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Evaluation of Three Mushroom Species Ethanol Extracts in the Treatment of Postrate Cancer in Wister Albino Rats and Phytochemical Analysis of the Fungi in Nigeria

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Abstract
This study was aimed at evaluating the phytochemistry and anti-postrate cancer activities of three mushroom species in adult male wistar rats. The crude extracts of *Ganoderma lucidum, Pleurotus porrigens and Pleurotus pulmonarius* was used in the treatment of postrate cancer in wistar albino rat. A significant reduction in the postrate specific antigen of the induced group with 0.21±0.1ng/ml compared to the other control groups. There were significant increase in the haemoglobin content with 31.80±1.6, white blood cell 5.30±0.9 and mole corpuscular volume 69.70±0.5FL in the treated group compared to the control. Furthermore, significant reductions in the stimulating hormones (GSH) superoxide dismutate (SOD) and increases in catalase (CAT) were observed in the treated postrate cancer animal compared to the control groups. Reduction in creatinine and increase in chlorine (Cl) and potassium level was observed in the treated group compared with control group. The result showed that the phytomedicine had a good antipostrate cancer activity and the result of phytochemical screening revealed the presence of medicinally important constituent in the fungi.

Keywords: Anti-postrate cancer; Natural drugs; Haemoglobin; *Ganoderma lucidum; Pleurotus pulmonarius; Pleurotus porrigens.*

1. Introduction
The use of plant and plant product as food is as old as human existence of mankind. Ancient man being very close to nature ate raw and fresh produce which contributed to his health living and long-life. The discovery that plant product could serve as therapeutic weapons to manage various diseases has made plant a sine qua non to human and animal lives. Plants are the main source of drug discovery and are used in traditional medicine in treatment and curing of different ailment. The treatment of postrate cancer with herbal or polyherbal and natural product drug is now gaining ground for numerous benefits that could be attributed to their uses. Surgical treatment the most commonly used now is found to be associated with loss of libido while herbal medicine has been found to increase libido while at the same time effecting cure [1]. Postrate cancer has become a major public health problem worldwide although the aetiology remains largely unknown [2]. Dietary factors, dietary supplement and physical activity might be important in the prevention of cancer. Prostate cancer is a form of cancer that develops in the prostate, a gland in the male reproductive system [3]. Most prostate cancers are slow growing; however, there are cases of aggressive prostate cancers. The cancer cells may metastasize spread from the prostate to other parts of the body, particularly the bones and lymph nodes. Prostate cancer may cause pain, difficulty in urinating, problems during sexual intercourse, or erectile dysfunction. Other symptoms can potentially develop during later stages of the disease. Rates of detection of prostate cancers vary widely across the world, with South and East Asia detecting less frequently than in Europe, and especially the United States. Prostate cancer tends to develop in men over the age of fifty [4]. Globally, it is the sixth leading cause of cancer-related death in men [5]. In the United States it is the second. Prostate cancer is most common in the developed world with increasing rates in the developing world. However, many men with prostate cancer never have symptoms, undergo no therapy, and eventually die of other unrelated causes [6]. Many factors, including genetics and diet, have been implicated in the development of prostate cancer. The presence of prostate cancer may be indicated by symptoms, physical examination, prostate-specific antigen (PSA), or biopsy [7]. Prostate-specific antigen testing increases cancer detection but does not decrease mortality [8].

The United States Preventive Services Task Force in 2012 recommended against screening for prostate cancer using the PSA testing, due to the risk of over-diagnosis and over-treatment with most prostate cancer remaining asymptomatic. The USPSTF concludes that the potential benefit of testing does not outweigh the expected harms.

Management strategies for prostate cancer should be guided by the severity of the disease. Many low-risk tumors can be safely followed with active surveillance. Curative treatment generally involves surgery, various forms of radiation therapy or less commonly, cryosurgery; hormonal therapy and chemotherapy are generally reserved for cases of advanced disease although hormonal therapy may be given with radiation in some cases [9]. Mushrooms are reported to cure several ailments in Asia [10]. This is a form of natural therapy which is reported to be most efficient in curing diseases. Therefore there is need to investigate the anti-postrate cancer activity of some Nigerian mushrooms.

2. Materials and Methods

The fungi used in this study are *Ganoderma lucidum, Pleurotus pulmonarius and Pleurotus porrigens* in the ratio of 3:2:2.

Ganoderma lucidum	300g
Pleurotus pulmonarius	200g
Pleurotus porrigens	200g

Ganoderma lucidum belong to the family Ganodermataceae. parasitic on living hardwood especially oaks and saprobic on deadwood of hardwood causing a white butt. it is locally called medicine of internal life. The fungus was collected from the lagoon front of the University of Lagos, Akoka and was authenticated by a mycologist in the department of Botany, University of Lagos, Akoka, Yaba. *P. pulmonaris* and *P. porrigens* were collected from Federal Institute of Medical Research, Oshodi and were authenticated by Mr. Akinyemi Federal Institute of Medical research, Oshodi, Nigeria. The plant material were dried at an ambient temperature between 35-40 ^{o}C in an oven for five days and powdered to coarse particles. Three hundred grammes of *Ganoderma lucidum, 200g* of *Pleurotus pulmonarius* and 200g of *Pleurotus porrigens* was macerated and soaked in 2.5 litres ethanol (80%) for seven days with frequent stirring. After filtration, the solvent was removed under pressure in a rotary evaporator at a temperature below 50 and dried to a constant of 36.3, 30.5 and 28.6g respectively.

2.1 Animals

Male Wistar Adult albino rat (160±20g) were obtained from the laboratory center, college of medicine of the University of Lagos, idiaraba and were kept under standard environmental condition of 12/12 hours light and dark cycle. They were housed in cages (5 animals per cage) maintained on standard animal food (livestock feed Nigeria Limited) and provided with water. They were allowed to acclimatize for 30days before commencement of the experiment. The use of the animal and the experimental protocol was approved by the experimental ethics committee on animal use of the College of Medicine of the University of Lagos, Nigeria.

2.2 Postrate cancer study

Postrate cancer was induced experimentally on the animals by administering intraperitoneally with Estradiol and Testosterone solution for 21days. The postrate level was monitored by sacrificing one of the normal rats and compared with the induced group. A total of seven groups were used. Five groups were induced while the remaining two groups were used as different controls and were treated daily for 45days.

Group I: Induced rat treated with 500mg/kgbwt of Mushroom extract
Group II: Induced rat treated with 250mg/kgbwt of Mushroom extract
Group III: Induced rat treated with 250mg/kgbwt of Mushroom extract
Group IV: Induced untreated
Group V: Induced treated with control drug (Fenasteride)
Group VI: Normal Control
Group VII Control given 0.2ml Acacia solution

Statistical Analysis

All data are expressed as mean± standard error of mean

3. Results

The effect of the herbal medicine on the postrate specific antigens (PSA) compared to the controls are shown in Table 1. There was significant difference in the postrate cancer animal treated with herbal medicine compared to the normal control and control groups. Also, no significant decrease was observed in the control groups. Table 2 summarized the result of the phytomedicine and Fenasteride effect on the biochemical parameters. The haemoglobin level of the postrate cancer rat treated with the drug and Fenasteride were significantly high compared to induce untreated. The drug proved to have a better high haemoglobin effect than Fenasteride.

There was a significant increase in the WBC and MCV level in the untreated postrate cancer animals. On the other hand the two heamatological parameters were observed to have increased markedly in levels of the postrate cancer rats treated with the drug. There was also a significant decrease in GSH and SOD while significant increase in CAT level was observed in the entire postrate cancer animal treated with the drug or Fenasteride. In contrast, the untreated animal showed a significant increase in both GSH and SOD level. There was no significant change observed in the creatinine levels in all the animals treated with different doses of the phytomedicine compared with the controls while a significant change was observed in chlorine and potassium content of the induced untreated animals compared with the control and normal controls.

Table 1: Effect of Extracts on Postrate Specific Antigen Compared with induced untreated and the controls.

Animal Grouping	Result (ng/ml)	Reference Range
Group 1	0.21 ± 0.1	0-4.0
Group 2	0.67 ± 1.5	0-4.0
Group 3	0.65±0.5	0-4.0
Group 4	0.85 ± 0.3	0-4.0
Group 5	0.32 ± 0.5	0-4.0
Group 6	0.00 ± 0.0	0-4.0
Group 7	0.00±0.0	0-4.0

Mean + SEM, (n = 5)
Group I: Induced rat treated with 500mg/kgbwt of Mushroom extracts
Group II: Induced rat treated with 250mg/kgbwt of Mushroom extracts
Group III: Induced rat treated with 250mg/kgbwt of Mushroom extracts
Group IV: Induced untreated
Group V: Induced treated with control drug (Fenasteride)
Group VI: Normal Control
Group VII Control given 0.2ml Acacia solution

Table 2: Effect of Extracts on Heamatological Parameters compared to Induced untreated and controls

Animal Grouping	HB (g/dl)	WBC x 10^3	MCV (FL)
Group 1	31.80 ± 1.6	5.30 ± 0.9	69.70 ± 0.5
Group 2	23.60 ± 0.8	3.90 ± 0.5	68.70 ± 0.2
Group 3	26.0 ± 0.9	5.10 ± 0.2	69.30 ± 0.1
Group 4	35.70 ± 0.4	5.60 ± 0.7	67.60 ± 0.2
Group 5	13.70 ± 2.1	16.25 ± 0.5	68.50 ± 0.5
Group 6	14.50 ± 2.5	9.00 ± 0.5	58.10 ± 0.3
Group 7	14.50±0.3	9.00±0.4	57.10±0.3

Mean ± SEM, (n = 5)
Group I: Induced rat treated with 500mg/kgbwt
Group II: Induced rat treated with 250mg/kgbwt
Group III: Induced rat treated with 250mg/kgbwt
Group IV: Induced untreated
Group V: Induced treated with control drug (Fenasteride)
Group VI: Normal Control
Group VII Control given 0.2ml Acacia solution

Table 3: Effect of Extracts on Antioxidant Enzymes

Animal Grouping	GSH (µ/mg)	SOD (µ/mg)	CAT (µ/mg)
Group 1	2.97 ± 0.01	5.54 ± 0.02	37.01 ± 0.20
Group 2	1.36 ± 0.25	4.58 ± 0.31	30.60 ± 0.15
Group 3	0.12 ± 0.95	1.49 ± 0.12	18.26 ± 0.01
Group 4	0.34 ± 0.1	1.72 ± 0.01	13.32 ± 0.03
Group 5	0.74 ± 0.05	0.68 ± 0.15	12.49 ± 0.17
Group 6	1.03 ± 0.15	1.39 ± 0.16	16.38 ± 0.14
Group 7	1.03±0.01	1.30±0.25	16.38±0.02

Mean ± SEM, (n = 5)
Group I: Induced rat treated with 500mg/kgbwt
Group II: Induced rat treated with 250mg/kgbwt
Group III: Induced rat treated with 250mg/kgbwt
Group IV: Induced untreated
Group V: Induced treated with control drug (Fenasteride)
Group VI: Normal Control
Group VII Control given 0.2ml Acacia solution

Table 4: Biochemical Profile of the Extracts compared to induced untreated and the controls

Animal Grouping	Creatinine	Chlorine (Cl)	Potassium (K)
Group 1	0.14 ± 0.25	4.70 ± 0.35	104.00 ± 0.13
Group 2	0.26 ± 0.02	4.70 ± 0.14	115.00 ± 0.18
Group 3	0.91 ± 0.15	4.20 ± 0.22	97.00 ± 0.01
Group 4	0.50 ± 0.05	5.30 ± 0.14	100.00 ± 0.02
Group 5	0.50 ± 0.21	3.20 ± 0.25	90.00 ± 0.05
Group 6	0.50 ± 0.18	3.90 ± 0.01	91.00 ± 0.01
Group 7	0.50±0.10	3.90±0.05	90.10±0.20

Mean ± SEM, (n = 5)
Group I: Induced rat treated with 500mg/kgbwt
Group II: Induced rat treated with 250mg/kgbwt
Group III: Induced rat treated with 250mg/kgbwt
Group IV: Induced untreated
Group V: Induced treated with control drug (Fenasteride)
Group VI: Normal Control
Group VII Control given 0.2ml Acacia solution

Table 5: Result of Phytochemical Analysis of the Ethanolic Extract of *Ganoderma lucidum*

TEST	OBSERVATION	INFERENCE
PHENOL		
Ganoderma lucidum (Ethanolic Extract)	Greenish black precipitate observed	Phenol present
TANNINS		
Ganoderma lucidum (Ethanolic Extract)	Greenish black colour observed	Tannins present
FLAVONOIDS		
Ganoderma lucidum (Ethanolic Extract)	Pink scarlet colour observed	Flavonoids present
SAPONINS		
Ganoderma lucidum (Ethanolic Extract)	Presence of foamy substance	Saponins present
GLYCOSIDES		
Ganoderma lucidum (Ethanolic Extract)	Violet blue or green colour observed	Glycosides present
STERIODS		
Ganoderma lucidum (Ethanolic Extract)	Red colour observed	Steroids present
ALKALOIDS		
Ganoderma lucidum (Ethanolic Extract)	Turbidity of the precipitate	Alkaloids present

Table 6: Result of Phytochemical Analysis of the Ethanolic Extract of *Pleurotus pulmonarius*

TEST	OBSERVATION	INFERENCE
PHENOL		
Pleurotus pulmonarius (Ethanolic Extract)	No greenish black precipitate observed	Phenol absent
TANNINS		
Pleurotus pulmonarius (Ethanolic Extract)	No greenish black colour observed	Tannins absent
FLAVONOIDS		
Pleurotus pulmonarius (Ethanolic Extract)	No pink scarlet colour observed	Flavonoids absent
SAPONINS		
Pleurotus pulmonarius (Ethanolic Extract)	Presence of foamy substance	Saponins present
GLYCOSIDES		
Pleurotus pulmonarius (Ethanolic Extract)	No violet blue green colour observed	Glycosides absent
STERIODS		
Pleurotus pulmonarius (Ethanolic Extract)	No red colour observed	Steroids absent
ALKALOIDS		
Pleurotus pulmonarius (Ethanolic Extract)	No cloudy precipitate observed	Alkaloids absent

Table 7: Result of Phytochemical Analysis of the Ethanolic Extract of *Pleurotus porrigens*

TEST	OBSERVATION	INFERENCE
PHENOL		
Pleurotus porrigens (Ethanolic Extract)	No greenish black precipitate observed	Phenol absent
TANNINS		
Pleurotus porrigens (Ethanolic Extract)	No greenish black precipitate observed	Tannins absent
FLAVONOIDS		
Pleurotus porrigens (Ethanolic Extract)	No pink scarlet colour observed	Flavonoids absent
SAPONINS		
Pleurotus porrigens (Ethanolic Extract)	Presence of foamy substances	Saponins present
GLYCOSIDES		
Pleurotus porrigens (Ethanolic Extract)	No violet blue green colour observed	Glycosides absent
STERIODS		
Pleurotus porrigens (Ethanolic Extract)	No red colour observed	Steroids absent
ALKALOIDS		
Pleurotus porrigens (Ethanolic Extract)	No cloudy precipitate observed	Alkaloids absent

Plate 1: Macrophotograph of *Ganoderma lucidum* Mag X1/6

Plate 2: Macrophotograph of *Pleurotus pulmonarius* Mag X 1/5

Plate 3: Macrophotograph *of Pleurotus porrigens* Mag X1/5

4. Discussion

Postrate cancer is now recognized as one of the major killer diseases worldwide claiming many lives world over. Herbal remedies, dietary supplement and physical activity might be important in the prevention of cancer. Rate of detection of postrate cancer vary widely across the world. Consequently, attention has been focused on the use of plant and herbal remedies believed to be safer and devoid of serious side effects as alternatives in the treatment of postrate cancer. The herbal preparation is one of such remedies prepared from various part of plant such as bark, fruit and herb used locally in the treatment of postrate cancer. Although, increase in appetite and water consumption was observed in the induced rats and normal animals treated with herbal medicine, there was no significant weight gain by the animals. The non-significant weight gain observed in the induced animal treated with the drug suggested that the herbal medicine might not have obesity forming tendency. The effect of the drug on the postrate specific antigens of the induced animal shows the phytomedicine is effective in reducing the PSA level which mean that the drug is as equally good for human consumption as well for commercial industry in treatment of postrate cancer in human. The herbal drug showed an increase in haemoglobin level in the group induced with postrate cancer and proved to have high haemoglobin formation effect than Fenasteride. The effective increase in haemoglobin level demonstrated by this drug support its local use as an antipostrate agent. Also, a significant increase in mole concentration per volume MCV and decrease in white blood cell of the induced compared to the control was observed. According to a report, WBC usually show increase in activity to toxic environment [11] The decrease in GSH and SOD in postrate cancer animals treated with different doses of the herbal medicine implied that the drug at the doses used did not produce any harmful effect on the stimulating hormone and as well as the superoxide dismutate. The high increase in catalase was also observed.

Appreciable recovery of chlorine and potassium level was recorded in the induced animals treated with herbal dug while creatinine showed a slight decrease. Phytochemical Analysis conducted on *Ganoderma* extract revealed the presence of phytochemicals such as Phenol, Tannins, Flavonoids, Glycosides, Steroids and Alkaloids. Phenolic Compounds are one of the largest and most ubiquitous group of plant metabolites [12]. They possess biological properties such as antiapotosis, antiaging, anticarcinogen, anti-inflammatory, antitherosclerosis, cardiovascular protection and improvement of endothelial function [13]. Several studies have described the antioxidant properties of medicinal plant which are rich in phenolic compounds [14]. Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plant in response to microbial infections and they have been found to be antimicrobial substances against a wide array of microorganism *In-* vitro. Their activity is probably due to ability to complex with extra cellular and soluble protein and also with bacterial cell wall [15]. They are also effective antioxidant and show a strong anticancer activity. The plant extracts were also revealed to contain Saponins which are known to produce inhibitory effect on inflammation [16]. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of Saponins include formation of forms in aqueous solutions, heamolytic activity, cholesterol binding properties and bitterness [17]. Steroids have been reported to have antibacterial properties and they are very important compound especially due to their relationship with compounds such as sex hormones [18]. In addition, alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [19]. Glycosides are known to lower the blood pressure according to many reports. The results obtained in this study suggest the identified phytochemical compound may be the bioactive constituent and these plant are proven to be an increasingly valuable reservoir of bioactive compound of substantial medicinal merit, just as suggested by Singh, *et al* [12]. Result of the phytochemical analysis on *Pleurotus pulmonaris* and *Pleurotus porrigens* revealed the presence of saponins alone with other active substances such as phenol, Tannins, Flavonoids Glycosides, Steroids and Alkaloids absent which shows that the potency of the extract may be a function of the number of substance it contained.

5. Conclusion

There was a clear indication that the extract preparation could be safe for use. The study showed that the extract preparation had some antipostrate cancer activity on the postrate specific antigens and also on other parameters examined. The study also revealed that the drug doses investigated did not provoke toxic effect to the animals. The result of the phytochemical screening shows the presence of medicinally important constituents in the plant studied. Therefore, extracts from these plants could be seen as good source of drugs most especially *Ganoderma lucidum*. The traditional medicine practice is recommended strongly for these plant as well as it is suggested that further work should be carried out to isolate, purify and characterize the active constituent responsible for the activity of these plants. Also, additional work is encouraged to elucidate the possible mechanism of action of these extract.

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