

STUDY OF ANABOLIC EFFECTS OF PEGANUM HARMALA OIL

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Peganum harmala is a perennial herb which can be planted in non-arable lands without disturbing useful crops. Seeds of *P. harmala* attracted attention of researchers as early as 1841 for their alkaloid contents, while oil was first reported by Ovejero (1947). The alkaloids are found in the husk while oil is present in the kernel (Siddiqui and Afza 1978).

Oil obtained from the kernel has been found non-toxic to rabbit skin (Mirza *et al* 1993). The present study aims at investigating its anabolic effect on broiler cockerels. Testosterone propionate has been used as standard drug (British Pharmacopoeia 1988). Although testosterone is an androgenic hormone but reason for its use in the analysis of anabolic activity is that no pure anabolic steroid without androgenic effects has ever been described. All the known actions of hormone are mediated by a single receptor protein (Goodman and Gilman 1985).

Extraction of oil. The moistened seeds were subjected to a kitchen chopper (10,000 rpm). The shaved off husk was immediately separated in a laboratory sieve shaker. Oil from the ground kernel was extracted in a 5 l batch Soxhlet extractor using *n*-hexane. The solvent was removed under vacuo. Free fatty acid ($6 \pm 1\%$) was neutralized using dilute sodium hydroxide. The oil was decolourized using charcoal.

Oral toxicity testing. Male and female adult Sprague-Dawley rats (weight 250-300 g) were selected for oral toxicity test. Animals were housed at a constant temperature of $22^\circ\text{C} \pm 2$ with a 12 h light/12 h dark photoperiod and allowed free access to food and water. They were housed in plastic cages with sliding perforated stainless steel covers. The dimension of cages were 30.0 x 21.5 cm at the top, 26.5 x 20.0 cm at bottom and 16.5 cm height. Testing was carried out during the light portion of the 12 h light/dark cycle. Toxicity was determined in two groups of six rats each (3 male + 3 female).

Peganum harmala oil was administered to the test group at 2 ml kg^{-1} body weight intragastrically (Griffith and Farris 1942; Clarke and Clarke 1975; Loomis 1978). An equivalent dose of the vehicle (normal saline) was administered to the control group. The test and control groups were observed for 72 h for behavioural effects and mortality for one week after treatment. The animals were kept in normal conditions, with free access to food and water. In the acute oral toxicity study, all rats under observation appeared normal with no symptoms of drug induced toxicity. Autopsy studies did not show any gross change in various organs i.e. heart, lungs, liver, stomach, spleen, kidneys, ovaries and testes.

Anabolic effects. Day old broiler cockerel (I.S.A. breed, K&N Poultry Breeding Farms, Karachi) were divided into three groups, each consisting of 20 chicks. All groups were fed on broiler starter mash supplied by Sindh Feed Ltd., Karachi, for four weeks and broiler finisher mash (normal feed) for three weeks. In addition, group 2 and 3 received *P. harmala* oil and testosterone respectively. *P. harmala* oil and testosterone diluted with olive oil having 0.05 ml of test drug and standard drug were administered into the comb using a microsyringe, according to "chick comb method", (Turner and Hebborn 1971). Slight modification was made to assess anabolic effects. Treatment continued daily for seven weeks. At the end of experiment body weights were taken and 24 h after the final dose, the chicks were anaesthetized with anaesthetic BP ether. Combs were cut off with the aid of scissors applied closely to the skull and weighed. The results are reported in Table 1.

Blood was drawn towards the end of the observation period from live chicks directly from heart using a sharp syringe and analysed for plasma protein, reducing sugars in plasma and haemoglobin contents. Test kits supplied by Boehringer and Mannheim were used for the blood analysis. Results are presented in Table 2.

Table 1
Response of the male chicks to testosterone propionate and *P. harmala* oil

S No	Drug	No. of Chicks	Mean body weight (g)	Mean weight of comb (mg)	Comb wt (mg) \pm S.E. Body wt (g)
1.	Control	20	2950	1814.175	0.61 \pm 0.02
2.	<i>P. harmala</i> oil (Test)	20	3209	2635.15	0.8211 \pm 0.04
3.	Testosterone Propionate (standard)	20	2830	2447.75	0.86 \pm 0.05

Both test and standard drugs were dissolved in olive oil; 0.05 ml applied daily for 7 weeks.

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Table 2
Comparison of blood parameters

S. No	Group of animals (20 birds in each group)	Mean value of plasma reducing sugar mg 100 ml ⁻¹	Mean value of Total plasma protein mg 100 ml ⁻¹	Mean value of haemoglobin contents g percent
1.	Control	220	4.93	9.2
2.	Treated with <i>P. harmala</i> oil	216	5.04	9.5
3.	Treated with Testosterone	224	5.10	9.3

Average weight of 20 chicks in group 2 i.e. those given *P. harmala* oil, was more as compared with those given normal diet or testosterone. This indicates that *harmala* oil helps in gaining weight. Similarly mean weight of chick's combs given *P. harmala* oil and testosterone was more than the control group. The ratio of combs' weight to body weight indicates androgenic effects. These ratios are closely similar to those of *P. harmala* oil and testosterone treated groups as compared with control group. In the control group the ratio is less, indicating anabolic and androgenic effects of testosterone propionate and *P. haramala* oil.

Table 2 shows that the mean value of total plasma protein for *P. harmala* oil and testosterone treated group is higher than control group, although it is within normal limits. The normal limit given by Sturkie (1965) is 5.14 g 100 ml⁻¹. The relative higher values of *P. harmala* oil and testosterone treated groups are also indicative of their anabolic effects. The well known anabolic function of the androgens is manifested in their capacity to promote synthesis of protein, not only in muscle but probably in most tissues of the body (Dorfman and Shiply 1956).

Testosterone propionate is well known standard marketed androgenic steroid. *P. harmala* oil shows increase in mean body weight, mean comb weight as compared with the control group. It can, therefore, be concluded that *P. harmala* oil also has anabolic properties similar to testosterone.

The autopsy findings indicated that there was no change in the colour, size or morphology of the internal organs of all the three groups except for the thick fat layers found in the abdomen of the chicks of test group.

The normal value of plasma reducing sugar of 7 weeks broiler chicks is 229 mg 100 ml⁻¹ (Tapper and Kare 1960) whereas the normal value of haemoglobin is 9.7 gram percent (Holmes *et al* 1993). The mean values of plasma reducing sugars and haemoglobin for all the three groups were within normal limits (Table 2).

The results indicate that all the chicks were normal and healthy. Moreover *P. harmala* oil increased anabolic effects in chicks.

Key words: *P. harmala*, Anabolic effects, Chicks.

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