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EFFECT OF *MUCUNA PRURIENS* ON BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS IN MPTP INDUCED MICE

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ABSTRACT

Mucuna pruriens has been known to possess valuable medicinal properties. The present study aims at investigating the effects of *M. pruriens* seeds against1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) induced mice on haematological and bio-chemical parameters. Male Swiss albino(Groups=7) mice with weights ranging from 25 to 30 g were fed with different concentration (200mg/kg, 400mg/kg) of *M. pruriens* seeds for one week. The result shows that the seeds improved the haematological and serum biochemical parameters with dose-dependent manner in MPTP induced mice. The effect of methanolic seed extract of *M. pruriens* seeds on Aspartate aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) activities were evaluated using spectrophotometric method. The results showed that the parameters of serum cholesterol, urea, creatinine, and triglycerides were normalized when the treated mice when compared to the control. In conclusion, the effect of biochemical and haematologic parameters of *Mucuna pruriens* seed extract were observed in MPTP treated mice.

KEYWORDS: Biochemical analysis, haematological parameters, *Mucuna pruriens*, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP).

INTRODUCTION

Mucuna pruriens is a tropical legume known as velvet bean, found in Africa, India and the Caribbean. It is also used for the management of several free radical-mediated diseases, such as

rheumatoid arthritis, diabetes, atherosclerosis, male infertility and nervous disorders, in the Ayurvedic system of medicine [Tripathi and Upadhyay, 2001]. Traditionally the seeds of *M. pruriens* are used as a tonic and aphrodisiac for male virility. The seed powder has recently been found to show the anti-Parkinsonism effects which are probably due to the presence of L-DOPA. It is well known that dopamine is the brain neurotransmitter. The dopamine content in the brain tissue gets reduced because of its blockade of crossing over the blood brain barrier to reach the site of action. As L-DOPA is the precursor of dopamine, it crosses the barrier and gets converted into dopamine resuming the neurotransmission **[**Kulhalli, 1999**]**. These important biological actions have led to the chemical investigations of *M. pruriens* seeds to isolate several fatty acids, amino acids besides L-DOPA **[**Siddhuraju et al., 1996**].** According to their report however, the activities of liver ALP, AST, and ALT were generally not significantly affected yet they concluded that results of their study suggest that the administration of the extract may adversely affect liver and kidney function; what a contradiction[Malomo et al., 2006]. Therefore, this medicinal plant may prove to be a rich source of compounds with possible antimicrobial activities but more pharmacological investigations are necessary [Ram and Mohammad, 2011]. Hence we planned to investigate the effects of *Mucuna pruriens* seed extract against MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) induced mice with biochemical and haematological parameters.

MATERIALS AND METHODS

Collection of seeds

Seed samples of *Mucuna* were collected from the Eastern and Western Ghats of South India.

Preparation of extract

Powdered seeds measuring 200g each were extracted in sterile distilled water on shaker (Stuart Scientific Orbital Shaker, UK) for 48 h. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was quickly frozen at -40 °C and dried for 48 h using a freeze dryer to give a yield of 30 g of dry extract. The resulting extract was reconstituted with sterile distilled water to desirable concentration and used throughout study.

Animals

Swiss albino male mice, weighing 25-30 gm were used. All animals were obtained from the Animal house, K M C H College of Pharmacy, Coimbatore, Tamil Nadu. They were allowed food and water *ad libitum* up to the experimentation period. Prior to use, the mice were housed in polypropylene cages in group of six to eight animals under natural light-dark cycle. Each

animal was used only once under standard laboratory conditions. All the observations were made at room temperature in a noiseless diffusely illuminated room and were made between 9.00 to 17.00 h in the experimental room. All the experimental protocols were approved by Institutional Animals Ethics Committee (IAEC) as per provisions of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) (KMCRET/PhD/04/2012-13), New Delhi, India.

Acute toxicity Study

This was performed for the extracts to ascertain safe dose by the acute toxic class method by the organization of Economic Co operation and Development (OECD 423guidelines). A single administration of starting dose of 2000mg kg^{-1} body weight p.o. of the water extract of *M. pruriens* seed was administrated to six mice and the mice were observed for three days to evaluate considerable changes in body weight and other signs of toxicity. There was no considerable change in body weight before and after treatment and no sign of toxicity were observed. When the experiment was repeated again with same dose level, 2000 mg $kg-^{1}$ body weight p.o. of seed extract was observed for 7 days no change was observed from the experiments.

Experimental Design

The animals were divided into six groups, each consisting of six mice. Group I served as vehicle control (10% Tween 80, p.o), Group II received MPTP (20 mg/kg, i.p) (Sigma-Aldrich) four consecutive days, Group III, IV received MPTP + crude extract (200, 400 mg/kg, p.o). Group V Crude extract (400mg/kg, p.o), VI MPTP+ Selegiline (10mg/kg, i.p), Group VII MPTP+ carbidopa + levodopa (100mg/kg, i.p) respectively. The treatment was given on the initial day, 30 min prior to first injection of MPTP and once a day for another six days of the experimental period.

Determination of serum biochemical parameters

The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by the method of Reitman and Frankel.[8] While alkaline phosphatase (ALP) was determined by Zhary and Denis.[9]

Assay for serum total cholesterol

The serum level of total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample as described by method of Stein (1987). Briefly, 1000: L of the reagent was added

to each of the sample and standard. This was incubated for 10 min at 20-25ºC after mixing and the absorbance of the sample and standard was measured against the reagent blank within 30 min at 546 nm. The value of TC present in serum was expressed in the unit of mg/dL.

TC concentration = A *sample* /A *standard* × 196.86 (mg/dL).

Assay for serum triglyceride

The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by method of Tietz (1990). 100µ L of the reagent was added to each of the sample and standard. This was incubated for 10 min at 20-25ºC after mixing and the absorbance of the sample and standard was measured against the reagent blank within 30 min at 546 nm. The value of triglyceride present in the serum was expressed in the unit of mg/dL.

TGL concentration = A *sample*/A *standard* × 194.0 (mg/dL).

Assay for serum high-density lipoprotein cholesterol

The serum level of HDL-C was measured by the method of Wacnic and Alber (1978). Low-density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample were precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 min at room temperature and centrifuged for 10 min at 4000 rpm. The supernatant represented the HDL-C fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant, was determined. The value of HDL-C was expressed in the unit of mg/dL.

Determination of serum low-density lipoprotein cholesterol

The serum level of (LDL-C) was measured according Friedewald *et al*. (1972) method using the equation below: The value was expressed in the unit of mg/dL.

LDL-C = TGL/5 - HDL-C.

Haematological parameters

The blood with anticoagulant was used for assay of the haematological parameters using the XF 9080 Animal Haematology Auto-analyzer (Hitachi, Germany). Parameters determined are packed cell volume (PCV), Red blood cells (RBC) count, White blood cells (WBC) count, mean corpuscular Haemoglobin concentration (MCHC), mean corpuscular volume (MCV) Lymphocytes, Monocytes and Neutrophils, using standard assay.

Statistical analysis

All the values expressed as Mean ± SE. The data was analyzed using analysis of variance (ANOVA) and the means were compared using the Dennett's test 3 using SPSS (version 17.0) at significance level of ($p < 0.05$ $p<0.01$, $p<0.001$).

RESULT AND DISCUSSION

Serum Biochemical Parameters

The effect of aqueous extract of *Mucuna pruriens* was mainly dose dependent significant ($P<0.05$) in serum AST, ALP and ALT activity. The MPTP-treated group recorded a maximum decrease ($p<0.05$) in the serum biochemical parameters level when compared to the control group as shown in Table-1a. The results of this present study show that the biomarker enzymes were decreased and were detected rapidly, hence, can be used for the prediction and diagnosis of metabolic insults. The membrane bound target enzymes, Aspartate Amino transaminase (AST) and Alanine Amino transaminase (ALT) significantly decreased in serum level when measured after one weeks of treatment in relation to the control. The reason for this decreased observation in enzyme level has been attributed to the fact that *M. pruriens* is a known antioxidant (Aguiyi *et al*., 1996; Tripathi and Upadhyay, 2001).

There was no significant change in the serum total cholesterol levels in the group when compared to the control group. Whereas significant reduction ($p<0.05$) in the serum total cholesterol level in the groups administered with 200 and 400 mg/kg.

There was a significant decrease ($p<0.05$) in the serum triglyceride level in the groups that received 200 and 400 mg/kg when compared to the control group. Whereas MPTP-treated group recorded a maximum decrease ($p<0.01$) in the serum triglyceride level when compared to the control group as shown in Table-1b.

In addition, the serum LDL-C level was significantly ($p<0.05$) depleted in the groups that received 200 and 400 mg/kg with a marked reduction ($p<0.05$) when compared to the control group as shown in Table-1b.

Effect of *Mucuna pruriens* Extract on some haematological parameters in albino mice

Table-2a shows that the present study showed in significant decrease in white blood cells, Red blood cells count in combination group as compared to control, however in other treated groups there was increase in white blood cells count, but mostly these changes were in normal

physiological range. However, there was significant ($p < 0.05$) increase in white blood cells count in *M.pruriens* treated group as compared to control. In combination group decrease in white blood cells count may be due to neutralizing effects of these drugs to one another. *Mucuna pruriens* seed extract produced a significant ($p < 0.05$) increase in white blood cells count in comparison with control rats after 6 weeks (Chukwadi *et al*, 2011). L-dihydroxyphenylacetic acid (L-dopa) is another key constituent in *Mucuna pruriens* that may likely be responsible for the observed findings in WBC total counts (Rajaram and Janardhana n, 1991).

There was significantly ($P<0.05$) increases in the haemoglobin, whereas PCV, plasmogen, Lymocytes, Monocytes and Eosinophils were decreases in combination of group as compared to the control group (Table 2b). When compared to the control, there was significant changes in MPTP treated groups.

Table: 1a.Effect of Extract of *Mucuna pruriens* on some biochemical parameters in Swiss albino mice*

Groups	ALT(UI)	AST(UI)	ALP(UI)
GP-I	37.33±0.58	137±1	383.33±4.16
GP-II	35.67±2.52	127.67±2.52	380.33±4.73
GP-III	35.33±3.06	125.67±4.93	351±2.65
GP-IV	27.33±2.08	119.67±0.58	340.67±7.37
GP-V	33.67±3.51	113.27±3.51	233.67±3.51
GP-VI	30.67±1.53	122.33±2.08	341.67±3.79
GPVII	24.33±4.04	109±1	231±9.64
GPVIII	25±3	111±1	246.67±4.16
GP-IX	20±4	96.67±3.06	205.33±1.15
GP-X	18.33±2.08	91.67±3.79	195±4.58

*Results represent Mean ± SE of six replicated experiments. They are significantly different at the $p< 0.05$ level of confidence.

GP I-Control**, GP II**-MPTP Toxin alone(20mg/kg), **GP III**-Crude extract alone(CE)(400mg/kg,), **GP IV**- F(100mg/kg), **GP V** -MPTP + CE (200mg/kg), **GP VI**-MPTP + CE (400mg/kg), , **GP VII**- MPTP + selegiline (10mg/kg,) **GP VIII**-MPTP + carbidopa (100mg/kg).

ALT – Alanine aminotransferase, AST – Aspartate aminotransferase, ALP – Alkaline Phosphatase.

Table: 1b. Effect of Extract of *Mucuna pruriens* on some biochemical parameters in Swiss albino mice*

Groups	TG	VLDL	HDL	TC	LDL
GP-I	159.93±0.42***	31.74±0.32**	75.83±0.88***	185.26±0.33***	28.03±0.45*
GP-II	104.93±0.45	20.94±0.12	12.33±1.26	76.67±0.58	14.70±0.74
GP-III	130.03±0.50**	66.35±0.75	55.63±0.75	179.00±0.50	60.35±0.75
GP-IV	127.43±0.75	63.68±1.04	42.50±1.04	165.33±0.76	58.62±0.76
GP-V	139.17±0.52	70.25±0.33	42.50±0.75	141.00±0.50	29.62±0.65
GP-VI	143.20±0.53	71.27±0.53	30.37±0.53	140.15±0.52	40.68±0.70
GP-VII	107.47±0.64	54.40±0.75	20.53±0.75	118.00±1.00	41.73±0.78
GP-VIII	107.30±0.56	52.98±0.86	18.63±0.86	106.00±1.00	32.38±0.76

*Results represent Mean ± SE of six replicated experiments. They are significantly different at the $p< 0.05$ level of confidence.

GP I-Control**, GP II**-MPTP Toxin alone(20mg/kg), **GP III**-Crude extract alone(CE)(400mg/kg,), **GP IV**- F(100mg/kg), **GP V** -MPTP + CE (200mg/kg), **GP VI**-MPTP + CE (400mg/kg), , **GP VII**- MPTP + selegiline (10mg/kg,) **GP VIII**-MPTP + carbidopa (100mg/kg).

Table: 2a.Effect of Extract of *Mucuna pruriens* on some haematological parameters in albino mice.

Groups	HB	PVC	WBC	RBC	PLASMOGEN	LYMPHOCTES	MONOCYTES	ES
Group – I	14.96±0.31	51.3±0.65	13.43±2.66	9.97±0.38	2±0.33	92.33±0.33	2.66±0.33	2.66±0.33
Group – II	12.36±0.13	48.46±4.01	7.4±0.87	9.14±0.95	4±0.57	90.66±1.76	3.33±0.88	2±0.57
Group – III	14.93±0.13	54.8±1.86	8.6±0.68	10.01±0.49	3±0.15	91±0.57	3.33±6.66	2.66±0.33
Group – IV	15.03±0.4	47.132.03	8.56±0.74	10.36±0.20	2.33±0.88	90±0.57	4±0.15	3±0.57
Group –V	16.03±0.49	53.26±4.44	6.51±0.56	8.83±0.85	7±1.52	84.33±5.17	5.33±0.20	5.33±1.2
Group –VI	14.08±1.6	62.33±1.2	10.4±0.15	9.74±0.15	4.66±0.66	76.66±3.52	7±1.0	4.66±1.76
Group –VII	14.66±0.8	45.26±3.65	7.76±1.06	8.12±0.60	3.33±1.33	89.66±2.4	4±0.57	3±0.57
Group-VIII	13.83±0.08	37.33±1.76	7.44±0.69	7.32±0.43	3.66±1.2	82.66±1.45	4.33±1.45	30.57

Results represent Mean ± SE of six replicated experiments. They are significantly different at the $p< 0.05$ level of confidence.

GP I-Control**, GP II**-MPTP Toxin alone(20mg/kg), **GP III**-Crude extract alone(CE)(400mg/kg,), **GP IV**- F(100mg/kg), **GP V** -MPTP + CE (200mg/kg), **GP VI**-MPTP + CE (400mg/kg), , **GP VII**- MPTP + selegiline (10mg/kg,) **GP VIII**-MPTP + carbidopa (100mg/kg).

Table: 2b. Effect of Extract of *Mucuna pruriens* on some haematological parameters in albino mice*

Groups	PC	MCV	MCH	MCHC	RDW	MPV
Group – I	259±82.71	51.8±2.65	15.1±0.9	29.16±0.29	22.53±0.49	8.7±0.11
Group – II	870.66±33.11	53.06±1.58	15.83±1.83	29.63±1.85	19.16±0.89	8.36±0.17
Group – III	1053.33±15.67	54.46±0.99	15.03±0.33	27.43±0.52	19.2±0.45	8.5±0.2
Group – IV	1319±69.15	53.33±1.45	16.26±0.63	25.33±0.88	22.41±1.15	7.76±0.39
Group –V	1121.33±189.09	58.16±1.29	16.6±0.4	29.6±0.92	27.6±4.87	8.93±0.34
Group –VI	1611.66±33.71	59.66±1.45	16.73±0.93	26.33±1.2	22.7±0.51	9.46±0.17
Group –VII	842.66±78.01	55.73±2.36	18.233±1.33	2.33±0.61	26.66±3.16	8.43±0.29
Group –VIII	939±20.1	56.66±0.66	15.33±0.88	24.79±2.06	20.33±0.88	7.83±0.66

Results represent Mean ± SE of six replicated experiments. They are significantly different at the $p< 0.05$ level of confidence.

GP I-Control**, GP II**-MPTP Toxin alone(20mg/kg), **GP III**-Crude extract alone(CE)(400mg/kg,), **GP IV**- F(100mg/kg), **GP V** -MPTP + CE (200mg/kg),

GP VI-MPTP + CE (400mg/kg), , **GP VII**- MPTP + selegiline (10mg/kg,) **GP VIII**-MPTP + carbidopa (100mg/kg).

CONCLUSION

Haematological and biochemical investigations showed that no significant difference (p>0.05) in the parameters measured for packed cell volume (PCV), haemoglobin (Hb), RBC and WBC differential counts. However, *Mucuna pruriens* was increased the total WBC count. Further studies are required to establish the histopathological studies and monoamino oxidase.

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