



Greener Journal of Biological Sciences

ISSN: 2276-7762 ICV: 5.99

Submitted: 18/05/2016

Accepted: 28/05/2016

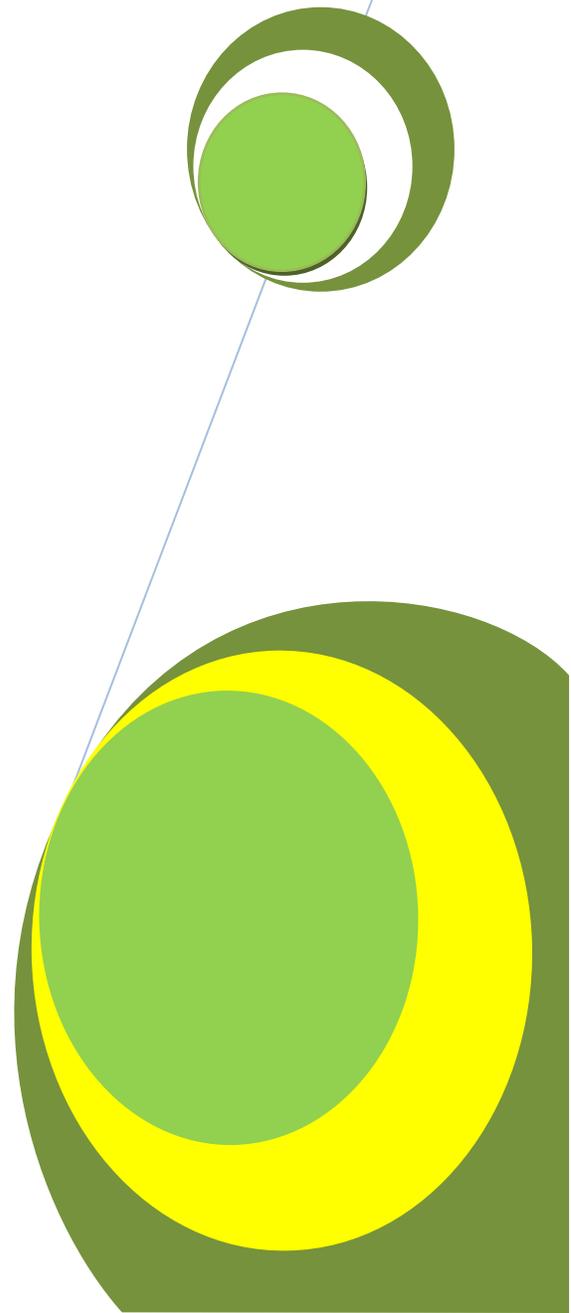
Published: 27/06/2016

DOI: <http://doi.org/10.15580/G.JBS.2016.3.051816091>

Anti-Inflammatory Effect of *Pleurotus ostreatus* (Oyster Mushroom) Aqueous Extracts on *Rattus norvegicus* (Albino Rats)

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Research Article (DOI: <http://doi.org/10.15580/GJBS.2016.3.051816091>)

Anti-Inflammatory Effect of *Pleurotus ostreatus* (Oyster Mushroom) Aqueous Extracts on *Rattus norvegicus* (Albino Rats)

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ABSTRACT

Anti-inflammatory effect of *Pleurotustostreatus* (oyster mushroom) aqueous extract was studied. The extract was prepared using a novel design of relative moisture content to determine the dry weight of the extract. Thirty-five albino rats (grouped into five groups of seven animals per group) were used for the experiment. The five groups were administered oral doses of *Pleurotus ostreatus* at the following dosages: Group one (control with 1ml normal saline), groups two, three and four had treatments with *Pleurotus ostreatus* at 100, 200, and 400mg/kg respectively, while group five was treated with Aspirin (a standard drug) at 15mg/kg as positive control. Egg albumin (0.1ml) was injected at the plantar surface of the right hind paw and with the aid of a locally constructed plethymograph (another novel approach), the paw volume was recorded at the 0,30,60,90 and 120 minutes after albumin injection. The result indicated that the aqueous extract has a significant ($P<0.05$) inhibitory effect on rats paw inflammation. The five groups were not significantly different at the time '0' but at 30 minutes, treatment 4 was significantly ($P<0.05$) different from the control (group one), while at 60 minutes to 120 minutes all the treatment groups were significantly ($P<0.05$) different from the control. During the analysis of percentage inhibition, it was observed that with 100mg/Kg treatment, there were 8.33, 53.84, 57.69 and 67.85% inhibitions at 30, 60, 90 and 120 minutes intervals respectively after albumin injection. Treatment with 200mg/kg of the extract resulted in a percentage inhibitions of 45.83, 76.92, 84.61 and 83.92 at 30, 60, 90 and 120 minutes intervals respectively. The highest inhibition was recorded with 400mg/kg with a 103% inhibition at 120 minutes after injection. The result buttressed that aqueous extract of *Pleurotus ostreatus* has a significant anti-inflammatory property on right hind paw of rats.

Key words: *Pleurotus ostreatus*, anti-inflammatory, Aspirin, inhibition, Egg albumin.

INTRODUCTION

The use of Mushroom as confectionery is dated back to prehistoric era because of its flavor; Though it's nutritional, and especially medicinal properties have just been unfolding in this era. With this new research into the health enhancement of mushroom, it has functioned, not only as confectionery, but also as source of vital nutrients and bioactive agents for therapeutic purposes against many health challenges like high blood pressure, cancer, high cholesterol, pains, inflammation (Wasser 2002). Anti-cataract potential of *Pleurotus florida* *in-vitro* experiment has been reported by Ganeshpurkar *et al* (2011). It's benefits in treating some life threatening illnesses have made it serve as functional food, what is commonly called "nutriceutical". There are many other bioactive and functional phytochemical extract derived from mushrooms (Mizuno *et al* 1995).

Pain and inflammation are among the most common indication of diseases in millions of people worldwide. Chronic inflammation can indicate the sign of various diseases, such as diseases like cardiovascular diseases, cancer, erythematosus, Alzheimer's, and Parkinson's diseases, (Karin *et al* 2006, Huang *et al*, 2010, and Sarkar and Fisher 2006). Synthetic drugs have been used, over the years for the treatment of these diseases; nevertheless, with the advent of new research adventures in traditional medical practices and the use of herbal medical technologies in the treatment of various ailments, including pain and inflammation, the use of mushrooms have been of great benefits in this regard (Huang *et al.*, 2010).

Ganodermalucidum is one of the common medicinal mushrooms. It has been widely used in China and other Asian countries for years to treat various diseases, including cancer. This mushroom is reported to have various bio-active components for the treatment of diseases such as tumor, cancer, pains, bacterial infections etc. [Wang et al., 1997, Yoon et al., 1994, and Eo et al., 2000]. It has also been reported to have an anti-inflammatory and hepatoprotective effect in rats (Lin, et al., 1995).

The objective of the study was to determine the anti-inflammatory effect of aqueous extract of *Pleurotus ostreatus* with a locally constructed plethymograph.

MATERIALS AND METHODS

Pleurotus ostreatus fruiting body was purchased from mushroom house, Faculty of Agriculture Demonstration Farm, University of Port Harcourt, Port Harcourt, Nigeria. It was developed through the process of tissue culture and spawn production to get mycelium mass, which was used to produce the fruiting bodies. These fruiting bodies were dried, pulverized before being used for the experiment.

The animals (*Rattus norvegicus*) (thirty-five) were purchased from the Animal House, Department of Animal and Environmental Biology Choba Campus. They were kept in plastic cages for two weeks to acclimatize to the laboratory condition. During this period, they were kept within an ambient temperature of 25°C with 12 hours daylight, 12 hours night cycle, and relative humidity 65-70%. They were covered with wire gauze and wood shaven used as bedding material. Clean drinking water and feed were provided *ad libitum*.

Preparation of aqueous extract of the *Pleurotus ostreatus*

The preparation of aqueous extract was carried out, using a new innovation to safeguard the protein denaturing in the extract. Five hundred grams of the dried and pulverized *Pleurotus ostreatus* was macerated in two liters of distilled water for 24 hours. After the 24 hours, the sample was decanted and filtered through the Whatman filter paper. The filtrate was measured and placed in a rotary evaporator for dehydration and recovery of the concentrated extract.

After concentration, a little fraction of the sample was measured and evaporated in an oven at a temperature of 105°C to have the weight of the dry extract. It was carried out to guard against the loss of essential constituents of the extracts. This is a novel approach to extract preparation in scientific research whereby the whole extract is not subjected to a high heat hence saving it from protein denaturing that would have occurred in course of subjecting the whole sample to high heat. The dry weight of the extract was then determined by relative moisture content of the two samples.

$$\text{Percentage moisture} = \frac{W_1 - W_2}{W_1} \times \frac{100}{1}$$

Where

W1 = weight of wet sample

W2 = weight of dry sample

The percentage determined was used to calculate the dry weight of the work extract.

From that, the extract concentration of 100, 200, and 400 mg/kg were prepared using an average animal weight of 250g (animals were between 230 and 270g)

Design and Construction of analog plethymograph

A novel concept on locally designed analog plethymograph for measurement of rat paw volume was developed in course of this study. The design and construction of this plethymograph arose from the fact that there were no functional conventional digital plethymograph and many have resorted to the use of thread to measure inflamed paw circumference rather than the inflamed volume (Johnny et al. 2011). Some also used the number of writhing as indicators of inflammation (Fernanda et al. (2008)). With this locally constructed equipment, these challenges were overcome.

It was constructed using locally available materials (sample bottle, a short catheter, a 5ml syringe and a retort stand with clips). A hole was drilled about 1cm from the top of the sample bottle and a catheter was used to connect the sample bottle through the hole to the syringe placed at a level below the sample bottle. The sample bottle was then clipped onto a retort stand (Plate 1). The sample bottle was filled to the level of the catheter with water, and then

the right hind paws of rats were dipped into the water in the sample bottle; any displaced water represented volume of the paw. The displaced water was read in the syringe to the sensitivity of 0.1ml.



Plate 1: Locally Constructed Analog Plethysmograph

Determination of anti-inflammatory potential of mushroom extracts

Albumin-induced paw edema method as described by Winter *et al* (1962) was adopted. 35 albino rats (*Rattus norvegicus*) were used. The animals were kept in a quiet room with a temperature and humidity $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$, respectively and a 12-hour light/dark cycle. Feed and water were given to them ad libitum. All efforts were made to minimize animal suffering.

The animals were fasted overnight, and in the morning they were grouped into five groups (group 1-5), with seven animals per group. Group one was used as the control and were administered 1ml of normal saline, while groups two to four were treated with 100mg/kg, 200mg/Kg and 400mg/Kg of aqueous extracts of *Pleurotus ostreatus* and group five treated with a standard, anti-inflammatory drug (Aspirin) at a dose of 15mg/kg. One hour later, 0.1ml of egg albumin was measured and administered into the subcutaneous tissue of the plantar surface of the right hind paw and the paw volume immediately measured as initial volume at 0 times. The paw volumes were later measured at 30minutes, 60minutes 90 and 120 minutes from the time of inflammation inducement. The percentage anti-inflammatory activity was calculated using the equation:

$$\text{Inhibition percentage (\%)} = \frac{V_t - V_c}{V_c} \times \frac{100}{1}$$

Where V_t is the average paw volume of the treated group and V_c is the average paw volume of the control group

RESULTS AND DISCUSSION

The result demonstrated that the *Pleurotus ostreatus* extracts at different concentrations (100, 200 and 400mg/kg) showed significant anti-inflammatory effects (Table 1) Though at the zero minutes, there was no significant difference between the different paw volumes among the treatment groups, there was a trend of relationships. At the 30minutes interval, there was a significant difference between the control and group four but there was no significant difference between the control and group two, three and five (Aspirin). However, there was also no significant difference ($p > 0.05$) in groups three, four and five. At 60 minutes interval, the control was significantly different ($p < 0.05$) from all the treatments. There was also a significant difference ($p < 0.05$) between group four and groups two and five. But there was no significant difference ($p > 0.05$) between group three and groups two, four and five. At that same interval, also, there was no significant difference ($p > 0.05$) between group two and group five.

At the 90 minutes interval, the control was significantly different from all the treatments, while group two and five showed no significant difference ($p > 0.05$) between them, group three was not significantly different ($p > 0.05$) from groups four and five, but significantly different ($p < 0.05$) from group two.

At the interval of 120minutes, the different samples were more significantly separated. The control was seen to be significantly different ($p < 0.05$) from all the treatment groups. Group four was significantly different ($p < 0.05$) from groups two and three, but it was not significantly different ($p > 0.05$) from group five. However, it was also observed that groups three and five were not significantly different ($p > 0.05$). (Table 1, fig 1)

The graphical representation showed that there was an increase in the volume of inflammation at the first thirty minutes; thereafter, the volume gradually reduced with time in all the treatment groups.

Table 1: Statistical Presentation of Paw Volumes Index at 30minutes Intervals(Mean± Standard Deviation)

Groups	Treatments	pv1 (0min)	pv2 (30min)	pv3 (60min)	pv4 (90min)	pv5 (120min)
Group1 (control)	1 ml normal saline	1.29±.25 ^a	1.97±.21 ^b	2.03 ±.21 ^c	2.03±.25 ^d	2.09 ±.25 ^d
Group 2	100mg/kg extract	1.37±.15 ^a	2.00±.23 ^b	1.71±.23 ^b	1.69±.09 ^c	1.63±.18 ^c
Group 3	200mg/kg extract	1.29±.19 ^a	1.66±.23 ^{ab}	1.46±.21 ^{ab}	1.40±.13 ^{ab}	1.41±.22 ^{bc}
Group 4	400mg/kg extract	1.11±.25 ^a	1.51±.25 ^a	1.20±.16 ^a	1.14±.15 ^a	1.09±.11 ^a
Group5	15mg/kg Aspirin	1.14±.22 ^a	1.70 ±.17 ^{ab}	1.54 ±.19 ^b	1.49±.20 ^{bc}	1.29±.17 ^{ab}

Values on the same column having the same superscript (a, ab, b, bc, c.) letter are not significantly different.

Increment in Paw Volume

Paw volume was observed to increase at a particular pattern. The control had 68.57% increase at the first 30minutes, while groups two, three, four and five had 62.86%, 37.14%, 40.00% and 55.71% respectively. However, at the end of 120minutes it was observed that the control still had a high percentage increase (80.00), while the groups two, three, four and five had percentage increase of 25.71, 12.86, -2.86 and 14.29%. This demonstrates the effect of the extract on the paw as an anti-inflammatory substance. It was especially seen that at group four, the paw volume was even less than the volume at the zero minute. This shows that even the effect of the initial volume of the injected egg albumin was not observed at 120 minutes time. (Table 2, Fig 2)

Table 2: Average percentage increase in paw volume

Groups	Treatments	Pv2-pv1 (30min) (%)	Pv3-pv1 (60min) (%)	Pv4-pv1 (90min) (%)	Pv5-pv1 (120min) (%)
Group1 (control)	1 ml normal saline	68.57	74.29	74.29	80.00
Group 2	100mg/kg extract	62.86	34.29	31.43	25.71
Group 3	200mg/kg extract	37.14	17.14	11.43	12.86
Group 4	400mg/kg extract	40.00	8.57	2.86	-2.86
Group5	15mg/kg Aspirin	55.71	40.00	34.29	14.29

Percentage paw inflammation inhibition by the *Pleurotus ostreatus* extracts

The percentage paw inflammation inhibition by the extract was analyzed and it was found that, compared to the control, the treatment groups have inhibitory effects as recorded in table3. From the result it was observed that at 30minutes interval, the aqueous extract has 8.33% effects on group two, while groups three, four and five (standard drug) had percentage inhibition of 45.83,41.67 and 18.75 respectively. While at 120minutes, the inhibition rates for groups two, three four and five drugs were 69.64,83.92,103.57and 82.14 respectively as they were compared to the control. (Table 3 Fig 2)

Table 3: Percentage inflammation inhibition by *Pleurotus ostreatus* extracts and Aspirin (standard drug)

Groups	Treatments	30min	60min	90min	120min
Group 2	100 mg/kg extract	8.33	53.85	57.69	69.64
Group 3	200mg/kg extract	45.83	76.92	84.62	83.92
Group 4	400mg/kg extract	41.67	88.46	96.15	103.57
Group 5	15mg/kg Aspirin	18.75	46.15	53.85	82.14

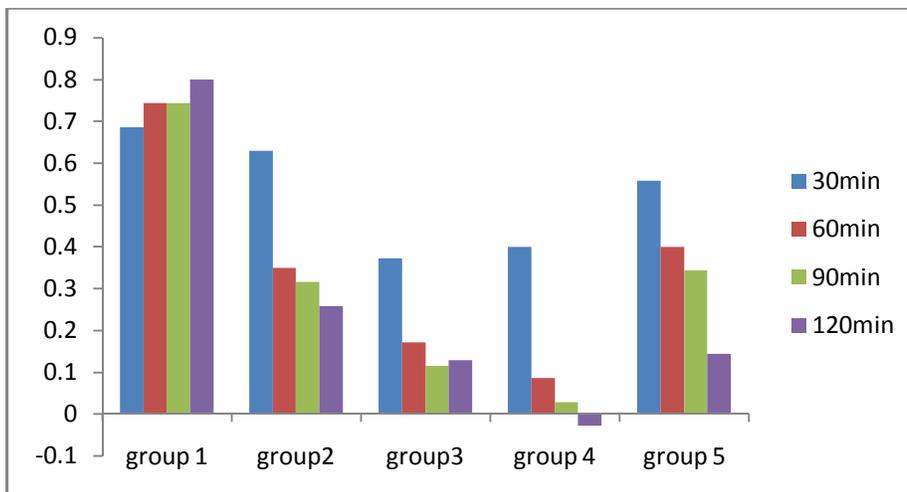


Fig.1: Average Increase in Volume

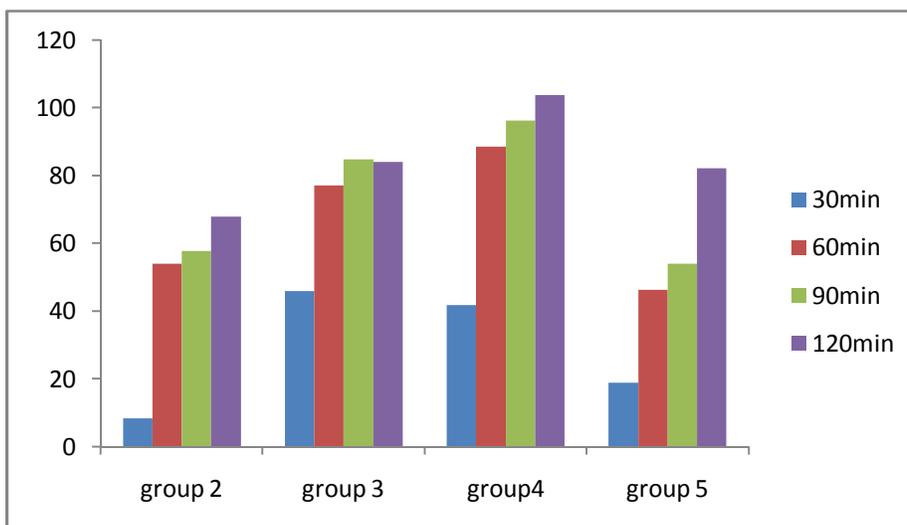


Fig. 2: Percentage Inflammation Inhibition of Aqueous Extract of *Pleurotus ostreatus*



Plate 2: Right Hind Limb Injected with Egg Albumin for Inflammation

DISCUSSION

This work has two novel approaches to scientific research. These novel ideas have been helpful in conducting this study. One of it is the process of extracting and dehydrating aqueous extract of *Pleurotus ostreatus*. It uses the principle of relative moisture analysis whereby, the entire extract was not subjected to high heat dehydration because high heat above certain temperature denatures protein hence, reducing its efficacy. With this novel approach, a little sample of the extract was subjected to high heat of 105°C (which most proteins will not remain viable) to really have its dry weight and then the solid matter of the fresh sample determined. This method preserves the protein and enhances much better result.

The second important and novel discovery in this work was the construction of analog plethymograph. This was design based on the scarcity of functional digital plethymograph. Lack of functional Plethymograph had made researchers to conduct anti-inflammatory studies using thread to measure inflammation which does not directly measure volume but the circumference of the inflamed paw (Johnny *et al.* 2011). With this new locally constructed analog Plethymograph, there could be great improvement in anti-inflammatory studies without necessarily resorting to the use of a sophisticated equipment and yet having the same result.

Result of this study revealed that the extract had a strong anti-inflammatory effect on the right hind paw of Wistar rats. It demonstrated that the extract at a concentration of 200mg/kg, had a higher percentage inhibition when compared to the standard drug at a normal dose of 15mg/kg. This supports the finding of Aditya and Gopal(2013)and many other earlier researchers who have found that mushroom extract has anti-inflammatory properties. The distinctively significant difference among the treatments is an indication that the extract really has active anti-inflammatory property. My observation of more than 100% inhibition in the 400mg/kg at 120 minutes could be trace to the fact that the egg albumin injected at the planter surface temporarily elicits some expansion, of which the extract also dried it up at the high dose. This may look debatable but could be traced to the fact that the extract has a stronger effect on the paw inflammation.

The result of the control suggested that without any anti-inflammatory drug, there were still some inherent anti-inflammatory properties in the rats which were expressed by a gradual reduction in inflammatory rate over the period under study. This was observed that within the first thirty minutes, the control had 68.75% inflammatory rate but in the following one hour thirty minutes, it only had 11.43 % inflammatory rate (Table 2).

CONCLUSION

From the result, it is found that the aqueous extract of *Pleurotus ostreatus*(oyster mushroom) has a significant anti-inflammatory property as expressed in the albino rats. This further demonstrated the medicinal properties of *Pleurotus ostreatus*. Another conclusion from this study is that researches with locally constructed equipment can give some degree of reliable result. It can also be said that this novel method of extract preparation and dehydration be viewed closely and generally adopted as a way of carrying out plant and animals extract preparation and dehydration.

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Cite this Article: Zitte LF, Konya RS and Akpiri RU (2016). Anti-Inflammatory Effect of *Pleurotus ostreatus* (Oyster Mushroom) Aqueous Extracts on *Rattus norvegicus* (Albino Rats). *Greener Journal of Biological Sciences*, 6(3):061-067, <http://doi.org/10.15580/GJBS.2016.3.051816091>