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Research Article

The influence of metabolites from wild type and mutant strains of *Schizophyllum commune* on *Escherichia coli* induced changes in Albino rats

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Abstract: The increasing emergence of several multi-resistant pathogenic microorganisms to synthetic drugs has drawn much attention of researchers to finding new, attractive and natural products such as mushroom species as neutraceuticals because they contain a tremendous variety of secondary metabolites. Hence this work is reported on the influence of metabolites from*Schizophyllum commune* wild type and mutants on *Escherichia coli* induced histopathological changes in Albino rats. *S. commune* mutant strains were generated by exposing the wild type fungus to ultraviolet rays at various time intervals of 30minutes, 60minutes and 90minutes coded as SCM1, SCM2 and SCM3 respectively. Metabolites from submerged fermentation of the *S.commune* strains were tested on Albino rats administered with pathogenic *E.coli.* Histopathological studies carried out on the testes, kidney and liver of the rats showed that SCM2 metabolite performed best in combating the virulence effects of pathogenic *E.coli* in the testes, kidney and liver tissues of the albino rats as compared to performance of SCM3, SCM1 and SCW respectively, hence SCM2 is recommended for use instead of the wild type in drug production.
Keywords: Metabolites, *Schizophyllum commune*, Albino rats, Submerged fermentation.

INTRODUCTION

Mushrooms are the reproductive structures (fruiting body or sporocarp) of certain fungi [1-2]. Prasad Y *et al.* have defined mushrooms as macrofungi with distinctive fruiting bodies that are large enough to be seen by the naked eye and to be picked by hand. It is estimated that there are approximately 1.5 million species of mushrooms in the world of which approximately 70,000 species are described [3].

Edible mushrooms are nutritionally endowed fungi (mostly ascomycetes and basidiomycetes) that grow naturally on the trunks, leaves and roots of trees as well as decaying woody materials [4]. Some mushrooms including edible mushrooms possess a new class of compounds with nutritional and medicinal features extractable from either the mycelium or the fruiting bodies of mushrooms referred to as "mushroom nutriceuticals" [5-6]. Medicinal mushrooms accumulate a wide variety of bioactive compounds including terpenoids, steroids, phenols, nucleotides and their derivatives glycoproteins and polysaccharides that display a broad range of biological activities [7-8]. These different bioactive compounds have been extracted from the fruiting body, mycelia and culture medium of various medicinal mushrooms such as *Lentinulaedodes, Ganodermalucidum, Schizophyllum commune, Trametesversicolor, Inonotusobliquus*and *Flammulinavelutipes* [9]

Schizophyllum commune fries is a higher fungus which belongs to family *Schizophyllaceae*, Order *Aphyllophorales*, phylum *Basidiomycota* of the kingdom fungi [10]. It is known to produce exopolysaccharides called schizophyllan. The family *Schizophyllaceae* contains only one genus; *Schizophyllum*and there is a single common worldwide species, although there are a few less common species of *Schizophyllum.* The genus name means "split gill," and thus it is called the split gill fungus. *S. commune* is one of the common gill-bearing bracket fungi of world-wide distribution [11].

S. commune mutants are the resulting strains after the wild type has been exposed to ultra-violet radiation for various time intervals. This in turn would cause a mutation that affects all the cell formation of the organism. The higher the exposure to the ultra-violet light, the more mutated the organism would be thus bringing about a major variation between the original organism and the mutated organism. In biology and especially genetics, a mutant is an individual, organism, or new genetic character arising or resulting from an instance of mutation, which is a base pair sequence change within the DNA of a gene or chromosome of an organism resulting in the creation of a new character or trait not found in the wild type [12]. There has also been a great concern among researchers about the increasing

appearance of several multi-resistant pathogenic microorganisms and tumour cells to the available antibiotics, which has become an interestingly important and pressing global problem. Due to this, the rate of drug discovery has dropped to dangerous proportions, the rate of nosocomial infections has risen and new diseases have evolved [13]. In the modern, drug safety evaluation has been practised in rodent and non rodent species widely since before the Second World War, there have been very few critical comparisons of the effect of drugs in man and those laboratory animals. Much potentially useful information still resides in archives of pharmaceutical companies and government agencies. Nevertheless, the available data suggests that the traditional approach using experimental pharmacology alongside conventional toxicology studies with pathology is usually sufficient to predict important adverse effects and to support the safe conduct of the first chemical studies in human. Indeed, dosing a rodent and non rodent species with a new drug up to one month identifies 90% of adverse effects that will never be detected in conventional animal studies [14]. This study aimed at the histopathological changes in the tissues of Albino rats using the pathogenic strains of *E.coli*and metabolites of *S. commune* wild type and mutants. Mutants from*S.commune* and their culture in natural substrates for the production of highly effective polysaccharides of pharmaceutical importance have not been explored hence the need for the present study.

Materials and methods

Collection of microbial sample

Schizophyllumcommune was collected from dead wood of *mangiferaindica* at Ogbomoso, Oyo State, Nigeria and identified by its characteristics using the descriptions of Zoberi MH [11] and Alexopoulos CJ *et al.* [15].

Sample preparation and establishment of mycelial cultures

Tissue culture was carried out on fresh carpophores of *S.commune* using the method of Jonathan SG *et al.* [16]. The mycelial thus generated were cultured on plates of potatoes dextrose agar (PDA).

Production of *Schizophyllum commune* mutants.

Various mutants of *Schizophyllum commune* were prepared as follows; Fresh plates of *Schizophyllum commune* were allowed to sporulate. The spores were removed with sterile distilled water and re-inoculated on three different freshly prepared plates. The plates were exposed to UV light at 260nm at various time intervals to induce mutation [17]. The first plate was exposed to UV for 30mins and labelled as *Schizophyllum commune* mutant1 (SCM 1).

The second plate was exposed to UV for 60mins and labelled as *Schizophyllum commune* mutant 2(SCM 2).The third plate was exposed to UV for 90mins and labelled as *Schizophyllum commune* mutant 3(SCM 3). Fresh plate of the organism before exposing to UV radiation was also prepared and labelled as *Schizophyllum commune* wild type (SCW).

Four different strains that eventually resulted were SCW, SCM1, SCM2 and SCM3.

Culture preparation for metabolites production

The basal medium used consisted of 100mls of *Hibiscus sabdariffa* solution added with 6g Glucose , 1.6g Malt extracts, 2g Peptone, 1.2g Yeast extracts, 0.8g KH_2PO4, 0.4g $MgSO4.7H_20$, 0.4g Urea and P^H adjusted to 5.8.(Yap and Ng 2001 with modification) *S. commune* wild type and mutants (i.e SCW,SCM1,SCM2 and SCM3) were initially sub-cultured on PDA plates and then 6mm of the vigorous growing agar plate culture(5- day old) was removed using sterile cork borer. The sterilized basal medium was inoculated with this mycelial disc of *S. commune* wild type and mutants. The fermentation experiments were set-up under aerobic condition with the use of aeration pumps to ensure continuous aeration and agitation. The fermentors were fixed to the hose connected to two aeration pumps to supply oxygen and to stimulate fermentation rate. Two fuel filters were fixed to the end of the aeration pumps so as to filter air coming to the media. The set-up was allowed to undergo batch fermentation at 28^0C for a period of 6 days with constant supply of electricity powering the pumps [15] with modifications.

Animal Experiments

One hundred white albino rats weighing between 100g and 180g were purchased from the Animal house of the Department of Veterinary Medicine, University of Ibadan. They were allowed to acclimatize for two weeks and being fed appropriately.

The animals were randomized into ten groups with ten rats in each group. The groupings and product administration were shown in table 1 below**:**

Table 1: Experimental design to investigate metabolites from *S. commune* wild type and mutants in the influence of *E.coli* toxicity in Testes, Kidney and Liver of Albino Rats

Animal groupings	Diet administered
Group one	0.2ml of 10^{-9} cells/ml of *E.coli*
Group two	1ml of distilled water only (Control group)
Group three	1mg/ml of precipitate from SCW
Group four	1mg/ml of SCW plus 0.2ml of 10^{-9} cells/ml of *E.coli*
Group five	1mg/ml of SCM_1 only
Group six	1mg/ml of SCM_1 and 0.2ml of 10^{-9} cells/ml of *E.coli*
Group seven	1mg/ml of SCM_2 only
Group eight	1mg/ml of SCM_2 and 0.2ml of 10^{-9} cells/ml of *E.coli*
Group nine	1mg/ml of SCM_3 only
Group ten	1mg/ml of SCMand 0.2ml of 10^{-9} cells/ml of *E.coli*

SCM_1 =*Schizophyllum commune* mutant one, SCM_2 = *Schizophyllum commune* mutant two, SCM_3 =*Schizophyllum commune* mutant three

The dosage administration was for 35days. Appropriate death was recorded as at when noticed during the dosage administration periods.

Sperm sampling

At the end of the 35 –day exposure period, sperm was collected for spermalgical studies such as sperm motility, sperm count and sperm morphology. The caudal epididymis was surgically removed and placed in a beaker. This site was chosen because it is generally considered preferable as this is the main sperm storage site in rat with optimized conditions.

Sperm motility Assay

The caudal epididymis was minced into a clean beaker containing 0.9ml of prewarmed normal saline (37^oC). A drop of sperm suspension was placed on the glass slide to analyze 200 motile sperm in four different fields. This was done microscopically within 2-4 min of their isolation from the epididymis and data expressed as percentages following the method of Dunn WL [18].

Sperm Count

Epididymis sperm obtained by mincing the epididymis into a clean beaker containing 0.9ml normal saline was left for 20 seconds for the sperm to swim and then counted in the Neubauer haemocytometer slide. The number of sperm in five squares (four corners and the centre) in the centre grid of both sides was counted and averaged following the methodology of Karmali MA; [19].

Sperm Morphology

A drop of the epididymis sperm was placed on a clean slide and equal volume of nigrosine and eosine were added to the slide. The cover slip was used to make a smear and allowed to air dry after which it was viewed under the light microscope [19].

Criteria for evaluating sperm assay

*Sperm count=$N/5\times10^6$ where N stands for viable sperm numbers

*% Motility= M/M+N×100 Where M=Motile sperm and N=Non motile sperm

*No of sperms morphologically examined per animal were 600

Histopathological Tests

Three animals each were selected from each group for histopathological tests. This was done by sacrificing the animals by cervical dislocation 24h after the last dosage administration. The Liver, kidney and Testes were collected and studied. The results were subjected to statistical analysis by one way ANOVA followed by Duncan test.

Collection of Liver, Kidney and Testes.

After the sacrifice of the animals by cervical dislocation under mild chloroform anaesthesia, the kidney, liver and testes were excised immediately and thoroughly washed in ice- cold saline.

Histopathology of the Liver, Kidney and Testes

The liver, kidney and testes were preserved in 20% formalin immediately after removal from the animals.

Tissue Processing

Liver, kidney and testes tissues were placed in 10% formalin (diluted to 10% with normal saline) for 1 hour to rectify shrinkage due to concentration of formalin. The tissues were dehydrated by ascending grades of isopropyl alcohol by immersing in 80% isopropanol overnight and 100% isopropyl alcohol for 1 hour. The dehydrated tissues were cleared in two changes of xylene, 1 h each. The wax impregnated tissues were embedded in paraffin blocks using the same grade wax. The paraffin blocks were cut with rotary microtome at 3 micron thickness. The sections were floated on a tissue floatation bath at 40°C and taken on glass slides and smeared with equal parts of egg albumin and glycerol. The sections were then melted in an incubator at 60°C and after 5min; the sections were allowed to cool

Tissue Staining

The sections were deparaffinised by immersing in xylene for 10 mins in horizontal staining jar. The deparaffinised sections were washed in 100% isopropyl alcohol and stained in Ehrlick's haematoxylin for 8mins in horizontal staining jar. After staining in haematoxylin, the sections were washed in tap water and dipped in acid alcohol to remove excess stain (8.3% HCl in 70% alcohol). The sections were then placed in running tap water for 10mins for blueing (alkalization). The sections were counter stained in 1% aqueous eosin (1 gram in 100ml tap water) for 1 min and the excess stain was washed in tap water and the sections were allowed to dry. Complete dehydration of stained section was ensured by placing the sections in the incubator at 60°C for 5mins. When the sections were cooled, they were mounted in DPX mount having the optical index of glass (the sections were wetted in xylene and inverted onto the mount and placed on the cover slip). The architecture was observed at low power objective under microscope. The cell injury and other aspects were observed under high power dry objective [20].

Effects of bioactives from wild type and mutants of *S. commune* on*E.coli* induced histopathological changes in liver and kidney of Albino rats

Preparation of *Escherichia coli* cell suspension

Highly pathogenic strain of *Escherichia coli* culture in slants was obtained from Babcock University Teaching Hospital, Ogun state. It was sub cultured to a freshly prepared Nutrient Agar plate. 10^{-9}cell suspension was prepared as follows; 18-hour broth culture of the organism was prepared and centrifuged. The 10^{-9} cell suspension was kept in the refrigerator from where 0.2ml each was being administered as at when needed.

Histopathology

Three animals each were selected from each group for histopathological test of the liver and kidney. The liver and kidney were collected and appropriate tissue staining as well as microscopy as previously described using the method of Dunn, 1974 was carried out.

RESULTS

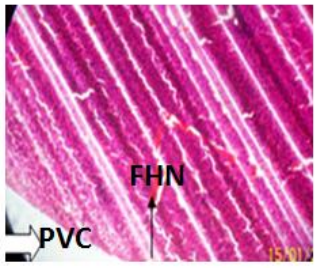

Plate 3.1a: Scw + 0.2ml of 10^{-9} cells/ml of *E.coli* [x 150]

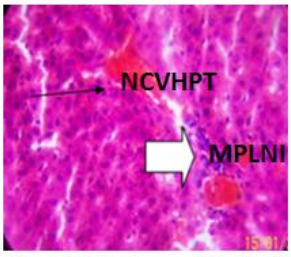

Plate 3.1b: Scm_1+0.2ml of 10^{-9} cells/ml of *E.coli* [x 150]

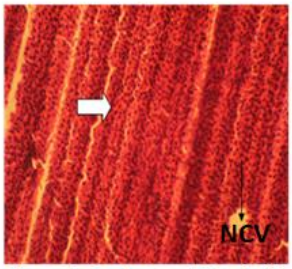

Plate 3.1c: Scm_2+ 0.2ml of 10^{-9} cells/ml of *E.coli* [x 150]

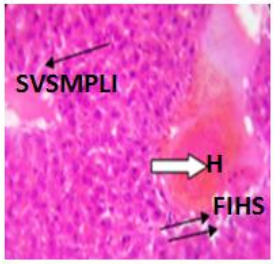

Plate 3.1d: Scm_3+ 0.2ml of 10^{-9} cells/ml of *E.coli* [x 150]

Plates 3.1(a-d):Histopathological appearance of liver tissues administered with 0.2ml of 10-9 cells/ml of E.coli and 1mg/ml of metabolites from Scw, Scm1, Scm2 and Scm3.

FHN: Focal Hepatic Necrosis, PVC: Portal Vascular Congestion, NCVHPT: Normal Central Vein and Hepatocyte, Portal Tracts, MPLNI: Mild Periportal Lymphocytic and Neutrophilic Infiltrate , NH: Normal Hepatocyte, NCV: Normal Central Vein , SVSMPLI: Severe Vascular and Sinosuidal and Mild Periportal , Lymphocytic Infiltrates, H: Haemorrhage, FIHS: Focal Areas of the Interlobular Hepatic Necrosis.

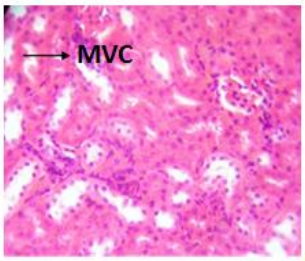

Plate 3.2a: Scw + 0.2ml of 10^{-9} cells/ml of *E.coli* [x 150]

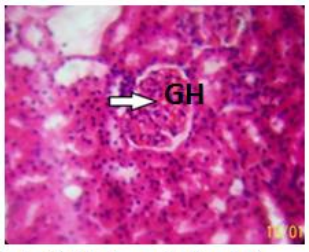

Plate 3.2b: Scm_1+0.2ml of 10^{-9} cells/ml of *E.coli* [x 150]

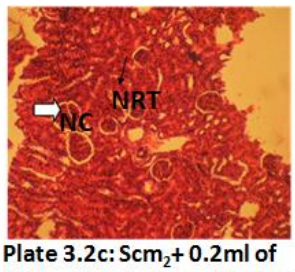

Plate 3.2c: Scm_2+ 0.2ml of 10^{-9} cells/ml of *E.coli* [x 150]

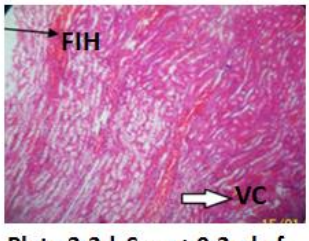

Plate 3.2d: Scm_3+ 0.2ml of 10^{-9} cells/ml of *E.coli* [x 150]

Plates 3.2(a-d) : Histopathological appearance of kidney tissues administered with 0.2ml of 10-9 cells/ml of E.coli and 1mg/ml of metabolites from Scw, Scm1, Scm2 and Scm3

MVC: Moderate Vascular Congestion , GH: Glomeruli Hypercellularity, NRT: Normal Renal Tubes, NC: Normal Corpuscle , FIH: Focal Interstitial Haemorrhage , VC: Vascular Congestion

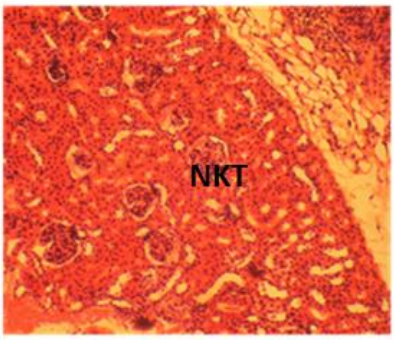

Plate 3.3a: Histological appearance of kidney tissue without 0.2ml of 10^{-9} cells/ml of *E.coli* [x150]

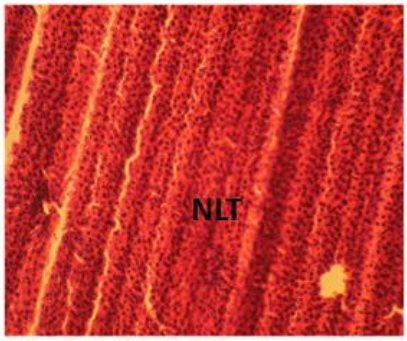

Plate 3.3b: Histological appearance of liver tissues without 0.2ml of 10^{-9} cells/ml of *E.coli* [x150]

NKT: Normal Kidney Tissue, NLT: Normal liver Tissue

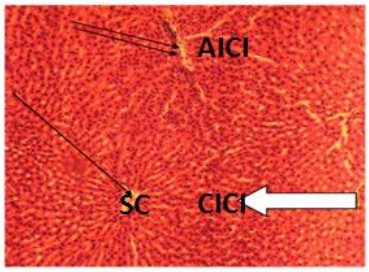

Plate 3.4a: Histopathological appearance of liver tissue administered with 0.2ml of 10^{-9} cells/ml of *E.coli* only[x 150]

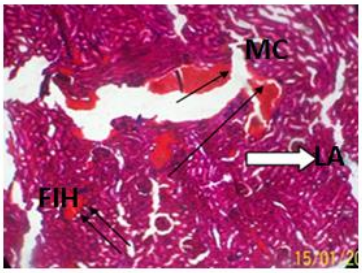

Plate 3.4b: Histopathological appearance of kidney tissue administered with 0.2ml of 10^{-9} cells/ml of *E.coli* only [x150]

AICI: Acute Inflammatory Cells Infiltrate , SC: Severe Congestion, CICI: Chronic inflammatory Cells Infiltrate, LA: Lymphocytic aggregates , FIH: Focal Interstitial Haemorrhage , MC: Marked Congestion

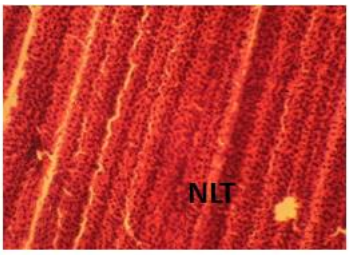

Plate 3.5a: liver tissue administered with 1mg/ml of metabolites from Scw only [x 150]

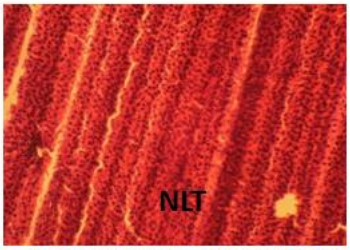

Plate 3.5b: liver tissue administered with 1mg/ml of metabolites from Scm_1 only [x 150]

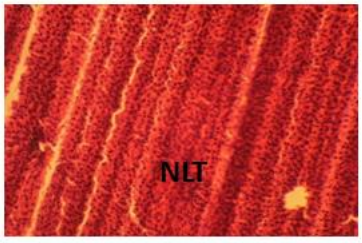

Plate 3.5c: liver tissue administered with 1mg/ml of metabolites from Scm_2 only [x 150]

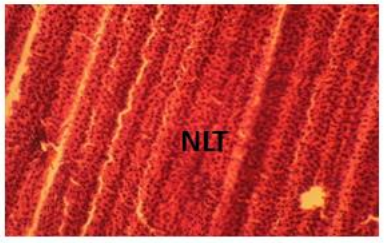

Plate 3.5d: liver tissue administered with 1mg/ml of metabolites from Scm_3 only [x 150]

Plate 3.5(a-d): Histological appearance of liver tissue administered with 1mg/ml of metabolites from Scw, Scm_1, Scm_2 and Scm_3 only.

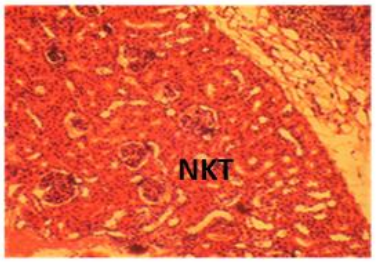

Plate 3.6a: kidney tissue administered with 1mg/ml of metabolites from Scw only [x 150]

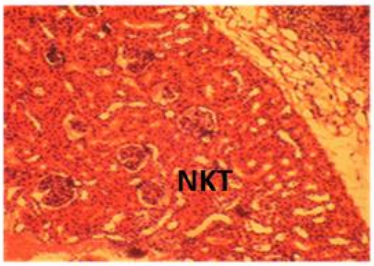

Plate 3.6b: kidney tissue administered with 1mg/ml of metabolites from Scm_1 only [x 150]

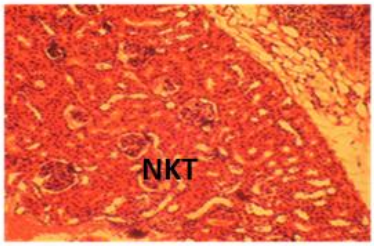

Plate 3.6c: kidney tissue administered with 1mg/ml of metabolites from Scm_2 only [x 150]

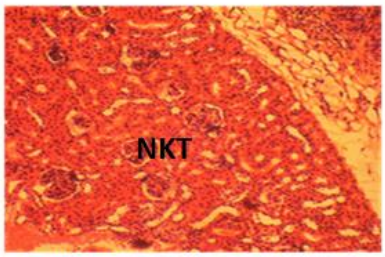

Plate 3.6d: kidney tissue administered with 1mg/ml of metabolites from Scm_3 only [x 150]

Plate 3.6(a-d): Histological appearance of kidney tissue administered with 1mg/ml of metabolites from Scw, Scm1, Scm2 and Scm3 only

NKT: Normal Kidney Tissues

DISCUSSION

The efficacy of extracted bioactives from wild type and mutants of *S. commune* were tested. The tests were carried out on their influence on enterotoxigenic*E. coli* induced histopathological changes in liver and kidney of albino rats. The results showed that bioactives from *S. commune* mutant II had the overall best influence on the pathogenic *E. coli* affected liver and kidney of albino rats. The results were presented above (Plate 3.1a-3.6d). Plate 3.1a (administration of metabolites from *S. commune* wild type together with *E. coli*) shows focal hepatic necrosis with portal vascular congestion of the liver tissue, Plate 3.1b (administration of metabolites from *S. commune* mutant I together with *E. coli*) shows normal central vein and hepatocyte with mild periportal lymphocytic and neutrophylic infiltrate while Plate 3.1c (administration of metabolites from *S. commune* mutant II together with *E. coli*) maintain normal central vein of the liver tissue. However, administration of metabolites from *S. commune* mutant III together with *E.coli* shows severe vascular and sinosuidal infiltrate with focal areas of interlobular hepatic necrosis (Plate 3.1d).

Plates 3.2a -3.2d where metabolites from *S. commune* wild, mutant I, mutant II and were administered respectively with *E.coli*, the kidney tissue of the rats revealed various abnormality ranging from moderate vascular congestion where metabolite from *S. commune* wild type was administered with *E. coli* ; glomerular hypercellularity where metabolite from mutant I and *E.coli* were administered; vascular congestion with focal interstitial haemorrage where mutant III and *E. coli* were administered except for metabolite from mutant II that influenced the effect of *E. coli* by maintaining normal corpuscle and normal renal tubes of the rat's kidney.

Also, the kidney and liver tissues of the rats where the metabolites from *S. commune* wild type, mutant I, II and III were administered separately without *E. coli* cell suspension, maintain their normal structures (Plates 3.5a-3.6d) whereas the kidney and liver tissues of rats administered with *E. coli* only show acute and chronic inflammatory cell infiltrate with severe congestion of the liver and focal interstitial haemorrage, lymphocytic aggregates with marked congestion of the kidney respectively.(Plates 3.4a and 3.4b).

According to Hughes MA *et al.* [21] and Vogt PK *et al.* [22], much of the pathogenicity associated with *E. coli* O157:H7 comes from the production of shiga toxins I and II (Stx1 and Stx2). Shiga toxin-producing *E. coli* (STEC) induces a condition known as hemorrhagic colitis, non-specific diarrhea and severe Haemolytic Uremic Syndrome (HUS). This contributes to renal dysfunction and mortality.

When shiga toxin is released from an *E. coli* O157:H7 bacterium infection induces colonization of the bowel and production of powerful Shiga-Like Toxins (SLTs), which are thought to enter the circulation system and to cause injury to the target endothelial cells in various organs. The report above is supported by the work of Miles PG *et al.* [23], who opined that the ability of the shiga toxins to pass through cell barriers is possibly due to the increased

permeability of the intestinal epithelial cells resulting from effects of the body's own immune system. The body increases permeability of cell barriers so that important cells of the immune system (neutrophils) can reach the *E. coli* infection. Shiga toxin may use this opportunity to break through the walls of the digestive tract, enter the blood stream, and bind white blood cells for transport to locations such as the kidney or brain [24].

The metabolites of *S .commune*played a major role by boosting the immune system of the rats, thus confirming the usefulness as well as the beneficial effect of *S. commune*. This is further supported by the work done by [25] on the nutritional value as well as the medicinal effect of mushrooms. According to the report of this present study, it can be deduced that the mutant created by exposure for 1h had its genetic constituent altered in support of active metabolites production able to combat the effect of pathogenic *E. coli*. This is in line with the work of [26] which showed that Schizophyllan produced by *Schizophyllum commune* is able to bind with mRNA poly (A) tail. The excellent recovery of renal function observed in experimental rats treated with *Schizophyllum commune* mutant II may also be explained by the regenerative capability of the metabolites produced by the mushroom. Similar work has been done by [27] when he observed the treatment of alloxan induced diabetic rats with*Trigonellafoenumgraecum* seed powder that would bring about the regenerative capability of the renal tubules. Thus, the findings of this present study shows that *Schizophyllum commune* mutant created by exposure to UV light for 1hour produced the best metabolites able to influence *Escherichia coli* induced toxicity in the kidney and liver of albino rats.

CONCLUSION

This work has shown that metabolites produced from exposure of *S. commune* wild type to ultraviolet rays for 60 minutes (SCM2) gave the best performance in restoring *Escherichialcoli* inducedhistopathological changes on liver and kidney of albino rats, hence, mutant strains of the organism should be explored in the pharmaceutical industries for the production of highly effective drugs to combat the emerging microbial diseases.

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