^{a}	75.00^{b}	75.00^{b}	0.00	100.00^{a}

Table 4: Survival rates of *Rattus norvegicus* used for therapeutic administration of the higher fungi against *Pseudomonas aeruginosa*

Day	FOM	PT	PS	Term	Lent	Mix	Control A	Control B
1	75.00^{b}	75.00^{b}	75.00^{b}	75.00^{b}	75.00^{b}	75.00^{b}	75.00^{b}	100.00^{a}
10	50.00^{c}	50.00^{c}	75.00^{b}	50.00^{c}	25.00^{d}	50.00^{c}	0.00	100.00^{a}
20	50.00^{c}	50.00^{c}	75.00^{b}	50.00^{c}	25.00^{d}	50.00^{c}	0.00	100.00^{a}
30	50.00^{c}	50.00^{c}	75.00^{b}	50.00^{c}	25.00^{d}	50.00^{c}	0.00	100.00^{a}

Table 5: Survival rates of *Rattus norvegicus* used for therapeutic administration of the higher fungi against *Streptococcus feacalis*

Day	FOM	PT	PS	Term	Lent	Mix	Control A	Control B
1	75.00^{b}	50.00^{c}	83.33^{ab}	83.33^{ab}	83.33^{ab}	75.00^{b}	50.00^{c}	100.00^{a}
10	75.00^{b}	41.67^{c}	75.00^{b}	75.00^{b}	75.00^{b}	50.00^{c}	0.00	100.00^{a}
20	75.00^{b}	41.67^{c}	75.00^{b}	75.00^{b}	75.00^{b}	50.00^{c}	0.00	100.00^{a}
30	75.00^{b}	41.67^{c}	75.00^{b}	75.00^{b}	75.00^{b}	50.00^{c}	0.00	100.00^{a}

Table 6: Survival rates of *Rattus norvegicus* used for therapeutic administration of higher fungi against *Bacillus subtilis*

Day	FOM	PT	PS	Term	Lent	Mix	Control A	Control B
1	83.33^{ab}	75.00^{b}	75.00^{b}	75.00^{b}	83.33^{ab}	100.00^{a}	75.00^{b}	100.00^{a}
10	75.00^{b}	75.00^{b}	50.00^{c}	75.00^{b}	75.00^{b}	75.00^{b}	0.00	100.00^{a}
20	75.00^{b}	75.00^{b}	25.00^{c}	75.00^{b}	75.00^{b}	75.00^{b}	0.00	100.00^{a}
30	75.00^{b}	75.00^{b}	25.00^{c}	75.00^{b}	75.00^{b}	75.00^{b}	0.00	100.00^{a}

Tables 7, 8, 9, and 10, showed the organs of the infected rates were seen with abnormalities such as mild cellular infiltration at the portal area (liver), marked portal congestion (kidney), diffused tubular necrosis (kidney) and marked bile duct proliferation (liver). These are indications that the infection or diseases have developed in the organs. Meanwhile, the administration of the higher fungi extracts brought 'normalization' process in the organs. This is considered therapeutic effects. On Table 9, no visible lesions were observed after the extract's administration, while on Tables 8 and 11, chronic condition became mild after treatment. The congestion of the portal area of the liver and the protein casts in the kidney tubules (Table 10) may be due to weak therapeutic effect of *L. subnudus* against *P. aeruginosa*. Similarly, on Table 7, the effect of the mixture extract was unnoticeable.

Table 7: Histological findings on tissues of rats infected with *Bacillus subtilis*, those treated with Mixture extract and control

Category	Tissue	Histological Findings
Rats administered with mixture extract 7 days before infection	Liver	Mild cellular infiltration
	Kidney	No visible lesions
Control (no infection)	Liver	No visible lesions
	Kidney	No visible lesions
Control (with infection)	Liver	Mild cellular infiltration at the portal area
	Kidney	No visible lesions seen

Table 8: Histological findings on tissues of rats infected with *E. coli*, those treated with *Fomes lignosus* and control

Category	Tissue	Histology Findings
Rats administered with *F. lignosus* extract 7 days after infection	Liver	No visible lesions
	Kidney	Mild portal fibrosis
Control (no infection)	Liver	No visible lesions
	Kidney	No visible lesions
Control (with infection)	Liver	No visible lesions
	Kidney	Marked portal congestion

Table 9: Histological findings on tissues of rats infected with *S. aureus*, those treated with *Pleurotus tuber-regium* and control

Category	Tissue	Histology Findings
Rats administered with *P. tuber-regium* 7 days before infection	Liver	No visible lesions seen
	Kidney	No visible lesions seen
Control (no infection)	Liver	No visible lesions seen
	Kidney	No visible lesions seen.
Control (with infection)	Liver	Cellular infiltration
	Kidney	Diffuse tubular necrosis.

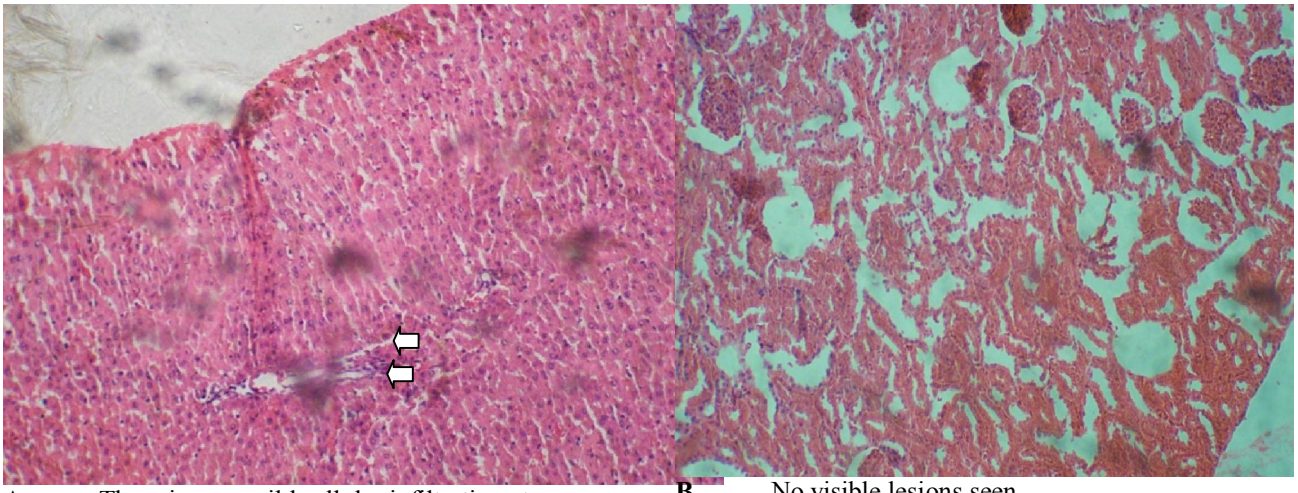
Table 10: Histological findings on tissues of rats infected with *Pseudomonas aeruginosa*, those treated with *Lentinus subnudus* and control

Category	Tissue	Histological Findings
Rats administered with *L. subnudus* 7 days after infection	Liver	Congestion of the portal area
	Kidney	Tubules with protein casts
Control (no infection)	Liver	No visible lesions
	Kidney	No visible lesions
Control (with infection)	Liver	Marked bile duct proliferation
	Kidney	No visible lesions seen

Table 11: Histological findings on tissues of rats infected with *Salmonella typhi,* those treated with *Termitomyces robustus* and control

Category	Tissue	Histological Findings
Rats administered with *T. robustus*	Liver	No visible lesions
7 days after infection	Kidney	Moderate renal congestion
Control (no infection)	Liver	No visible lesions seen
	Kidney	No visible lesions seen
Control (with infection)	Liver	Severe congestion of sinusoids
	Kidney	Numerous tubules with protein casts

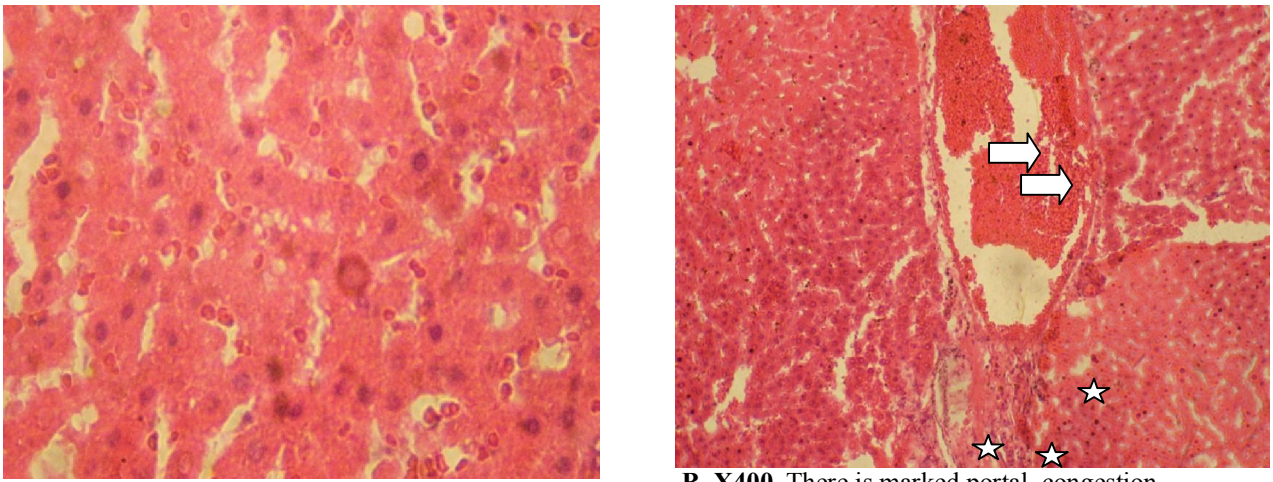
The histological findings are shown on Plates 1, 2, 3, 4, and 5 respectively. However, on the overall, the test fungi extracts have therapeutic abilities and can be used against the infections caused by the test bacteria.



A There is very mild cellular infiltration at the portal area.

B No visible lesions seen.

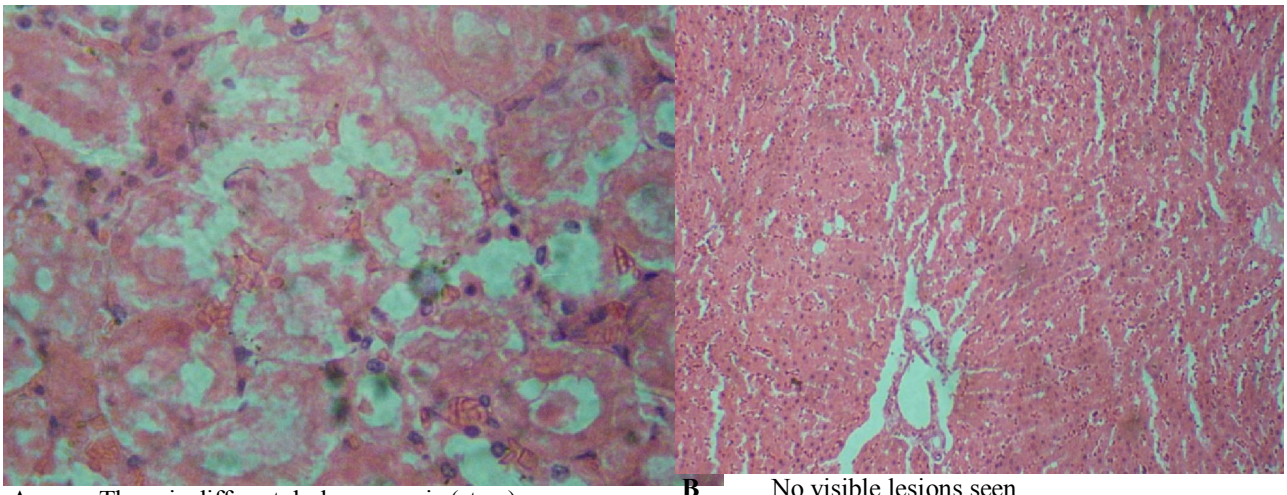
Plate 1: Photomicrograph of rats' livers infected with *Bacillus subtilis* (A) Mild cellular infiltration (B) The kidney of infected rates with no visible lesions.



A No visible lesions

B X400. There is marked portal congestion (arrows) and mild portal fibrosis (stars).

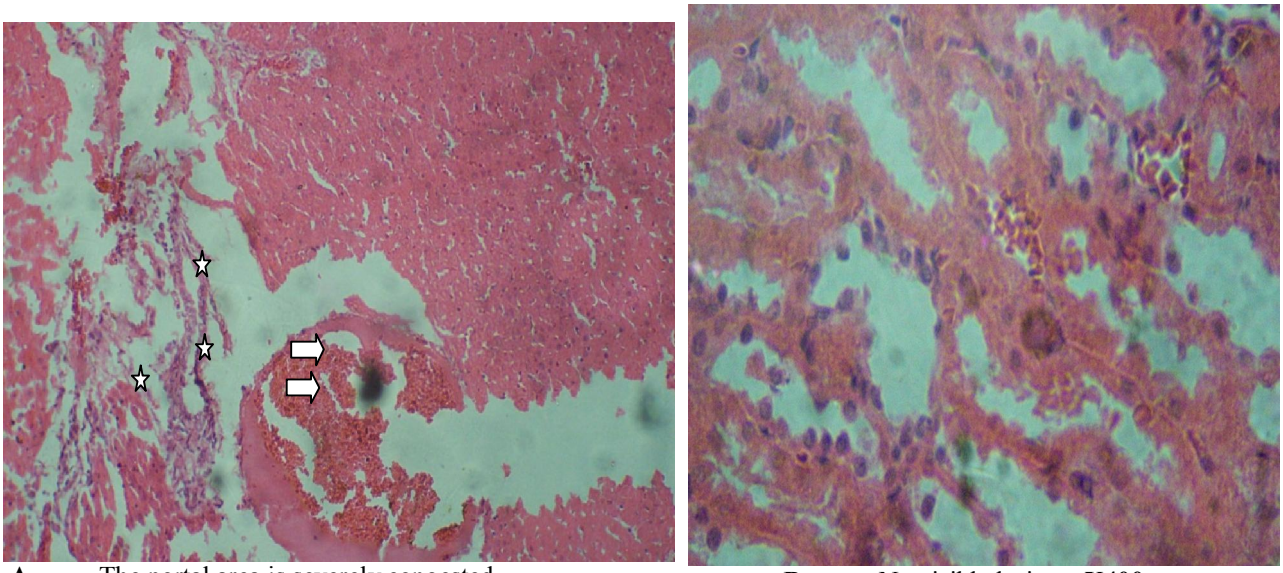
Plate 2: **Photomicrograph of the liver of rats infected with *Escherichia coli* with no visible lesions (A) and the kidney with marked portal congestion (B)**



A There is diffuse tubular necrosis (stars) with protein casts in the tubular lumen (arrows)

B No visible lesions seen **X400**

Plate 3: Photomicrograph of the kidney of rates infected with *Staphylococcus aureus* showing diffuse tubular necrosis (A) and the liver of control rats with no visible lesions (B)



A The portal area is severely congested (arrows). There is marked bile duct proliferation (stars).

B No visible lesions **X400**

Plate 4: Photomicrography of rats' livers infected with *Pseudomonas aeruginosa* (A) Congested portal area (B) the kidney of control rats (uninfected)

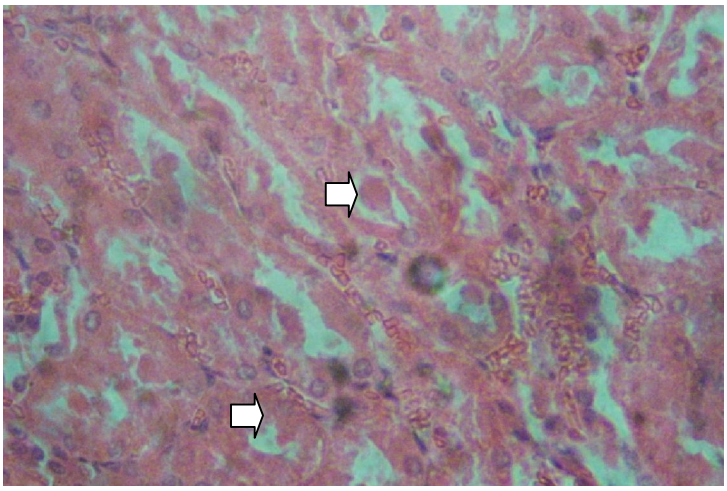


Plate 5: Photomicrograph of rat's kidney infected with *Salmonella typhi* with moderate renal congestion, numerous tubules with protein casts.

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