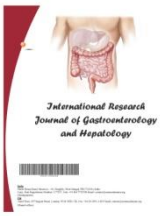

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Alterations in Hepatic Functions in Administration of *Pennisetum puerperium* to Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Part of its metabolic function, the liver; which is a vital organ of all vertebrates is exposed to numerous toxins that may alter its physiologic functions. This functional tendency is often implicated via specific markers whose levels can be assayed to ascertain the functional capacity of the organ. Current study examined the changes in levels of liver enzymes following administration of *Pennisetum puerperium (P. puparium)* aqueous leaf extract to albino wistar rats. Twenty (20) adult wistar rats weighing between 130 g – 150 g were used. The rats were grouped into four (4) of five rats each. Group I received normal rat feed, groups II to IV received 200 mg/kg, 300 mg/kg and 400 mg/kg body weight of aqueous *P. puparium* leaf extract respectively for a period of After the period of administration of the extract, rats were euthanized and blood samples for biochemical assay of

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Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP) activities. The relative body and liver weights were also determined. Findings of the research study showed that the administration of doses of the plant extract (200, 300 and 400 mg/kg body weight) to animals significantly produced an increased ($p < 0.05$) serum levels of liver enzyme markers (AST, ALT and ALP) when compared with the control group. There was also a significantly increased relative body and liver weights following administration of all doses of the plant extract to animals when compared with the control group. It can be concluded that *P. puparium* aqueous extract may possess deleterious effects on the hepatic functions of the animals at the various doses of the plant extract tested in this present study.

Keywords: Liver; Pennisetum puparium; biomarker.

1. INTRODUCTION

The liver is an important organ of vertebrates and few other lower animals [1,2] its array of functions such as detoxification, protein synthesis and production of biochemicals necessitate its key significance in digestion process (es). In mammals, the liver is vital for survival in life with no currently known way of compensating for its functional absence in long term, although there appears to be new techniques to circumvent this in short term [3].

The liver's main job is to filter the blood coming from the digestive tract before passing it to rest of the body, the liver also detoxifies chemical and metabolizes drugs as in the intestine, the liver also makes protein important for blood clothing and other functions [4,5].

Recently, a good number of traditional herbs have been implicated in the prevention and treatment of liver related ailments; as well as in the improvements of hepatic functions. *Artemisia annua* [6], the leaves of *Guinesis* unripe fruit of *capsicum frutescenee,* stem bark of *chrysophyllum albidum* [7], *kaya grandifolia* [8]*, Azadirchta indica (Dogon yaro)* [9]*, Zingiber officinale* [10], *Vernonia amygdalina* [6] and *Garcina kola* [7], to mention a few.

Pennisetum purpureum is a robust grass with perennial stems. The plants produce short, creeping rhizomes 15 to 25 cm long with fine roots at the nodes and culms that are from 2 to 8 m in height, up to 2.5 cm in diameter at the base, and has a solid center [9]. Older culms may branch several times. Leaf blades are 50 to 90 cm long and 1 to 3 cm wide, flat, and have a white midrib. Leaves of new, vigorous growth have wide, robust leaves; older culms have finer, narrow leaves. Leaf margins are rough (fine-toothed). The inflorescence is a compact, erect, bristly tawny or purplish spike 8 to 30 cm long and 1.5 to 3 cm wide.

The plant *Pennisetum puerperium* has been reportedly used as a diuretic in anuria or oliguria and also as a source of medicinal salt [11]. It has also been suggested that *Pennisetum purpureum* possess hepato-protective effects, even though no scientific research has been published to validate this claim [12]. Hence, this study was undertaken to evaluate the effect of *Pennisetum puerperium* on liver functions of wistar rat over a long period of time.

1.1 Aim of Study

Current study determined the effect of *Pennisetum puerperium* on liver functions of wistar rat over a long period of time. Specifically, the study evaluated the effects of *Pennisetum puerperium* general body weights of wistar rat. Study also investigated the effect of *Pennisetum puerperium* on the activities of selected liver markers; Alanine Amino-Transferase (ALT), Aspartale Amino-Tranferase (AST), and Alkaline Phosphate (ALP).

2. MATERIALS AND METHODS

2.1 Study Design

Due to the invasive nature of the research, study was designed to be suited for rats, and centred on examining the effects that aqueous extract of *Pennisetum puerperium* has on selected markers of liver functions; Alanine Amino-Transferase (ALT), Aspartale Amino-Tranferase (AST) and Alkaline Phosphate (ALP). First, twenty (20) rats of average body weights between 130–150 g were procured and acclimatized for four (4) weeks in the Animal house of the Ambrose Alli University, Ekpoma, Edo State; following which they were grouped into four (4) of five rats each.

With group I receiving normal rat feed and water for 28 days (4 weeks), groups II to IV received 200mg/kg, 300 mg/kg and 400 mg/kg of aqueous *Pennisetum puerperium* leaf extract respectively.

2.2 Procurement, Preparation and Identification of Plant

Fresh *Pennisetum puerperium* leaf was obtained from local farms in Ekpoma, thereafter; it was identified by expert taxonomists from the Department of Botany, Ambrose Alli University, Ekpoma, Edo State. The leafs were then dried for two weeks using sunlight, and then, crushed (with pestle and mortar) and grinded into powder for subsequent addition to rat feeds following standardization.

2.3 Standardization of *Pennisetum puerperium*

Three different doses of *Pennisetum puerperium* extract were made into stock solutions of Low, medium, and high doses, by weighing 30 g, 40 g and 50 g of dry *Pennisetum puerperium* with the aid of an electronic weighing balance. This was then homogenized by dissolving in 100 ml of distilled water after filtering with the Wattmann filter paper. These gave stock solutions of 200 mg/ml, 300 mg/ml, and 4000 mg/ml that were fed to different groups at different doses.

2.4 Blood Sample Collection

After two (2) weeks of extract administration, animals were humanely sacrificed via cervical dislocation with blood samples obtained via cardiac puncture, using a 5ml syringe. Thereafter, collected samples were then kept in an EDTA container to prevent coagulation; and then, transported to laboratory for liver function test.

2.5 Determination of Body Weight

Rats' body weights were determined weekly using a digital balance, this was recorded in grams.

2.6 Determination of ALT and AST Activities

The activities of the aminotransferases were determined using Randox laboratories reagent kits based on the method of Reitman and Frankel (1957). It involves the transamination of the amino acids by the enzymes. The keto acids produced will then form a reaction with 2, 4, dinitrophenylhydrazine, the corresponding coloured hyrazones which was read at a wavelength of 45 mn using a spectrophotometer (spectrophotometer AJ – 105 made in England). The activities of the enzymes were extrapolated from a standard curve and expressed as unit 1ml in which one unit is defined as the amount of enzyme activity in 1 ml of serum that will lower the absorbance by 0.001 in 1 minutes under the condition of the method.

2.7 Determination of Alkaline Phosphate Activity

Alkaline phosphatase activity was determined by the method of Annino and Gese (1976). When p-nitrophenolphosphate is incubated with alkaline phosphate, it is hydrolysed to p-nitrophenol (with a change in colour from almost colourless to yellow I alkaline solution). Addition of sodium hydroxide stops the enzyme reaction and brings out the colour of the p-nitrophenol which was estimated at 410 nm using spectrophotometer. The activity of the enzyme is expressed as units 1ml and one unit is the amount of the enzyme activity that will liberate 1 micro mole of p-nitrophenol per hour under the conditions of the method. The concentration of plasma creatinine, urea were also determined by the methods of Annino and Gese (1976).

2.8 Ethical Issues

Experimental protocols were executed in strict compliance with the commendations and guides for the care and use of laboratory animals. Study adhered to the code of conduct stipulated by the Institute for Laboratory Animal Research [13].

2.9 Statistical Analysis

Results were expressed as mean± SEM (standard error of the mean) and the statistical significance of the treatment and the control groups of the animals was determined using the ANOVA. While the t-test was used for the statistical determination of the relative body to organ weights of the experimental and control groups. The data of p-values ≤ 0.05 were considered to be statistically significant.

3. RESULTS

See tables below for detailed presentation of results following data collection.

Table 1. Change in body weight of *Pennisetum puerperium* administration to Wistar rats

Body weight (g)			
Groups	**Initial**	**Final**	**Weight change**
1.	184.00	203.00	19
2.	137.10	157.30	20.2
3.	129.30	160.10	30.8
4.	123.90	146.80	22.9
Average	143.58±13.75	166.80±12.40	23.23±2.65

Values are expressed as mean ± Standard of mean (S.E.M), n=5

Table 2. Change in liver weight of *Pennisetum puerperium* administration to Wistar rats

Liver weight (g)			
Groups	**Initial**	**Final**	**Weight change**
1.	18.00	20.00	2.00
2.	23.10	17.30	6.20
3.	19.30	16.10	3.20
4.	23.90	16.80	7.10
Average	21.08±2.87	17.55±1.71	4.63±2.42

Values are expressed as mean ± Standard of mean (SD), n=4

Table 3. Statistical comparison of liver weight changes in *Pennisetum puerperium* administration to Wistar rats

Liver organ weight (g)							
	Control	***P. puerperium* extract**	**t Stat**	**P(T<t)**	**t-crit**	**p-value**	**Remark**
Liver $Weight_{(g)}$	5.1000±0.49160	7.1000±0.62849	2.709	0.0366	2.353	0.05	Sign

Values are expressed as mean ± Standard error of mean (S.E.M), n=5. P<0.05: Significant as determined by student t-test: paired two sample for means. Significant difference p-value < 0.05 = increase when compared control group

Table 4. Comparative changes in selective liver enzymes in *Pennisetum puerperium* administration to Wistar rats

Serum liver enzymes (mg/dl)								
Groups	**M±SD**	**Parameters**	***Pennisetum puerperium* extract**	**t Stat**	**P(T <= t)**	**t-crit**	**p-value**	**Remark**
2	90.35±3.00	ALT	89.12±3.27	0.224	0.419	2.353	0.056	Sig.
3	16.75±1.11	AST	17.50±0.87	0.634	0.285	2.353	0.023	Sig.
4	62.60±0.72	ALP	66.49±4.39	0.892	0.219	2.353	0.031	Sig.

Significant difference p-value < 0.05 = increase when compared control group. M = Mean, SD = Standard Deviation

4. DISCUSSION

P. Puerperium makes up the bulk of the diet of forest elephants in West Cameroon (Tchamba and Seme 1993). It is an important forage and pasture grass in its native Africa and throughout the Tropics, especially for cattle. Overtime, Extract from the species has been employed successfully as strong diuretics, and are used for that purpose in Africa. It is also used in a number of other herbal remedies (Burkill, 1994). The seeds are eaten by many bird species. However, because of the aggressive spread of the species, it is a menace to native vegetation in the Galapagos Islands (Mauchamp, 1997) and at the margins of swamps and streams in Florida (Miami-Dade County 2002).

P. Puerperium is herbaceous weed widely distributed throughout the world; it is known indigenous tribes of Nigeria [14-16]. The plant has been used as vegetables and for medical purpose for hundred of years, the ancient Egyptians use it for heart failure and heart diseases. It has been used as antibacterial, antidiabic and pain relieve in hearmorrehoid and

whit low [13]. Despite the intake of *P. Puerperium* it is edible because it serves as vegetable to the Europe and Middle East, Asia and Mexico it also serves as omega-3 fatty acids than any other leafy vegetables plant.

P. purpurum generally contain diterpenoids, triterpenoids, alkaloids, flavonoids, lignoids and proanthocyanidins [17], which have strong antiplasmodial activity. Therefore, the antiplasmodial activity observed in this study may be attributed to the presence of these bioactive compounds. This result is similar with that obtained from previous publications [18-20]. To this point, current study investigated the effect of the leaf extract on selected liver enzymes. The study also examined body weight changes due to administration of extract on albino wistar rats.

Findings from current study showed that data was subject to statistical analysis using students t-test. It shows that in control group initial weight shows moderate significant increase when compared to final weight and P*ennisetum puerperium* aqueous leaf extract treated groups initial show no significant when compared to final. From current study, both high (400 mg/kg) and low dose (200 mg/kg) of *Pennisetum purpurum* leaf extracts exhibited a statistically significant increase on all liver markers that were examined (Table 4). However, only the low dose (200mg/kg) of the leaf extract showed a significantly decreased effect on body weight of group III treated rats. Thus, result of this study may justify the traditional use of the plant for hepato-protection therapy in humans [21-22].

A careful look at Table 4 shows the comparative changes in liver AST, AST and ALP levels in *P. Puerperium* administration to wistar rats as against the control. From the table, average ALT level sis seen for *P. Puerperium* treated group as compared with control. This proved to have significantly increased ($p < 0.05$) in comparison with other assayed liver enzymes across groups, implicating *P. Puerperium* alcohol extract to cause an increase in ALT level than other enzymes assayed. Frederich et al. (2008) had reported a similar result on the effect of *P. Puerperium* aqueous extract on selected liver markers. Result from this work concurs with that of Frederich et al, however disagreeing with most of other similar reports on hepato-protective effects of *P. Puerperium* aqueous extract.

Again from Table 2, a higher average weight is observed for group IV rats as compared with those of other groups. This weight change was almost slightly accompanied by increase in liver enzymes for all test groups as compared with control. This also implicates *P. Puerperium* as a potent incrementer of body weight. Even though its exact mechanism of doing this may not fully be explained. However, chances are that it increases metabolism of lipids to increase triglyceride build-ups in adipocytes [6].

5. CONCLUSION

From this study, it can be concluded that at different doses (200 mg/kg and 400 mg/kg), aqueous leaf extract of *Pennisetum purpurum* may increase liver markers and enzyme activity as evidenced by the statistically significant increase seen in fed rats (Table 4) above in a dose dependent manner, which may partly justify the claim by traditional practitioners about the use of this plant in hepato-protection.

6. RECOMMENDATIONS

We recommend further evaluation of the plant with more sophisticated approach, especially dwelling on its active phytochemicals. Also a need to identify its active ingredients responsible for the observed increment in liver enzyme activity is imperative.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic Committee approval has been collected and preserved by the author.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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