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RESEARCH ARTICLE

Phytochemical Screening and Effect of *Phyllanthus amarus* Roots on Sodium Arsenate Induced Hepatic Cell Damage

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ABSTRACT

LD_{50} and effect of ethyl acetate root extract of phyllanthus amarus on arsenate induced hepatic cell damage in wistar rats was assessed. Arsenate (10mg/kg body weight) was used to induce hepatic cell damage, whereas LD_{50} was determined using standard methods. Results of phytochemical screening identified presence of glycosides, steroids, tannins, alkaloids, saponins and terpenoids but not flavonoids and phlobatanins. The study revealed that Phyllanthus amarus root extract at high dose of 700mg/kg extract is physiologically safe. The 30-albino rat of wistar strain (180-200g) was used for the studies and was divided into six groups of five rats per each. Group A serves as Positive control and was treated with distilled water of treatment equivalence, Group B serves as Arsenate induction only, Group C serves as pre-treatment group and was treated with phyllanthus amarus root extract at the dose of 500mg/kg body weight for 4 days before arsenate induction, while Group D serve as post-treatment group which involves Arsenate induction at 10mg/kg body weight for 4 days before extract administration, Group E serve as immediate group which involves Arsenate induction followed by extract administration for 4 days and Group F serves as extract administration (500mg/kg) only for four days. The administration lasted for 8 days period after which the animals were sacrificed and blood serum was obtained for biochemical, Hematological and enzyme assay. ALT, AST and ALP each were significantly ($p<0.05$) increased in the arsenate induced group compared with normal control. There were significantly decrease in pretreatment, post treatment and immediate treatment groups compared with arsenate-induced group. Hematological and Biochemical parameters follow the same trend. The result obtained revealed that ethyl acetate crude extract of phyllanthus amarus roots could have ameliorative property on hepatic cells.

KEYWORDS

Phyllanthus Amarus, Lethal Dose, Arsenate Induction, Hepatic Cells, Ameliorative Property

INTRODUCTION

Plants are a source of fuel, building materials, craftwork material, dyes, food supplements and medicine for people, all over the world.

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Plants used for medicine contain a wide range of substances that can be used to treat chronic illness as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance (Diallo, 1999). The medicinal value of plants lies in some chemical

substances or group of compounds that produce a definite physiological action in the human body. These chemical substances are called secondary metabolites. The most important of these bioactive groups of plants are alkaloids, terpenoids, steroids, flavonoids, tannins and phenolic compounds (Edeoga, 2005). Western medicine is well known and in use, but at the same time it has created problems due to some side effects such as carcinogenicity caused by synthetic drugs (Lamar, 1999). This has enhanced the interest in search for natural products with medicinal property such as antioxidants and antibiotics for use in foods and medicine. Therefore, phytotherapy has been considered an alternative to alleviate side effects associated with synthetic drugs (Sanchez, 1999). Information on the chemical constituents does not only aid in discovering new therapeutic drugs, but such information can also help in disclosing new sources of economic materials such as tannins, oils, gums, that are precursors for the synthesis of complex chemical substances (Fansworth, 1996).

According to World Health Organization (WHO), traditional medicine is estimated to be used by up to 80% of the population of most developing countries. These plant-based medicines are used for primary health care needs (Bulletin WHO, 2002). Traditional medicine of late has been viewed by the pharmaceutical industry as a source of "qualified leads" in the identification of bioactive agents for use in the production of synthetic modem drugs. Between 25-50% of modern drugs are derived from plants. Although plants are unique in their activities but it has also been found that different tribes or countries for different ailments may use a particular plant, this shows that plants possess a very wide range of healing powers, which are attributed to their chemical composition. Despite the wealth of human experience and folklore concerning the medicinal uses of plants, proper scientific investigation has only been applied to a small fraction of the world's plants. This is a cause of concern as plant species continue to disappear (Raj, 1995).

The demand for medicinal plants is increasing in both developing and developed countries. A response to this situation is urgently needed to prevent the disappearance of plant species and the ethno-pharmacological knowledge that accompanies them (Silva, 1997).

Brief Description of *Phyllanthus amarus* Root

Phyllanthus is a genus of the family Euphorbiaceae that was first identified in central and southern India 18th century. It is commonly called carry me seed, stonebreaker, windbreaker, gulf leaf flower or gala of wind (Bharatiya, 1992). There are over 300 genera with over 5000 species in the Euphorbiaceae worldwide. Phyllanthus is one of the genuses that falls under this enormous family with about 750-800 species, found in tropical and subtropical regions worldwide. It is an erect annual herb of not more than one and half feet tall and has small leaves and yellow flowers. It is subdivided into 10 or 11 subgenera: *Botryanthus, Cicca, Co-nani, Emblica, Ericocus, Gomphidium, Isocladus, Kirganelia, Phyllanthodendron, Phyllanthus* and *Xylophylla* (Unander *et al.,* 1995 and Calixto *et al.,* 1998). About 22 species are native to Southern Africa (Archer, 2000). The Southern Africa species are common through Zimbabwe, Malawi, Zambia, Mozambique, South-West Africa, Angola and Nigeria. This plant can be found in low altitude hot dry scrubland and the medatriandra grassland with scattered trees, on fixed coastal sand dunes sea level to 900 m.

In folk medicine, *phyllanthus amarus* has reportedly been used to treat jaundice, diabetes, otitis, diarrhea, swelling, skin ulcer, gastrointestinal disturbances and block DNA polymerase in the case of hepatitis B virus during replication (Oluwafemi and Debiri, 2008). The beneficial medicinal effects of this plant material typically result from the combinations of secondary products present in the plant (Joseph and Raj, 2010). Several compounds including alkaloids, flavonoids, phenols and terpenes were isolated from this plant and some of them interact with the key enzymes. In traditional medicines, it is used for its hepatoprotective, anti-diabetic antihypertensive, analgesic, anti-inflammatory

and anti-microbial properties (Adeneye, 2006). The plant is also used in the treatment of stomach disorders, skin diseases and cold (Kokwaro, 1976; Iwu, 1993). It has anti- diarrhea effect (Odetola and Akojenu, 2000). Its anti-viral activity against hepatitis B virus has been established (Thyagarajan, 1988; Wang, 1995, and anti-carcinogenic (Joy and Kuttan, 1998).

Toxicity of Arsenate

Arsenate, one of the most harmful metalloids, is ubiquitous in the environment. The present study was carried out to investigate the protective role of *phyllanthus amarus* root extract on arsenate induced hepatic cell damage. In the study, Arsenate was chosen as the source of arsenic acid. Arsenate and many of its compounds are especially potent poisons and they disrupt ATP production through several mechanisms. At the level of the citric acid cycle, arsenate inhibits pyruvate dehydrogenase by competing for phosphate. It uncouples oxidative phosphorylation, thus inhibiting energy-linked reduction of $NAD^{+,}$ mitochondrial respiration, and ATP synthesis. Hydrogen peroxide production is also increased, which might form reactive oxygen species and oxidative stress. (Santra, 1999). Exposure of Arsenate through drinking of contaminated water may cause conjugated hyperbilirubinemia with simultaneous rise in serum alkaline phosphatase. Elevation of ALP, AST and ALT was noticed as an effect of Arsenate exposure in human. Laboratory test also observed that Arsenate induced stress may cause Hepatic cell damage. The studies of Arsenate toxicity involving human subjects were carried out only on exposed individuals, although reports of Arsenate induced hepatic toxicity are available on Murine model, using mice control (Mazumder *et al*., 1999).

MATERIAL AND METHODS

Plant Collection

Mature whole plant sample were collected from the Mkar hills around University of Mkar, Mkar Benue state Nigeria. The plant was authenticated at the Federal College of Forestry Jos, Plateau State. The plant roots were detached from the whole plant, dried at room temperature and used for the extract prepared.

Preparation of Crude Extract of *Phyllanthus amarus* Roots

Fresh root of *phyllanthus amarus* were collected and air-dried for 14days until constant weight was obtained. They were pulverized using a blender machine and forty grams (40g) of the powder root was dissolved in 250ml of 70% ethyl acetate as solvent, shaken for 10 minutes and then allowed to stay for 3days at room temperature to achieve maximum extraction (Ujah *et al*., 2013 and Soumya *et al*., 2013). The solution was filtered using whatman No 1 filter paper and the filtrate concentrated in water bath at 45°C. The dried extract was later weighed and reconstituted in distilled water to the required dosage for administration. The extract was tested for phytochemical constituents.

Experimental Animals

Albino rats were obtained from the animal holding unit, Department of Chemical Sciences University of Mkar, Mkar. The animals were allowed to undergo acclimatization period of seven days and were housed in a wooden cage with good ventilation. They were kept at room temperature 28±2°C and relative humidity 70% with 12 hours natural light and dark cycle. The rats were allowed free access to standard feeds bought from Vital Feeds at Gboko, Benue State and supplied with portable water. The experimental protocol was followed as approved by Institutional Animals Ethics Committee (IAEC) and animal care was taken as per the guidelines of the European Convention for the Protection of Vertebrate animals and other scientific purposes. (European Treaty Series, 2005)

Experimental Design

This research work was organized into two phases: phase I and phase II. Phase I was conducted according to the method of Loke (1983) for the determination of Media Lethal Dose (LD_{50}) using 24 mice divided into eight groups of 4 mice each. The second phase consist of five rats per group, each with their respective

tails marked for easy identification. The animals in their various group exception of

Group A. (known as positive control which was treated with fed and water only) were treated inter-peritoneal with Sodium Arsenate (10mg/kg body weight respectively) as shown below;

Group B. Arsenate induction administered for 4 days at 10mg/kg body weight only.

Group C. (Pre-treatment group) *phyllanthus* root extract administration at the dose of 500mg/kg body weight for four days, followed by Arsenate induction for 4 days.

Group D. (Post-treatment group) Arsenate induction at 10mg/kg body weight for 4 days before extract administration, for another 4 days

Group E. (Immediate treatment) Arsenate induction was immediately followed by extract administration for 4 days.

Group F. Extract administration (500mg/kg) only for 4 days.

Determination of LD_{50}

Preliminary LD_{50} was performed to identify the toxicity level of *phyllanthus amarus* root on wistar mice to see if the plant extract can be used for further research, Loke (1983) method was used. The experimental animals were divided into eight (8) groups of 4 mice each, which are A,B,C,D, E, F, G and H. The various doses of the plant extract were administered to the experimental animals according to their body weight per kg.

Qualitative Phytochemical Screening

Preliminary phytochemical screening was performed to identify phytochemicals in ethyl acetate root extract of *phyllanthus amarus* used in this study. Phytochemicals such as flavonoids, tannins, steroids, phlobatannins, saponins, terpenoids, cardiac glycosides, alkaloids, quinones, and phenols were screened by chemical method (Trease and Evans, 1989), modified by Harbone (1996) and Sofowara (2008).

Determination of Hematological and Biochemical Parameters

Whole blood was collected from the heart by cardiac puncture using sterile syringe and needle. The blood samples were put in ethylene diamine tetra acetate (EDTA) treated sample tubes. The packed cell volume or the haematocrit and White blood cell count (WBC) was determined by the method of Baker and Silverton (1998), Hemoglobin (Hb) concentration was determined according to Jain (1986), using the cyanomethemoglobin method, while platelets were determined by the method of Mitruka and Rawnsley (1977). Biochemical assay was carried out as follows: Billirubin content was measured by the method described by Jendrassik and Grof (1938), Total proteins assay was conducted according to Tietz (1995), while serum albumin level was examined by the method described by Grant, (1987).

Determination of Serum Enzyme Assay

Alanine transaminase (ALT) and Aspartate transaminase (AST) activities were assayed using the method of Reitman and Franke (1957) and Alkaline Phosphatase (ALP) serum level was estimated by the principle of Tietz, (1995). All the above biochemical parameter was determined in the plasma using the Randox kits by Cypress diagnostics (Belgium).

Statistical Analysis

Data obtained were expressed as mean ± standard error of mean using Analysis of Variance (ANOVA) (Armitage *et al*., 2008), and Statistical Package for Social Scientist (SPSS) version 20. $P<0.05$ was regarded as significant compared with appropriate controls.

RESULTS

Phytochemicals of *Phyllanthus Amarus* Root Extract

The phytochemical analysis of the extract revealed the presence of glycosides, tannins, steroids, alkaloids, saponins, and terpenoids, but no flavonoids and phlobatannins, in the ethyl acetate extract as shown in Table 1.

Table 1: The Phytochemical Composition of the Crude Extract of *Phyllanthus amarus R*oot

Phytochemicals	Crude Extracts
Flavonoids	-
Glycosides	+
Tannins	+
Steroids	+
Alkaloids	+
Phlobatannins	-
Saponins	+
Terpenoids	+

Where: (+) mean detected (-) mean not detected

LD_{50} Determination

The acute toxicity of *Phyllanthus amarus* was determined by the method of Loke (1983). The toxicity study was carried out in accordance with Organization for Economic Co-operation and Development (OECD) guideline in Swiss mice weighing 18 to 30 g.

The doses of 100, 200, 300, 500, 7000, 1000, 1500 and 2000gm/kg body weight of the extract were administered orally. The groups were continuously observed for mortality and behavioral changes during the period of 24 hours. The changes in body weight, food and water intake as well as activeness were monitored.

There was no abnormality observed in any of the groups A to E. However, there were some signs of weakness and rough hairy body observed in groups F and G while death of two mice occurred at group H with a dose of 2000mg.The table below shows the result of the LD_{50} and the median lethal dose calculated to be 693mg/kg.

Table 2: Mortality Recorded in Lethal Dose (LD_{50}) Determination for *Phyllanthus amarus* Root

Groups	Dose (mg/kg	No. of mice	No. of death recorded
A	100	4	None
B	200	4	None
C	300	4	None
D	500	4	None
E	700	4	None
F	1000	4	None
G	1500	4	None
H	2000	4	2/4

For the hematological parameters in table 5 above, statistical analysis showed that the ethyl acetate extract of *Phyllanthus amarus* root extract recorded a decrease ($P<0.05$) for RBC, Hb, PCV, exception of WBC, Platelets and Lymph levels in Group B (6.03±0.11, 7.25±0.32, 13.13±0.53, 46.81±1.96, 516.71±31.22, 86.64±3.09) compared to Group A (4.06±0.16, 18.54±10.41, 15.97±0.89, 52.09±0.45, 286.43±6.66, 81.84±2.73). However, there was significant increase observed for RBC, Hb, in group C and E (9.80±0.18, 17.03±0.2; 15.27±0.34) compared to B (Arsenate induction group). The result is further shown in chart 2 and 3.

Chart 2 and 3 shows the effect of crude extract of phyllanthus amarus on serum hematological parameters of arsenate induced hepatic cell damages in albino wistar rats.

Table 3: Effect of Crude Extract of *Phyllanthus amarus* Roots on Serum Enzyme Activities of Sodium Arsenate Induced Hepatic Cell Damage in Albino Wistar Rats

GROUPS	AST (u/l)	ALT (u/)	ALP (u/l)
A. Normal control	24.14±0.51	123.00±11.46	130.43±5.6.58
B. Arsenate induction (10mg/kg)	47.14±0.88*	149.57±1.51*	182.57±0.65*
C. Pretreatment (500mg/kg)	30.00±2.16*[a]	129.43±1.56*[a]	152.00±2.72[a]
D. Post treatment (500mg/kg)	43±1.13a b	143.71±2.68*[a]	161.57±17.84[a]
E. Immediate treatment (500mg/kg)	31.57±1.13*[abc]	131.00±2.52*[a]	168.00±13.88[a]
F. Extract treatment (500mg/kg)	24.59±1.06*[ad]	120.00±1.41*[abcd]	131.43±22.52*[abcd]

Results are expressed in mean ± SEM (n=5). *Significant at P<0.05 compared with group A; [a]Significant at P<0.05 compared group B; [b]Significant at P< 0.05 compared with group C; [c]Significant at P <0.05 compared with group D; [d]Significant at P<0.05 compared with group E.

Table 4: Effect of Crude extract of Phyllanthus amarus Roots on Serum Biochemical Parameters of Arsenate Induced Hepatic Cell Damage in Albino Wister Rats

Groups	Total protein(g/l)	Albumin (g/l)	Billirubin (u/l)
A Normal control	10.41±0.49	76.43±2.30	57.00±2.22*
B Arsenate induction (10mg/kg)	7.01±0.22*	94.00±1.1*	72.57±1.00*
C Pretreatment (500mg/kg)	9.06±0.12*[a]	75.00±0.82[a]	66.43±1.00*
D Post treatment (500mg/kg)	5.64±0.15*[ab]	67.71±1.66*	69.71±0.87*
E Immediate treatment (500mg/kg)	9.19±0.28*[abc]	75.14±1.47[ac]	59.86±1.30*[ab]
F Extract treatment (500mg/kg)	10.39±0.28*[cd]	76.33±2.36[a]	56.01±1.15*[ab]

Results are expressed in mean ± SEM (n=5). *Significant at P<0.05 compared with group A; [a]Significant at P<0.05 compared group B; [b]Significant at P <0.05 compared with group C; [c]Significant at P <0.05 compared with group D; [d]Significant at P<0.05 compared with group E.

Table 5: Effect of Crude Extract of *Phyllanthus amarus* Roots on Serum Hematological Parameters of Arsenate Induced Hepatic Cells Damage in Albino Wister Rats

GROUPS	WBC (10^9/l)	RBC	Hb (%)	PCV (%)	PLATELET	LYMPH(10^5/µL)
A. Normal control	4.06±0.16	18.54±10.41	15.97±0.89*	52.09±0.45	286.43±6.66	81.84±2.73
B .Arsenate induction (10mg/kg)	6.03±0.11	7.25±0.32	13.13±0.53*	46.81±1.96*	516.71±31.22*	86.64±3.09
C. Pretreatment (500mg/kg)	3.80±0.39[a]	9.80±0.18[a]	13.82±0.27*	44.26±1.62*	464.57±35.43*	80.30±2.0
D. Post treatment (500mg/kg)	6.46±0.46*[ab]	7.81±0.22	16.16±0.64[s]	46.80±1.53*	428.86±27.62*	84.7±2.43
E. Immediate treatment (500mg/kg)	5.97±0.53*[abc]	9.03±0.13[a]	15.27±0.34*	42.91±1.51*	328.00±46.04 [abc]	86.00±1.80
F. Extract treatment (500mg/kg)	4.25±0.32[cd]	17.03±0.21	15.72±0.33	52.01±2.23*	435. 43±40.81*[d]	84.47±1.44*[cd]

Results are expressed in mean ± SEM (n=5). *Significant at P<0.05 compared with group A; [a]Significant at P<0.05 compared group B; [b]Significant at P<0.05 compared with group C; [c]Significant at P <0.05 compared with group D; [d]Significant at P< 0.05 compared with group E

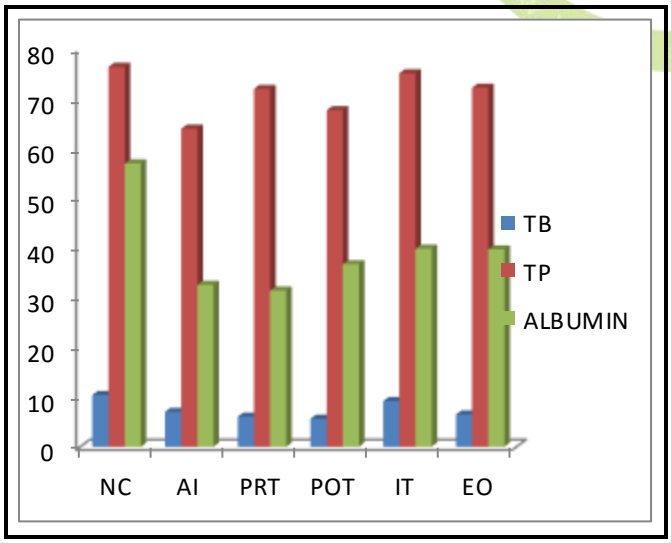

Chart 1: Shows the Effect of Crude Extract of *Phyllanthus amarus* on Serum Biochemical Parameters of Arsenate Induced Hepatic Cell Damage in Albino Wistar Rats

(TB-TOTAL BILIRUBIN, TP- TOTAL PROTEIN, NC-NORMAL CONTROL, AI- ARSENATE INDUCTION, PRT- PRE TREATMENT, IT- IMMEDIATE TREATMENT, EO- EXTRACT ONLY).

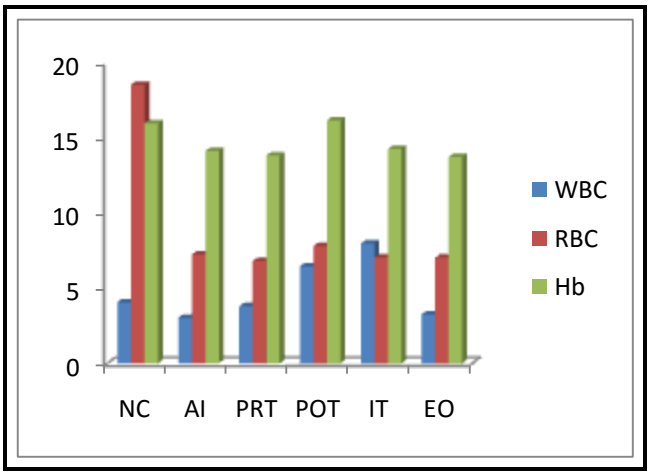

Chart 2: WBC-White blood cell, RBC- Red blood cell, Hb- Hemoglobin

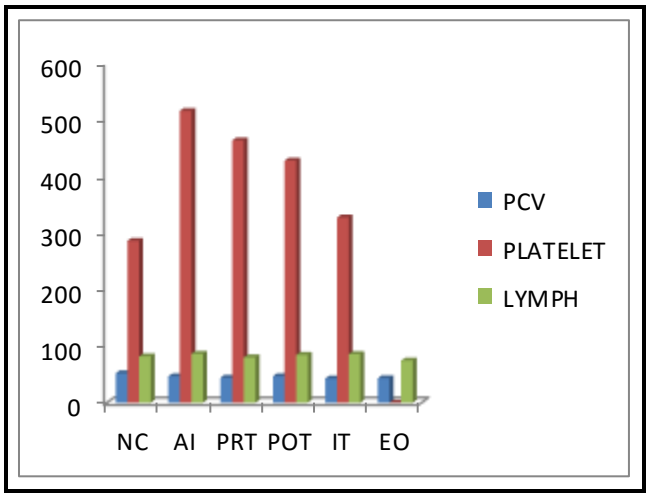

Chart 3: PCV-Packed cell volume

DISCUSSION

The presence of tannins, steroids, saponin, terpenoids, cardiac glycosides and alkaloids as revealed in the results of phytochemical analysis in *Phyllanthus amarus* root extract suggests its usage for various medicinal purposes in folk medicine. Alkaloids are the most efficient therapeutic plant substance. Both natural and synthetic alkaloids are used as basic medicinal agent because of their analgesic, antispasmodic and antibacterial properties. (Stray, 1998).

Effect of Crude Extract of *Phyllanthus amarus* Roots on Serum Enzyme Activities of Arsenate Induced Hepatic Cell Damage in Wistar Rats

The result showed that arsenate caused an elevation in the serum content of ALT. AST, and ALP. This indicates liver dysfunction especially the rise in ALT activity (Doumas, 1971 and Ngaha 1989). AST and ALT are reliable determinants of liver injury (Wang, 2001). Hence, serum levels of these enzymes were used as indices for monitoring chemically induced tissues damages. From the result of this study, it was observed that treatment of albino wistar rats with ethyl acetate root extract of *Phyllanthus amarus* roots in pretreatment and immediate treatment with Arsenate induction caused a significant reduction in enzymes levels. This is evidence in marked decreased serum ALT, AST, and ALP activities of those treated with *P. amarus* extracts roots at ($p<0.05$) relative to the group treated with Arsenate alone. The marked decrease in the activities of these three marker enzymes (ALT, AST and ALP) agrees with the studies carried out by other researchers on hepatic cell damage of other herbal plants. (Dacie, 1991). Moreover, this could be due to some major compounds found to exist, such as glycosides and many of these were gallic acid derivatives and free epicatechin, which are present in the ethyl acetate-soluble fraction and could be a potent antioxidant, which can be used for the prevention of diseases related to oxidative stress as suggested by Soumya *et al.*, 2013.

Effect of Crude Extract of *Phyllanthus amarus* Roots on Serum Biochemical Parameters of Arsenate Induced Hepatic Cell Damage in Wister Rats

From the result of this study, it was observed a significant decrease in Total Protein, but increased Albumin and Billirubin level in Arsenate induced group when compared with normal group respectively. However, there was significant increase in Total Protein, but a decreased Albumin, and Billirubin level in-group C, E and F compared with group B. It has been revealed that increase in blood albumin typically is a sign of severe dehydration (Rajbar, 1999). This shows that *Phyllanthus amarus* roots have ameliorative property because there was a decrease in Albumin, Billirubin and increased Total protein levels in all the treated groups and this probably indicate also, that the buffering capacity of the blood and body fluid have been enhance (Ujah *et al*, 2014). This is clearly seen in both the pretreatment and post treated groups.

Effect of Crude Extract of *Phyllanthus amarus* Roots on Serum Hematological Parameters of Arsenate Induction

The result obtained in the statistical analysis of hematological parameters showed that the ethyl acetate extract of *Phyllanthus amarus* root extract recorded a decrease for WBC, RBC, Hb, PCV, exception of Platelets and Lymph levels in Group B compared to Group A which indicates an infection due to destruction of red blood cell as recorded in the studies of Heller and Clermont, 2003. However, there was significant increase observed in post treatment group, pretreatment group and immediate treatment group compared

to group B and this shows that the ethyl acetate extract of *phyllanthus amarus* root has hematopoietic property and can be recommended to patient with hepatic cell damage caused by arsenate.

CONCLUSION

In conclusion, this research work has shown that ethyl acetate extract of *Phyllanthus amarus* roots could have ameliorative property on damage hepatic cells because the levels of ALT, AST and ALP in the pretreatment and post treatment group recorded a significant decrease which means a patient with hepatic cell damage caused by arsenate can be given post treatment or immediate treatment with *phyllanthus amarus.* People with occupational exposure can be advice to add *phyllanthus* roots extract to their deity for its antioxidant activity, to help prevent them from the effect of arsenate. Besides, it also has hematopoietic property and likely enhances the buffering capacity of the body fluid. However, dosage is very important.

RECOMMENDATION

Further study on lipid peroxidation products should be carried out and the urea, creatinine and other electrolyte levels should be determined, backed up with histological examination of the liver cells to affirm the therapeutic safety of the plant. The active ingredient should be isolated, purified, and used for the research.

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