IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008.Volume 5, Issue 4 (Mar. – Apr. 2013), PP 81-86
www.iosrjournals.org

Sub-Chronic Effects of MethanolicBenth Bark Extract Of *BrideliaFerruginea*on Some Selected Biochemical Indicesin Rats

*[1, 2]Salau, B. A., Adeleke Atunnise[1], Oluwaseyi OSodipo[1], Oluwafemi E. Kale[3], E. O. Ajani[4]

[1]*Department of Biochemistry, ObafemiAwolowo College of Health Sciences, Ikenne Campus, OlabisiOnabanjo University, Ago-Iwoye, Ogun State.*
[2]*Department of Chemical Sciences, College of Natural Science, Redeemer's University, Km 46, Lagos/Ibadan Expressway, PMB 3005, Redemption City Mowe, Ogun State, Nigeria*
3Department of Chemical Pharmacology, ObafemiAwolowo College of Health Sciences, Ikenne Campus, OlabisiOnabanjo University, Ago-Iwoye, Ogun State.
[4]*Department of Bioscience and Biotechnology, College of pure and Applied Sciences, Kwara State University, Ilorin, Nigeria*

Abstract:*Recently, there have been growing concerns over the reliance and safety of untested and unregulated plant remedies. Brideliaferruginea is one of the herbal plants commonly used, of which chronic toxicity studies are yet to be examined. Sub-chronic toxicological effects of methanolicbenth bark extract of brideliaferruginea on biochemical indices in rats were evaluated. Three groups of six animals each were used in this study. Group 1 (control)received no treatment. Group 2 and 3 (treated)received calculated doses of 250mg/kg and 500mg/kg of the extract orally for four weeks. All animals were sacrificedusingdiethylether anesthesia 24hours after the last dosing and blood samples taken. Periodic weights and packed cells volume(PCV) were measured, portions of the heart and brain were excised, homogenized and used for bioassays. From our results, we observedsignificant decrease ($p<0.05$) in PCV; superoxide dismutase in Heart and Brain; and brain catalase. No significant effect ($p>0.05$) was observed in heart and brain;reduced glutathione;catalase, also no effect was observed in lipid peroxidation of heart and brain. However, the two doses kept the weight constant for a period of four (4) weeks. Overall, thebark extract of brideliaferrugineamay affect antioxidant and blood status as well as keeping body weight constant. We suggest therefore thatdoses approaching or above 250mg/kg body weight should be avoided by individuals or in herbal preparations.*

Keywords:*Herbal plants, Brideliaferrugineabenth bark, Antioxidants, PCV and Body weight.*

I. Introduction

Traditionally, herbs and herbal products have been considered to be non-toxic and have been used to treat a range of ailments(Bodeker et al., 2005) however, about three billion of the world's population, living in developing countries now consume herbal medicines as their source of primary health care (Bodeker et al., 2005; Ernst,2000;Farnsworth et al., 1984). Commonpractice among the populace is the use of herbal preparations as prophylactic measure even when apparent disorders or symptoms are absent. Studies have shownacute toxicities resulting from the use of herbs on many occasions. Still, the potential toxicity of several herbs and herbal products has not been recognized (Chan, 2003).Convincingly, with the many ongoing pharmacological screening tests, ithas beenfound that pharmacologically inactive substances may interfere with or enhance the potency of activecompounds (Jowell, 1999; Chan, 2003), which may affect the body adversely.

B*rideliaferruginea*, which is the commonest savannah *bridelia*, has attracted several importance to itself and the populace. Studies have shown its anagelisics and antipyretic(Akuodoret al., 2011) antimicrobial activity (Adeoye et al., 1998) and water purification properties (Kolawole and Olayemi 2003).Itsleaves and fruits were used locally as purgative and vermifuges (Cimanga et al., 1999). Iwu (1984) describeditsmolluscidal activity.Other studies described its anti-diabetic properties(Adewale and Oloyede, 2012;Lotikar and Rajarama-Rao, 1966) among other usefulness.

However, in spite all these, it is evident that the fact that something is natural does not necessarily make it totally safe or effective. This is because majority of the active ingredients of plant extracts are also chemicals and as thus, are similar to those in purified medications. Therefore, they may due to chance by no exceptions possess the same potentials to cause serious adverse effects (Cupp, 1999; D'Arcy, 1993; D'Arcy, 1991).

Till now, studies involving biochemical changes of the methanolic bark extract of *brideliaferruginea*on vital organs such as heart and brain have not being reported. Thus, in this present study, we evaluated the possible effects of the methanolic bark extract of *brideliaferruginea*on biochemical indicesduring a sub-chronic dosing in rats in order to ascertain the *in vivo*safety and efficacies of biochemicalactivities of this plant.

II. Material And Methods

2.1) PLANT MATERIALS AND PREPARATIN OF EXTRACTS

Some fresh bark*brideliaferruginea* were collected at around 3:30 pm from the Awolowo mini-market, some few miles away from the College of health Sciences, OlabisiOnabanjo University, Sagamu, Ogun state, Nigeria. The identification and authenticity of the plant was done at the Forestry Research Institute, Ibadan, Nigeria.
Exactly 200g of the pulverized sucker was soaked in 70% methanol for 48hours, the extract was filtered and then concentrated with rotary evaporator. The dried product was kept in air tight contain and the required dose was reconstituted in daily basis and administered.

2.2) EXPERIMENTAL SUBJECTS AND MANAGEMENT

Eighteen (18) albino rats (males and females) of average weight 200g were obtained from the animal house of the University of Ibadan, Oyo-State, Nigeria. The rats were housed within the experimental animal handling facility of the Department of Biochemistry, Faculty of Basic Medical Sciences, OlabisiOnabanjo University, Ogun State, Nigeria at ambient temperature and humidity with a 12 h light/12 h dark schedule. These rats were placed on rat chowduring the two weeks of acclimatization and experimental period. Study was in accordance with established guidelines for care and use of the laboratory animals in biomedical research.

2.3) ANIMAL GROUPING

G1: Normal control
G2: Group administered with 250mg/kg body weight of methanolic extract of *brideliaferruginea*
G3: Group administered with 500mg/kg body weight of methanolic extract of *brideliaferruginea*
All administration was oral, as a single dose using orally intubator on a daily basis for a period of four (4) weeks.

2.4) LABORATORY ANALYSIS

Body weight of the rats were checked on weekly basis for the period of four (4) weeks.Packed Cell Volume (PCV) was measured by using method of Dacie and Lewis (1991); superoxide dismutase (SOD) activities was analyzed using method of Marklundand Marklund 1974 whilst reduced glutathione (GSH), catalase activities and lipid peroxidation (LPO) were carried out using methods of Beutler et al. (1963), Sinha(1972) and Varshney and Kale (1990) respectively.

2.5) STATISTICAL ANALYSIS

The data were analyzed using one-way ANOVA, Level of significance was assessed using Duncan Multiple range (DMRT) at $p<0.05$ (SPSS 14.0 software was used for data analysis).

PHYTOCHEMICAL TESTS

The methanolic bark extracts of *brideliaferruginea*were tested for the presence of some vital plant active ingredients.As described bySofowora (1982)

III. RESULTS

TABLE 1: Phytochemical screening tests of the methanolic bark extracts of *Brideliaferruginea*

ACTIVE CONSTITUENTS	rf. Value	CONCENTRATIONS
Alkaloids	0.46	++
Tannins	0.30	++
Saponins	0.85	+
Terpenoids	0.39	++
Steroid	0.56	+
Phlobatannins		++
Glycosides, Flavonoids	0.30-0.85	-
Anthraquinones	0.30-0.85	-

Keys: ++ = Highly present; + = Present; - = Not present; rf. indicates retention factor with respect to concentration.

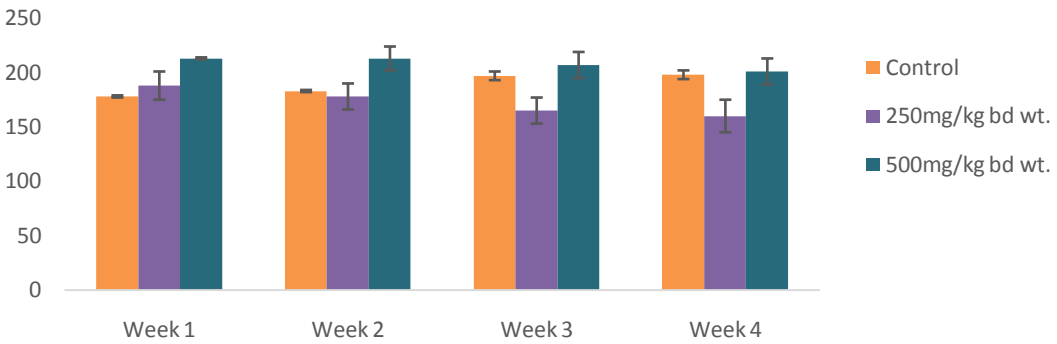

Fig 1: Effect of different concentrations of Methanolic extract of *Bridelia Ferruginea* extract on body weight in gram (g)

TABLE 2: Effects of sub-chronic administration of methanolic bark extracts of *brideliaferruginea*on packed cells volume in rats.

Rat group	PCV%	% change from control
G1	51.10±3.64	
G2	41.00±0.84[a]	-19.77
G3	42.8±2.22[a]	-16.24

Means±SEM for 6 rats per group, Values within a column with superscripts are significantly different at $p<0.05$ when compared with control (G1).

TABLE 3: Effects of sub-chronic administration of methanolic bark extracts of *brideliaferruginea*on superoxide dismutase (SOD) activities in heart and brain in rats.

	SOD (units/g)			
Rat group	Heart	% difference from Control	Brain	% difference from Control
G1	30.18±4.30		21.11±3.22	
G2	15.32±4.32[a]	-49.24	16.67±0.42[a]	-21.03
G3	13.18±2.04[a]	-56.33	12.66±1.34[a]	-40.03

Means±SEM for 6 rats per group, Values within a column with superscripts are significantly different at $p<0.05$ when compared with control (G1).

TABLE 4: Effects of sub-chronic administration of methanolic bark extracts of *brideliaferruginea*on reduced glutathione (GSH) levels in heart and brain in rats.

	GSH (µmol/mg protein)	
Rat group	Heart	Brain
G1	1.90±0.54	1.42±0.31
G3	1.84±0.21	2.50±0.35
G4	2.39±0.25	2.31±0.28

Means±SEM for 6 rats per group.

TABLE 5: Effects of sub-chronic administration of methanolic bark extracts of *brideliaferruginea*on catalase (CAT)activities in heart and brain in rats.

Rat group	CAT ($\mu molH_2O_2/min$) Heart	% difference from Control	Brain	% difference from Control
G1	3.00±0.66		55.83±14.62	
G2	5.16±1.10	72.0	14.01±0.60[a]	-74.91
G3	3.5±0.50	16.67	15.48±0.36[a]	-72.27

Means±SEM for 6 rats per group, Values within a column with superscripts are significantly different at $p<0.05$ when compared with control (G1).

TABLE 6: Effects of sub-chronic administration of methanolic bark extracts of *brideliaferruginea*on lipid peroxidation (LPO) levels in heart and brain in rats.

Rat group	LPO (µmol/mg protein) Heart	Brain
G1	4.23±1.06	1.74±1.02
G2	4.58±0.72	4.01±1.06
G3	6.30±0.46	2.29±1.33

Means±SEM for 6 rats per group.

As shown in table 1: The methanolic bark extracts of *brideliaferruginea*were tested for the presence of some vital plant active ingredients. We vary the retention factor (rf.) from 0.30, 0.39, 0.46, 0.56 to 0.85; alkaloids, tannins and terpenoids were highly present than flavonoids,saponins and steroid in this extract at varying rf.Values as indicated by table 1. Other active ingredients which tested negative were glycosides, anthraquinones, as seen in this study.

Figure 1showed body weights in grams forthe period of four weeks. Increase ($p<0.05$) in weight was observed when the initial and final weights of the control group were compared. However, no significant ($p>0.05$) difference was observed with the initial and final weights of the extract treated groups.

Table 2showed the PCV of control and experimental groups treated with *methanolic* extract of brideliaferruginea. A significant decrease ($p<0.05$) was observed when the experimental groups (G2: 41.00±0.84; G3: 42.80±2.22) were compared with the control group (51.10±3.64%).

Table3, is the Superoxide dismutase, significant decrease ($p<0.05$) was observed in Heart and Brain when the experimental groups were compared with the Control group.

Table 4showed the reduced glutathione of control and experimental groups *brideliaferruginae*, No significant difference ($p>0.05$) was observed in both control and treated groups.

Shown in table 5 is the catalase activities levels of control and *brideliaferruginea*methanolic extract treated groups. Significant decrease was observed in catalase activities in the brain when control group was compared with experimental groups. However, such effect was not observed ($p>0.05$) in the heart tissues.

Table 6 depicts lipid peroxidation of rats treated with *brideliaferruginae*methanolic extract, no significant difference was observed ($p>0.05$) when the control groups were compared with experimental groups in the heart and brain tissues.

IV. Discussion

Chemicals, whether synthetic or natural has the capacity to be toxicand plants are no exception. The use of phytomedicineexpecially in developing countries is now increasing in leaps and bounds with apparent confidence that the natural is less toxic. However, this resurgence use of herbal drugs has been reported to be accompanied by increasing risks of adverse effects associated with herbal active ingredients (Chan, 2003). In our study, we evaluated the possibleeffects of methanolic bark extract of *brideliaferruginea*on biochemical indicesduring a sub-chronic oral administration in rats. From the result of this study, the phytochemical

screening of methanolic bark extracts of *brideliaferruginea*, showed the presence of bioactive compounds which may singularly or synergeticallyaffect the biochemical parameters observed.
The constancy in weight of the *brideliaferruginea*methanolic treated rats may be as a result of components such as saponin (Gogelein and Huby, 1984)and high level of tannin (Mukuru et al., 1992) which have been shown to be growth depressant by complexing protein. Thus, its intake may be of benefit for weight maintenance and reduction, but may have adverse effect on growing children worse still the teratogenic effect on growing foetusis plausible if used during pregnancy.

Reduction in PCV observed ($p<0.05$) could be attributed to the presence of tannins which binds metals such as ferrous required for red blood cell formation (Karamac et al., 2009) and saponin which may interfere with the red blood cell status (Takechi and Tanakar, 1995), thusmay cause aenemia in a long term.However, moderate use of the extract may improve haemorrhological status of the blood as a result of reduced PCV (Lowe, 1993) which may have positive effect on cardiovascular health.
The significant decrease in superoxide dismutase activities observed in rat (heart and brain) treated with Brideliaferrugineamethanolic extract could be as a result of possible decrease in blood protein that might have been caused by saponin and tannin present in extract, moreover, the chelating effect of tannin on Copper and Zinc (Karamac, 2009) may adversely affects the level of SOD both in cytosol and mitochondria.
Catalase is one of the first line antioxidant enzymes in the defense against free radicals (Lammi-Keefe *et al* 1984).Its level is affected by feed intake and free radicals concentration (de Castro1 et al., 2009). The plausible reduction in catalase observed in brain tissue may be as a result of toxic effect of saponin that may complex or reduce protein utilization (Rashef et al., 2006). And high content of tannin and phlobtanin which reduce the level of iron that is necessary for production of catalase (Lavin, 2010).
Insignificant difference ($p>0.05$) observed in lipid peroxidation might indicate the level of free radical production or reduction in antioxidant activities which does not reach a critical stage that may affect the body adversely. However, the reduction in PCV and lack of increase in weight may have either positive or negative impact.

V. Conclusion

This result demonstrated the effect of methanolic extract of Brideliaferruginea at different concentrations which may reduce PCV, catalase in the brain; prevents body weight increase and may not have adverse effect on superoxide dismutase, reduced glutathione and lipid peroxidation.

AKNOWLEDGEMENT

We acknowledgment the following people AinaOlubusayoOmotoyosi, DosumuSekinatMonilola, OdubanjoMorenikeTitilope and UmezurikeOgadinma Theresa for their support during sample collection and laboratory analysis, also Ti-OluwaniSalau for typing the manuscripts.

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