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RESEARCH ARTICLE

Testosterone like Activity of Ethanolic and Aqueous Extracts of *Mucuna pruriens* Seeds and its Effects on Serum Biochemical Metabolites in Immature Male Rats

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ABSTRACT

Testosterone like activity of seeds of *Mucuna pruriens* and its effects on serum biochemical metabolites in immature male rats were investigated. Forty eight immature male rats were divided into four equal groups. Rats of groups A and B were orally given ethanolic and aqueous extracts of *Mucuna pruriens* seeds daily at the dose rate of 500 mg/kg body weight, respectively, for 14 days. Rats of group C were injected with testosterone at the dose rate of 2.5 mg/kg body weight daily, while rats of group D served as controls. After 7 days, six rats from each group were euthanized, while the remaining six rats from each group were euthanized after 14 days of treatment. Rats given ethanolic extract gained higher weight compared to controls ($P<0.05$). Testis weight was the highest in rats treated with testosterone. The effect of treatments on the weight of the liver and the kidneys was non significant. Rats given ethanolic or aqueous extract had higher serum testosterone concentration than controls. Similarly, rats given ethanolic or aqueous extract had higher serum total proteins, total cholesterol and HDL cholesterol compared to controls. Moreover, ethanolic extract treated rats also had higher total cholesterol and HDL cholesterol than aqueous extract treated rats. However, differences in serum total proteins, total cholesterol and HDL cholesterol between control and testosterone injected rats were non significant. Serum triglycerides, LDL cholesterol and ALT activity did not differ among rats of four groups. Serum AST activity and urea were lower in rats treated with ethanolic or aqueous extract compared to controls. Thus, seeds of *Mucuna pruriens* had testosterone like activity and increased serum total proteins, total cholesterol and HDL cholesterol, with no adverse effects on the serum LDL cholesterol, liver or kidney functions.

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INTRODUCTION

Pakistan has a rich source of herbs and indigenous medicinal plants which have been used since ages for the treatment of various ailments in man and animals. These plants have been shown to possess various therapeutic activities, such as antipyretic, analgesic and anti-inflammatory. *Calotropis procera* possesses anthelmintic (Basu and Chaudhuri, 1991; Iqbal *et al.*, 2005) and analgesic activity (Dewan *et al*., 2000). This plant can also be used as a purgative, anticancer, as well as for the treatment of leucoderma, ulcers, piles and diseases of spleen (Jain *et al*., 1996). Many indigenous medicinal plants have also been shown to possess sex hormones or like activities. Estrogen like activity has been seen in *Pithecellobium dulce* (Saxena and Singal, 1998) and *Colebrookia appositifolia* (Gupta *et al*., 2001).

Mucuna pruriens is an herbaceous twining annual. This plant, also known as Kaunch, is native of many countries including Indo-Pakistan subcontinent. It is a leguminous plant, having antidiabetic, aphrodisiac (Suresh and Prakash, 2011) and central depressant effects (Amin *et al*., 1993). It is still used to increase libido in men, to treat depression and nervous disorders, and to help improve mental alertness. Mucuna has been used for generations in India to treat Parkinson's disease. In infertile men, seeds of *Mucuna pruriens* have been shown to rectify the perturbed seminal plasma contents of

alanine, citrate, glyceryl phosphoryl choline (GPC), histidine and phenyl alanine, improve semen quality, increase serum dopamine, adrenaline, nor adrenaline, testosterone and LH levels (Shukla *et al*., 2009; Gupta *et al*., 2011), re-activate anti-oxidant defense system and reduce stress (Shukla *et al*., 2010). The present paper describes testosterone like activity of the ethanolic and aqueous extracts of *Mucuna pruriens* seeds and its possible effects on the serum biochemical metabolites in immature male rats.

MATERIALS AND METHODS

Collection and extraction of plant material: Seeds of *Mucuna pruriens* were purchased from the local market, grinded into fine powder and used for extraction. The ethanolic and aqueous extracts of powdered material were prepared, using the Soxhlet's apparatus (Fisher Scientific), as described earlier (Ahmad *et al*., 2009; Karcıoglu *et al*., 2011). Briefly, suitable amount of the powdered plant material was soaked in reagent grade ethanol (90%) and soxhlation was done at 70°C for 72 hours to isolate ethanolic extract. For aqueous extracts, deionized water was used instead of ethanol and the soxhlation was carried out at 100°C for 72 hours. In this way, a golden brown extract was obtained which was dried in a rotary evaporator.

Experimental rats: Forty eight immature male rats (3-4 weeks of age) were procured from the National Institute of Health, Islamabad, Pakistan. These rats were maintained under naturally prevailing climatic conditions with proper arrangements for protection against severe weather conditions. They were provided with feed and water *ad libitum*. These rats were acclimatized for 3-5 days before start of the experimental treatments.

Treatments & post-treatment monitoring: Experimental rats were randomly divided into four groups A, B, C and D, with 12 rats in each group. Rats of groups A and B were orally given ethanolic and aqueous extracts of *Mucuna pruriens* seeds at the dose rate of 500 mg/kg body weight, respectively, for 14 days. Rats of group C were given testosterone (Testoveron Depot; Medipham Pvt Limited, Lahore, Pakistan) subcutaneously at the dose rate of 2.5 mg/kg body weight for 14 days, while rats of group D served as control.

After 7 days, six rats from each group were euthanized, while the remaining rats were euthanized after 14 days of treatment. Body weight & weights of the testis, liver & kidneys were recorded. Serum samples collected from these rats were analyzed for the concentrations of testosterone, total proteins, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, ALT activity, AST activity and urea, using commercially available kits (Randox Laboratories Ltd., UK). Testicular tissues were used for the determination of testis cholesterol contents.

Serum biochemistry: Serum total protein contents were measured using total protein kit, Catalogue No. TP-245. The concentration of total proteins in the standard was 6.0g/dl. Serum cholesterol contents were measured following enzymatic endpoint method, using cholesterol kit (Catalogue No. CH-200). The concentration of cholesterol in the standard was 200 mg/dl. Triglyceride contents in serum were determined by GPO-PAP method, using triglycerides kit, Catalogue No. TR-210. The concentration of triglycerides in the standard was 200 mg/dl. For the determination of serum HDL-cholesterol, analytical kit (Catalogue No. CH-203) was used. The concentration of HDL-cholesterol in the standard was 200 mg/dl. LDL-cholesterol contents in the serum were computed by using the following formula:

LDL cholesterol (mg/dl) = Total cholesterol – HDL cholesterol–Triglycerides/5

Serum ALT activity was measured with ALT kit (Cat # AL-146), while AST kit (Cat # AS-147) was used for the determination of serum AST activity. The concentration of these enzymes in respective kit was 2.0 mmol/L. Serum urea concentrations were determined through enzymatic kinetic method, using analytical kit (Cat # UR-445). The concentration of the standard in the kit was 80 mg/dl.

Hormone assays: Serum testosterone concentrations were determined, using solid phase enzyme-liked immuno-sorbent assay (ELISA), using commercially available Testosterone ELISA kit (Cat # EIA-1559, DRG Diagnostics, Germany). The concentrations of standards were 0, 0.2, 0.5, 1.0, 2.0, 6.0 and 16.0 ng/ml. The analytical sensitivity of the assay was 0.083 ng/ml. The cross reactivity for other steroids was <3.3%. The intra-assay and inter-assay coefficients of variation were <5 and <10%, respectively.

Determination of testis cholesterol contents: For the determination of testis cholesterol contents, 100 mg tissue was homogenized in 500 µL chloroform: methanol (2:1). After homogenization, 125 µL of 0.15M sodium chloride was added and samples were centrifuged at 2000g for 15 minutes. The lower phase was used for the determination of cholesterol contents using the analytical kit method.

Phytochemical screening: The powdered material of *Mucuna pruriens* seeds was screened for the presence of various phytochemical constituents. These constituents included: tannins, alkaloids, glycosides, saponins, reducing sugars, steroids and carbohydrates. The protocol already described by Evans (1996) was followed for tannins, alkaloids steroids and saponins, while tests for glycosides, reducing sugars and carbohydrates were based on the report of Parekh and Chanda (2007).

Statistical analysis: Mean (± SE) values were computed for various parameters for rats of each group. In order to ascertain the magnitude of differences in these parameters among various groups, the data were analyzed statistically through two way Analysis of Variance procedure, following completely randomized design. Duncan's multiple range test was applied for multiple means comparisons, where necessary.

RESULTS

As shown in Table 1, rats given ethanolic extract gained significantly higher body weight compared to

controls (71.67±14.84 versus 44.25±4.39g; P<0.05). Rats treated with aqueous extract showed the lowest weight gain (34.17±5.65g). Weight of the testis did not differ between rats given plant extracts and controls, but it was higher in rats injected with testosterone (P<0.05). The effect of treatments on the weight of the liver and kidneys was non significant. Serum testosterone concentrations were the lowest (P<0.05) in control group (0.45±0.05 ng/ml). The rats given ethanolic (2.20±0.17 ng/ml) or aqueous extract (2.22±0.25 ng/ml) of *Mucuna pruriens* had higher serum testosterone concentrations than controls (P<0.05) but lower compared to rats given testosterone injection (8.67±0.59 ng/ml). Testis cholesterol contents did not vary among rats of four groups, although the mean value was lowest in rats of control group.

Serum total proteins were lowest in control rats (Table 2). Rats given ethanolic (49.18±1.75 g/L) or aqueous extract (51.17±1.76 g/L) of *Mucuna pruriens* had higher (P<0.05) serum total proteins compared to controls (46.31±1.05 g/L); however, rats given testosterone injection had non-significantly higher serum total proteins compared to controls. Serum total cholesterol contents were higher in rats treated with ethanolic (119.71±3.18 mg/dL) or aqueous extract (103.34±4.02 mg/dL) compared to control group (83.44±2.92 mg/dL). Moreover, ethanolic extract treated rats also had higher (P<0.05) total cholesterol than aqueous extract treated rats. However, difference in serum total cholesterol between control rats and testosterone injected rats was non significant. Serum triglycerides, LDL cholesterol and ALT activity did not differ among rats of four groups. Serum HDL cholesterol was higher in rats treated with ethanolic or aqueous extract compared to control group. Moreover, ethanolic extract treated rats also had higher (P<0.05) serum HDL cholesterol than aqueous extract treated rats. However, difference in serum HDL cholesterol between control and testosterone injected rats

Table 1: Weights of reproductive organs and serum hormone concentrations in male rats given ethanolic and aqueous extracts of *Mucuna pruriens* (mean±SE)

Parameters	Days of treatment	Group A (ethanolic extract)	Group B (aqueous extract)	Group C (testoste-rone)	Group D (control)	Overall mean
Body weight gain (g)	7	22.83±2.24	22.33±4.32	47.00±7.27	35.50±3.94	31.92±3.08B
	14	1 20.50±3.12	46.00±8.10	52.83±8.64	53.00±6.23	68.08±7.10A
	Mean	71.67±14.84A	34.17±5.65C	49.92±5.45B	44.25±4.39BC	50.00±4.65
Testis weight (g)	7	0.43±0.02	0.43±0.03	0.47±0.03	0.43±0.03	0.44±0.01
	14	0.63±0.03	0.53±0.02	0.69±0.04	0.53±0.05	0.60±0.02
	Mean	0.53±0.03AB	0.48±0.02B	0.58±0.04A	0.48±0.03B	0.52±0.02
Weight of the liver (g)	7	3.58±0.24	3.27±0.37	4.00±0.54	4.36±0.31	3.80±0.20B
	14	5.66±0.25	3.67±0.50	4.04±0.54	5.24±0.97	4.65±0.34A
	Mean	4.62±0.35	3.47±0.30	4.02±0.36	4.80±0.51	4.23±0.20
Weight of the kidney (g)	7	0.36±0.01	0.37±0.02	0.37±0.03	0.40±0.03	0.38±0.01B
	14	0.56±0.03	0.43±0.02	0.48±0.04	0.53±0.06	0.50±0.02A
	Mean	0.46±0.03	0.40±0.02	0.43±0.03	0.46±0.04	0.44±0.01
Serum testosterone (ng/ml)	7	2.33±0.27	2.18±0.35	8.62±0.69	0.34±0.07	3.37±0.68
	14	2.07±0.22	2.25±0.39	8.72±1.04	0.56±0.05	3.39±0.71
	Mean	2.20±0.17B	2.22±0.25B	8.67±0.59A	0.45±0.05C	3.38±0.48
Testis cholesterol (mg/dL)	7	18.79±2.11	19.11±0.61	17.93±0.52	18.21±0.62	18.51±0.55A
	14	20.45±0.43	19.88±0.54	21.74±1.58	19.09±0.39	20.29±0.46B
	Mean	19.62±1.06	19.49±0.41	19.84±0.98	18.65±0.38	19.40±0.38

Values with different letters within a row or a column for each parameter differ significantly (P<0.05).

Table 2: Concentrations of various biochemical metabolites in immature male rats given ethanolic & aqueous extracts of *Mucuna pruriens* (mean±SE)

Parameters	Days of treatment	Group A (ethanolic extract)	Group B (aqueous extract)	Group C (testosterone)	Group D (control)	Overall mean
Total proteins (g/L)	7	44.19±1.03	46.99±1.12	47.38±1.32	43.75±1.36	45.58±0.66B
	14	54.17±1.59	55.34±2.31	48.12±0.73	48.87±0.59	51.63±0.96A
	Mean	49.18±1.75AB	51.17±1.76A	47.75±0.73BC	46.31±1.05C	48.60±0.72
Total cholesterol	7	126.94±1.34	104.37±5.83	87.79±5.06	81.63±5.66	100.18±4.29
(mg/dL)	14	112.47±4.66	102.31±6.05	90.73±2.74	85.25±2.01	97.69±2.93
	Mean	119.71±3.18A	103.34±4.02B	89.26±2.78C	83.44±2.92C	98.94±2.58
Triglycerides (mg/dL)	7	73.11±4.31	62.97±6.90	70.66±6.51	103.11±8.46	77.46±4.46B
	14	84.97±3.46	88.05±11.94	99.69±5.96	87.84±9.92	90.14±4.13A
	Mean	79.04±3.17	75.51±7.58	85.17±6.07	95.48±6.63	83.80±3.15
HDL cholesterol	7	79.14±2.91	58.51±2.10	45.57±2.05	46.99±2.18	57.55±3.01
(mg/dL)	14	70.43±3.93	56.66±3.53	46.20±1.02	42.89±2.99	54.04±2.66
	Mean	74.78±2.67A	57.58±1.98B	45.89±1.09C	44.94±1.87C	55.80±2.00
LDL cholesterol	7	33.21±3.54	33.24±5.16	28.69±3.86	18.14±3.31	28.32±2.28
(mg/dL)	14	24.30±2.44	28.05±3.83	24.58±3.12	24.79±1.41	25.43±1.36
	Mean	28.76±2.45	30.65±3.16	26.64±2.45	21.47±1.98	26.88±1.33
AST activity (U/L)	7	15.29±1.17	15.51±1.16	19.62±0.63	20.87±1.41	17.82±0.74
	14	17.91±1.29	15.66±1.18	19.41±1.48	19.65±0.48	18.16±0.64
	Mean	16.60±0.92B	15.58±0.79B	19.51±0.77A	20.26±0.74A	17.99±0.48
ALT activity (U/L)	7	19.84±1.78	21.54±1.65	22.04±1.57	22.55±2.39	21.49±0.90
	14	23.16±1.99	19.91±2.00	24.38±2.17	23.54±1.48	22.75±0.96
	Mean	21.50±1.37	20.73±1.26	23.21±1.32	23.05±1.35	22.12±0.66
Urea (mg/dL)	7	17.48±0.77	22.61±1.62	34.27±2.83	34.95±1.51	27.33±1.78
	14	19.44±1.31	25.87±0.96	34.29±2.37	34.98±2.11	28.64±1.57
	Mean	18.46±0.78C	24.24±1.02B	34.28±1.76A	34.96±1.24A	27.98±1.18

Values with different letters within a row or column for each parameter differ significantly (P<0.05).

was non significant. Serum AST activity was lower ($P<0.05$) in rats treated with ethanolic or aqueous extract compared to testosterone injected or control rats. The differences between the former two and the latter two groups were non significant. Similarly, serum urea was lower in rats given ethanolic or aqueous extract than in rats given testosterone or the controls, the difference between the latter two groups was non significant. Rats given ethanolic extract also had lower serum urea than those given aqueous extract (Table 2).

Upon phytochemical screening, seeds of *Mucuna pruriens* showed positive reaction for tannins, alkaloids, glycosides, saponins, steroids and carbohydrates. However, they showed negative reaction for reducing sugars.

DISCUSSION

In the present study, rats given ethanolic as well as aqueous extract of *Mucuna pruriens* seeds showed higher serum testosterone levels compared to controls. This indicates that both ethanolic and aqueous extracts of *Mucuna pruriens* possess androgenic activity. Moreover, rats given ethanolic extract of *Mucuna pruriens* gained significantly higher body weight compared to controls. However, this was not seen in rats given aqueous extract of *Mucuna pruriens*. Thus, the increase in body weight of rats given ethanolic extract can be attributed to androgenic activity of *Mucuna pruriens*. According to Rao and Alice (2001), plant extracts can cause changes in the general metabolic status, affecting the body or organ weight of these animals. Androgenic activities of plants have been reported to increase the anabolic activity which resulted in increased body weight of treated rats (Johnson and Everitt, 1988). Perhaps *Mucuna pruriens* increases the production of Human Growth hormone (HGH) and testosterone levels. This in turn increases the body's ability to build lean muscles and breakdown fat. According to Gauthaman *et al.* (2003), this increase in the body weight can be attributed to the androgenic effect of the extract, secondarily contributing to appetite stimulation.

In an earlier study, seeds of *Mucuna pruriens* have been shown to significantly improve mating behaviour, potency and libido of normal male rats (Amin *et al*., 1996; Suresh *et al*., 2009). This improvement in the sexual function of male rats due to the use of *Mucuna pruriens* was attributed, at least in part, to its androgenic activity. *Mucuna pruriens* seeds at a dose level of 200 mg/kg body weight have also been shown to improve sexual behaviour, libido and potency and daily sperm production in streptozotocin-induced diabetic male rats (Suresh and Prakash, 2011).

Phytochemically, seeds of *Mucuna pruriens* possessed tannins, alkaloids, glycosides, saponins, steroids and carbohydrates but no reducing sugars. Previous reports (Anonymous, 2007) also indicate that *Mucuna pruriens* contain L-Dopa, as well as a number of bioactive alkaloids (mucunine, mucunadine, mucuadinine, pruriendine and nicotine). Moreover, seed coat has also been shown to contain various bioactive substances including steroids.

Both ethanolic and aqueous extracts of *Mucuna pruriens* increased serum total protein contents compared to controls. Since androgens have protein anabolic effects (Reeves, 2003), this increase in serum total protein contents would have been due to anabolic effects of androgens present in *Mucuna pruriens* and is necessary for the increased growth and weight gain.

Serum total cholesterol was higher in rats given ethanolic or aqueous extract of *Mucuna pruriens* compared to controls. Moreover, rats given ethanolic extract had higher serum total cholesterol compared to those treated with aqueous extract. However, testosterone injection did not increase serum total cholesterol over controls. It is known that cholesterol in the precursor of all steroids (Reeves, 2003). Increased serum cholesterol in rats given extracts of *Mucuna pruriens* might be used for the synthesis of testosterone.

Moreover, serum HDL cholesterol contents were higher in rats given ethanolic or aqueous extract of *Mucuna pruriens* compared to controls. However, there was no difference in serum triglycerides as well as serum LDL cholesterol contents between rats of treated and control groups. This indicates that the increase in serum total cholesterol contents was due to increased serum HDL cholesterol, while serum LDL cholesterol was not affected by these extracts. In an earlier study, Guan *et al*. (2006) observed that ovariectomized hamsters orally given soybean and kudzu phytoestrogen extract for 7 weeks had significantly decreased serum total cholesterol and non-high density lipoprotein cholesterol, with HDL cholesterol being unaffected. In our study, the treatments were given for only 14 days. It is possible that long term treatment of rats in the present study would have reduced the serum total cholesterol levels.

The present results showed that there was no difference in serum ALT activity among rats of different groups. Serum AST activity and urea levels were lower in rats given ethanolic or aqueous extract of *Mucuna pruriens* compared to controls. This indicates that extracts of *Mucuna pruriens* had no adverse effects on the liver or the kidney functions in rats.

In conclusion, seeds of *Mucuna pruriens* had testosterone like activity & increased serum total proteins, total cholesterol & HDL cholesterol. However, they had no adverse effects on the serum LDL cholesterol, liver or kidney functions.

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