

PROTECTIVE EFFECT OF AQUEOUS EXTRACT OF *Phyllanthus amarus* ON THE HAEMATOBIOCHEMICAL PARAMETERS OF GLYPHOSATE INDUCED TOXICITY IN WISTAR RATS

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ABSTRACT

Glyphosate is an organophosphorus herbicide widely used in agricultural practices. The present study was conducted to find out the protective effect of P. amarus aqueous extract against hematobiochemical alterations caused by glyphosate toxicity in Wistar rats. About 60 Wistar rats were randomly (n=12) divided into five groups: Group 1 (Control group), Group 2 (Glyphosate (GLP)-500 mg/kg), Group 3 (Phyllanthus amarus (PA)-100 mg/kg aqueous extract), Group 4 (Glyphosate 500 mg/kg + Phyllanthus amarus 100 mg/kg aqueous extract-GLP + PA prophylactic) and Group 5 (Glyphosate 500 mg/kg (Upto 9 days) + Phyllanthus amarus 100 mg/kg aqueous extract-GLP + PA therapeutic (10th to 28th day)). Haematological parameters, liver specific enzymes (ALT, AST and ALP), kidney function tests (BUN and creatinine), serum electrolyte and minerals were studied in glyphosate induced toxicity. Phyllanthus amarus extract (Prophylactic and Therapeutic) showed highly significant reduction (P<0.01) in the Haemoglobin, PCV and TEC compared with glyphosate group. Numerical reduction in the values of ALT, AST and ALP in the treatment groups compared with glyphosate group. Significant (P<0.05) reduction in the values of BUN and creatinine observed in treatment groups compared with glyphosate group. Significant (P<0.05) reduction in the value of sodium observed in treatment groups compared with glyphosate group. The results indicated that P. amarus had partially protective effect on haematobiochemical changes caused by glyphosate toxicity in Wistar rats.

Keywords: Glyphosate toxicity, Haematobiochemical parameters, *Phyllanthus amarus*, Wistar rats

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INTRODUCTION

The modernization of agriculture in India resulted in the manifold increase in the use of chemical fertilizers, which

may also accumulate in the environment affecting non target species including humans. Glyphosate is the isopropylamine salt of N-(Phosphonomethyl) glycine. It is an organophosphate herbicide. Clinical signs of glyphosate toxicity includes multi organ toxicity with hepatotoxicity, nephrotoxicity, gastrointestinal and cardiovascular system (Lee *et al.*, 2009; Sribanditmongkol *et al.*, 2012). Glyphosate is an endocrine disruptor which affects the male reproductive system of rats resulting in the disruption of aromatase enzyme, increased testosterone levels in the serum. Animal studies stated that glyphosate may enhance Adenosine Tri Phosphatase (ATPase) activity and uncouple mitochondrial oxidative phosphorylation (Olorunsogo, 1982).

Phyllanthus amarus is a broad spectrum medicinal plant that has received worldwide recognition. The entire plant is used in India to treat hepatitis, dysentery, irritating sores and jaundice. The *P.amarus* extracts and the compounds isolated from, *P.amarus* shows a wide spectrum of pharmacological activities including antiviral, antimalarial, antimicrobial, anticancer, antidiabetic, hypolipidemic, antioxidant, hepatoprotective, nephroprotective and diuretic properties (Patel *et al.*, 2011). Phyllanthin, a lignan present in the aqueous extract showed significant antioxidant effect. Hence the present study was undertaken to find out the protective effect of aqueous extract of *Phyllanthus amarus* against experimentally induced glyphosate toxicity in Wistar rats.

MATERIALS AND METHODS

Animal study

Sixty male and female 4 – 6 weeks old Wistar rats were obtained from the Lab Animal Medicine, Madhavaram after getting approval from the Institutional Animal Ethics Committee (Approval Lr. No.3701DFBS/IAEC/2021, Dated: 16/08/2021). Aqueous extract of *Phyllanthus amarus* dry powder was purchased from Chemiloids, a pharmaceutical company and 71% glyphosate (FLITE 71) was purchased locally. Control group was given with *ad libitum* water and normal rat feed. The second group (GLP) was given 500 mg/kg of glyphosate through oral gavage after one week of acclimatization. The third group (PA) was given 100 mg/kg of aqueous extract of *Phyllanthus amarus*. The fourth group (GLP+PA prophylactic) was given 500 mg/kg of glyphosate and 100 mg/kg of aqueous extract of *Phyllanthus amarus* together and the fifth group (GLP +PA therapeutic) was given 500 mg/kg of glyphosate for 9 days and 100 mg/kg of aqueous extract of *Phyllanthus amarus* from the 10th to 28th day. Animals were sacrificed on 29th day of trial. Blood samples were collected by retrobulbar plexus method in EDTA and plain vacutainers for haematological and serum biochemical studies respectively.

Haematology

The haematological parameters such as Packed Cell Volume (PCV), Haemoglobin (Hb), Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Differential Leukocyte Count (DLC) and Platelet Count (PLTs), neutrophils, lymphocytes, eosinophils

and basophils were enumerated in the EDTA samples analyzed by auto analyzer BC vet 2800 (Mindray).

Serum biochemistry

The blood collected in plain vacutainer with clot activator allowed to settle for 30 minutes and centrifuged at 2500 rpm for five minutes. The separated serum was collected and subjected to serum biochemical evaluation in A15 BioSystem auto biochemical analyzer (BioSystem). The parameters analysed were liver specific enzymes of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), Blood Urea Nitrogen (BUN), Creatinine, Sodium, Potassium, Calcium and Phosphorus.

Statistical analysis

The data generated from the different parameters of the experimental study were subjected to one way analysis of variance (ANOVA) test using statistical package for the social sciences (SPSS) software version 20 for the Window (Snedecor and Cochran, 1983).

RESULTS AND DISCUSSION

All the experimental animals of the different groups showed no appreciable clinical signs and mortality. The results of the haematological parameters (Table 1) of Haemoglobin (Hb), Packed Cell Volume (PCV) and Total Erythrocyte Count (TEC) showed highly significant ($P < 0.01$) reduction in glyphosate group when compared to control and treated groups of *P. amarus* (prophylaxis and treatment) indicating that exposure to glyphosate might induce anaemia (Decreased

PCV and Hb) caused by glyphosate extravascular hemolysis or due to its property to induce oxidative stress (Modesto and Martinez, 2010). The erythrocytic membrane is susceptible to oxidative stress (Ambali *et al.*, 2010). *P. amarus* treated group showed improvement in these values when compared to glyphosate group due to its antioxidant property (Patel *et al.*, 2011).

The total leucocyte count, MCV, MCH, MCHC platelet, neutrophil, lymphocyte, eosinophils, basophils showed no significant difference between control and treatment groups.

Serum liver enzymes ALT, AST and ALP showed no significant difference between control and treated groups (Table 2). In addition to that numerical elevation was observed in glyphosate group compared to treatment groups and it could be due to oxidative stress caused by glyphosate (Turkmen and Dogan, 2019) and there was numerically slight reduction in these values observed in treatment group of *P. amarus* caused by its antioxidant property (Patel *et al.*, 2011).

Serum total protein, albumin, globulin values in the present study showed no significant difference between control and treatment groups (Table 2). The results revealed numerical elevation observed in glyphosate group compared to treatment groups and it indicated that oxidative stress induced by hepatic damage caused by glyphosate (Turkmen and Dogan, 2019) who conducted glyphosate toxicity studies in male Wistar rats at the rate of 375 mg/kg for a period of 28 days. It was reversed in the treatment

Table 1. Mean (\pm SE) haematological parameters in 28 days study in Glyphosate toxicity induced in Wistar rat model (n=12)

Groups	Control	GLP	PA	(GLP+PA prophylactic)	(GLP+PA therapeutic)	F Value	Sig
Hb (g/dL)	14.45 ^a \pm 0.26	12.85 ^a \pm 0.33	13.1 ^{ab} \pm 0.20	13.7 ^{bc} \pm 0.96	13.35 ^{ab} \pm 0.39	5.11	**
PCV (%)	39.75 ^a \pm 0.72	33.86 ^b \pm 0.12	41.83 ^a \pm 4.51	43.54 ^a \pm 0.84	39.6 ^a \pm 0.82	2.93	**
MCV (fL)	52.60 \pm 0.65	50.90 \pm 0.63	52.4 \pm 0.94	52.18 \pm 0.55	52.07 \pm 0.76	0.85	NS
MCH (pg)	17.32 \pm 0.21	16.81 \pm 0.22	17.36 \pm 0.45	17.31 \pm 0.16	17.54 \pm 0.53	0.60	NS
MCHC (g/dL)	33.64 \pm 0.56	32.94 \pm 0.15	33.04 \pm 0.25	33.09 \pm 0.41	33.19 \pm 0.08	0.64	NS
TEC ($\times 10^6/\mu\text{L}$)	8.34 ^b \pm 0.08	7.48 ^a \pm 0.26	7.81 ^a \pm 0.13	7.97 ^{ab} \pm 0.11	7.64 ^a \pm 0.18	4.03	**
WBC ($\times 10^3/\mu\text{L}$)	18.39 \pm 1.32	21.25 \pm 2.32	19.51 \pm 1.46	21.12 \pm 2.16	18.10 \pm 1.84	7.131	NS
Platelet ($\times 10^3/\mu\text{L}$)	1065.4 \pm 95.79	1999.5 \pm 899.3	1146 \pm 84.49	1278.50 \pm 53.788	1086.83 \pm 85.08	0.89	NS
Neutrophils (%)	13.91 \pm 1.28	16.00 \pm 1.59	13.50 \pm 0.48	13.58 \pm 1.21	15.66 \pm 1.19	1.46	NS
Lymphocytes (%)	82.58 \pm 0.57	82.08 \pm 1.38	79.90 \pm 1.73	80.33 \pm 1.32	82.33 \pm 0.71	1.03	NS
Monocytes (%)	2.91 \pm 0.14	3.00 \pm 0.21	3.09 \pm 0.21	3.00 \pm 0.17	3.00 \pm 0.17	0.10	NS
Eosinophils (%)	1.14 \pm 0.14	1.26 \pm 0.10	1.10 \pm 0.07	1.12 \pm 0.14	1.06 \pm 0.14	0.53	NS

Mean bearing similar superscripts do not differ significantly within a row -One-way ANOVA-Duncan's test; NS -Non significant; ** Significant (P<0.01); GLP-Glyphosate; PA-*P. amarus*

Table 2. Mean (\pm SE) serum biochemical parameters in 28 days study in Glyphosate toxicity induced in Wistar rat model (n=12)

Groups	Control	GLP	PA	(GLP+PA prophylactic)	(GLP+PA therapeutic)	F Value	Sig
ALT (U L ⁻¹)	140.50 \pm 20.43	162.00 \pm 13.74	144.9 \pm 15.7	157.83 \pm 10.43	154.91 \pm 13.9	0.35	NS
AST (U L ⁻¹)	113.83 \pm 21.40	183.08 \pm 27.82	126 \pm 17.6	169.5 \pm 11.90	133.6 \pm 22.59	1.99	NS
ALP (U L ⁻¹)	242.66 \pm 15.25	323.83 \pm 49.83	253.1 \pm 34.9	276.83 \pm 28.09	272.00 \pm 27.71	1.06	NS
Total Protein (g dL⁻¹)	6.79 \pm 0.16	7.15 \pm 0.20	7.04 \pm 0.20	6.95 \pm 0.10	6.80 \pm 0.17	0.78	NS
Albumin (g dL⁻¹)	3.05 \pm 0.22	4.07 \pm 0.21	3.58 \pm 0.23	3.758 \pm 1.05	3.71 \pm 0.23	2.35	NS
Globulin (g dL⁻¹)	3.07 \pm 0.21	3.74 \pm 0.24	3.45 \pm 0.15	3.20 \pm 0.29	3.30 \pm 0.10	1.55	NS
BUN (mg dL⁻¹)	21.2 ^a \pm 1.30	28.14 ^b \pm 1.77	20.73 ^a \pm 1.28	23.28 ^a \pm 0.79	23.16 ^a \pm 1.84	3.10	*
Creatinine (mg dL⁻¹)	0.51 ^a \pm 0.67	0.96 ^b \pm 0.14	0.50 ^a \pm 0.11	0.63 ^a \pm 0.05	0.61 ^a \pm 0.10	2.82	*

Means bearing similar superscripts do not differ significantly within a row -One-way ANOVA-Duncan's test; NS -Non significant; * Significant (P<0.05); GLP-Glyphosate; PA-P. *amarus*

Table 3. Mean (\pm SE) serum biochemical parameters in 28 days study in Glyphosate toxicity induced in Wistar rat model (n=12)

Groups	Control	GLP	PA	(GLP+PA prophylactic)	(GLP+PA therapeutic)	F Value	Sig
Ca (mmol/dL)	13.98 \pm 0.77	14.59 \pm 0.88	13.17 \pm 0.79	13.20 \pm 0.98	13.07 \pm 1.07	5.119	NS
P (mmol/dL)	7.82 \pm 0.68	9.81 \pm 0.55	8.07 \pm 0.54	9.52 \pm 0.55	8.52 \pm 0.88	2.935	NS
Na (mmol/dL)	93.02 ^b \pm 22.10	155.60 ^a \pm 1.49	97.93 ^b \pm 4.52	153.30 ^a \pm 2.03	153.69 ^a \pm 3.31	4.037	*
K (mmol/dL)	6.69 \pm 0.11	11.14 \pm 2.83	7.36 \pm 2.25	10.47 \pm 2.58	9.76 \pm 3.07	7.131	NS
Cl (mmol/dL)	111.97 \pm 8.66	121.05 \pm 1.46	114.60 \pm 1.33	120.00 \pm 2.00	119.42 \pm 2.10	0.898	NS

Means bearing similar superscripts do not differ significantly within a row -One-way ANOVA-Duncan's test; NS -Non significant; *Significant (P<0.05); GLP-Glyphosate; PA-*Phyllanthus amarus*

group due to antioxidant property of *P. amarus* (Patel *et al.*, 2011).

Kidney function tests of BUN and creatinine showed significant ($P < 0.05$) difference between the glyphosate and other groups (Table 2). Significant elevation of BUN and creatinine were observed in glyphosate group compared to the control and treatment groups indicating that glyphosate produces kidney damage reflected as renal injury specific markers of creatinine and BUN (Sugam *et al.*, 2008; Turkmen and Dogan, 2019). These levels were reduced in the treatment groups (Prophylactic and Therapeutic) due to removal of the oxidative stress caused by glyphosate due to the action of *P. amarus* antioxidant property (Patel *et al.*, 2011).

Serum electrolyte value of sodium (Table 3) in the present study observed that significant ($P < 0.05$) difference was observed between glyphosate group compared to control group. Significant elevation in the glyphosate group could be due to renal damage (Sugam *et al.*, 2008). The sodium value was reduced in the treatment groups compared to glyphosate indicating the nephroprotective property of *P. amarus* (Patel *et al.*, 2011). The serum calcium, phosphorus, potassium and chloride values in the present study did not reveal no significant difference between control, glyphosate and other treated groups.

To conclude *P. amarus* had partially protective effect against glyphosate toxicity caused haematobiochemical changes.

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