Steroidal and Metabolic Effect of *Kaknaj* (*Physalis alkekengi* Linn)

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Abstract

In the present study the hydroalcoholic extract of fruits of *Physalis alkekengi* was investigated for its steroidal and metabolic activity in albino rats of either sex in two different tests. In both the tests, the animals were treated with the test drug (450 mg/kg/p.o.) twice a day for three days and were sacrificed subsequently on day 4. In the test for steroidal activity, thymus gland was dissected out and weighed while in the test designed for metabolic activity, liver was dissected out for glycogen estimation and blood was collected for the estimation of blood sugar, serum protein and serum cholesterol. The test drug reduced the thymus weight significantly ($p<0.01$) as compared to the plain control. It also induced hyperproteinemic and liver glycogen increasing effect and moderately increased the blood glucose level. The findings suggest that the hydroalcoholic extract of fruits of *Physalis alkekengi* possesses marked steroidal and metabolic activity. Steroidal effect may be one of the bases for its use in kidney diseases especially nephrotic syndrome like condition.

***Keywords*:** Steroidal activity, Metabolic activity, *Physalis alkekengi*, Unani Medicine.

Introduction

The fruit of *Physalis alkekengi*, Linn (Fam. Solanaceae) commonly known as *Kaknaj* is in use in Unani Medicine (*Tibb-e-Unani*) since ancient times to ameliorate various renal diseases (Ghani 1920; Aawan, 1993; Aziz, 1948). It's attributed effects in Unani literature such as anti-inflammatory, diuretic, nephroprotective and tonic to kidney etc are considered instrumental for its efficacy in various renal diseases (Aawan, 1993; Ghani 1920; Aziz, 1948; Dymock, 1891; Nadkarni, 2000; Chopra *et al*., 1956; Trease and Evan, 2002). An injury caused by mechanical or chemical stimuli to the kidneys and the urinary tract are also described to be brought about by its oral administration (Ibn Sina, 1906). Ethnobotanical reports suggest almost similar effects and indicate its therapeutic application in different kidney diseases such as kidney failure, stones; injury to kidney and bladder etc and also to protect the kidney from chemical and physical stimuli (Anonymous, 1996; Dymock, 1891). In a recent study it has been shown to possess significant nephroprotective effect against gentamicin induced nephrotoxicity in experimental animals. It also improved a condition which was simulating with the symptoms of nephrotic syndrome (Wasim et al., 2010). It was hypothesized therefore that

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the steroidal effect (immunological/anti-inflammatory) may be one of the reasons for its efficacy in such a condition. Therefore the hydroalcoholic extract of *Kaknaj* (30:70) was studied for steroidal effect by Thymus Regression Test (Stephenson, 1954). Further since the steroidal agents induce certain metabolic effects therefore metabolic activity was also studied by observing its effect on liver glycogen, serum glucose, serum protein and serum cholesterol levels.

Materials and methods

Preparation of ethanol extract

The fruits of *Physalis alkekengi,* Linn were procured from Dawakhana Tibbiya College, Aligarh Muslim University (AMU), Aligarh, India. Prof S.H. Afaq and Dr. M. Inamuddin (Pharmacognosists), Department of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh confirmed the identity of the drug. A voucher specimen (No. WA/2005/3) has been deposited in the museum of the department of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh, India for future reference.

The fruits were dried at room temperature and reduced to coarse powder by grinding. Powdered drug was macerated in 70% ethanol and left for 12 h at room temperature. It was then extracted for 6 h in a Soxhlet apparatus at 82± 2 ^{0}C. 100 g of powder was extracted in 400 ml of solvent. The extract was filtered using Whatman filter paper and the filtrate was concentrated over a water bath. The yield of the extract was found to be 30% of crude drug (w/w). The extract was reconstituted a fresh in distilled water whenever it was intended to be administrated to the animals.

Experimental Animals

Wistar Albino rats of either sex weighing 40-50 g (Thymus regression test) and 100-150 g (Metabolic test), divided into three groups of six animals each were used. They were maintained on standard diet and water *ad libitum* and housed in clean polypropylene cages at room temperature (25-30 ^{o}C) with a 12 h light: 12 h dark cycle.

Treatment Schedule

The dose of *Kaknaj* for albino rats was calculated by multiplying the human therapeutic dose, described and practiced in Unani Medicine (Ghani, 1920; Husain, 1872; Nabi, 1901; Singh, 1974) by conversion factor of 7 (Freidrich et al., 1966). The dose thus calculated was found to be 450 mg/kg. The test drug

suspended in distilled water was administered to the animals intragastrically with the help of a gastric canula twice a day.

Test for steroidal activity

The test drug was studied for steroidal effect by the method of Stephenson (1954) and Amin *et al*. (1994).

Albino rats of either sex, weighing 40-50 gm were divided into 3 groups of 6 animals each having equal distribution of sexes and such that the total weight of animals in various groups were approximately the same. The animals in Group I served as plain control and received 3 ml of distilled water by oral route, twice a day, for 3 days. The animals in Group II serving as standard control were treated with Hydrocortisone 33.33 microgram/100 g, twice a day, for 3 days, by subcutaneous injection. While the animals in Group III were treated with the hydroalcoholic extract of fruits of *Kaknaj* at a dose of 450 mg/kg, twice a day, for 3 days, by oral route and served as test group. The concentrated extract was reconstituted in suspension form with distilled water (450 mg/3 ml, w/v) and 2% gum acacia, before the administration.

On the 4th day all the animals were sacrificed by overdosing of anaesthetic ether, administered by inhalation and the thymus gland was dissected out. The body weight and the weight of the thymus gland were recorded. The results were expressed as mg of thymus gland/100 gm of body weight.

Test for metabolic activity

The metabolic effect of the test drug was studied on liver glycogen by the method of Montgomery (1957), serum glucose by the method of Hultman (1959), serum protein by the method of Dumas (1971) and serum cholesterol by the method of Wybenga (1974) in albino rats.

Albino rats of either sex, weighing 100-150 gm were divided into 3 groups of 6 animals and treated in the same way as in the previous test. On the 4th day all the animals were sacrificed by overdosing of anaesthetic ether, administered by inhalation, and blood sample was collected by cutting the throat for the estimation of blood sugar, serum protein and serum cholesterol, while the liver was dissected out for glycogen estimation.

2.4. Statistical Analysis

The results were given as mean ± S.E.M. Significance was determined by using the Student's '*t*' test. *P*-value equal to or less than 0.05 showed significance.

Results

Steroidal effect

In plain control group the mean thymus weight was found to be 231.40 ± 0.314 mg/100 gm of body weight while in the standard group treated with hydrocortisone, 33.33 µg/100 g, it decreased to 146.54±0.355 mg/100 gm of body weight ($p<0.001$). The weight of thymus gland in the animals treated with the extract of fruit of *Physalis alkekengi,* Linn (*Kaknaj*) was found to be 178.34±0.310 mg/100 gm ($p<0.01$). The results are presented in Table-1.

Table-1. Effect of *Kaknaj* on the weight of thymus gland

Groups	Thymus Weight (mg/100 gm) (Mean±S.E.M.)
Group I (plain control)	231.40 ± 0.314
Group II (standard control)	146.54 ± 0.355a3
Group III (test group)	178.34 ± 0.310a1b1

n = 6

a = against plain control b = against standard control

1 = $p < 0.05$ 2 = $p < 0.01$ 3 = $p < 0.001$

Metabolic effect

Effect of test drug on liver glycogen

The liver glycogen was found to be 14.12±0.43 mg/gm in plain control group while it increased to 28.89±0.23 mg/gm ($p<0.001$) in the standard group treated with hydrocortisone, 33.33 µg/100 gm of body weight. In the animals treated with the test drug it increased to 20.14±0.62 mg/gm ($p<0.01$).

Effect of test drug on serum glucose

Serum glucose was found to be 76.32±0.45 mg/dl in the plain control group. It increased to 114.74±0.86 mg/dl ($p<0.001$) and 90.38±0.32 mg/dl ($p<0.05$) in standard and test groups, respectively. Glucose level was significantly lower ($p<0.01$) in test group as compared to standard group.

Effect of test drug on serum protein

Serum protein was found to be 5.74±0.49 gm/100 ml of serum in plain control group while in hydrocortisone treated group it amounted to 9.76±0.79 gm/100

ml of serum ($p<0.01$). In the animals treated with the extract of test drug, it was found to be 6.53±0.62 gm/100 ml of serum showing a significant increase as compared to plain control ($p<0.05$).

Effect of test drug on serum cholesterol

Serum cholesterol was found to be 173.54±0.52 mg/dl in plain control group. It increased to 201.33±0.42 mg/dl ($p<0.05$) in hydrocortisone treated group. However it decreased slightly to 169.66±0.48 mg/dl in the group treated with the test drug but was not found significant statistically. The results of metabolic effects are presented in Table-2.

Table-2. Effect of fruit of *Kaknaj* on metabolic parameters

Groups	Liver Glycogen (mg/gm) (Mean ± S.E.M.)	S. Glucose (mg/dl) (Mean ± S.E.M.)	S. Protein (g/100 ml) (Mean ± S.E.M.)	S. Cholesterol (mg/dl) (Mean ± S.E.M.)
Group I (Plain control)	14.12±0.43	76.32±0.45	5.74±0.49	173.54±2.52
Group II (standard control)	28.89±0.23a3	114.74±0.86a2	9.76±0.79a2	201.33±0242a1
Group III (test drug)	20.14±0.62a2b1	90.38±0.32a1b2	6.53±0.62a1b2	169.66±1.48b1

N = 6

a = against plain control b = against standard control

1 = $p<0.05$ 2 = $p<0.01$ 3 = $p<0.001$

Discussion

The study reveals that the test drug *Physalis alkekengi* possesses significant steroidal activity. It was found to reduce the thymus weight to an extent which was only moderately lesser than the regression produced by hydrocortisone (Table.1). The thymolytic activity of hydrocortisone and its analogues particularly in immature animals is well documented (Stephenson, 1960). In an *in vitro* study it has been shown that basophilic cells normally found in 12 and 13-day embryonic thymus glands disappeared after steroid treatment (Younan, 1968). Thymus regression effect of steroids was also evident from the findings of the present study where the weight of thymus gland was found to be decreased significantly ($p<0.001$) under the influence of hydrocortisone.

Similarly, the test drug by decreasing the weight of thymus gland significantly ($p<0.01$) indicated having thymolytic and thereby steroidal effect. Since, the steroids have an immunosuppressant effect, which is the basis of their therapeutic application in nephrotic syndrome therefore the efficacy of test drug in nephrotic syndrome like condition for which it has been recommended in Unani literature and validated in an experimental study (Wasim et al., 2010), may be attributed at least partially to its steroidal effect. This finding is also suggestive of its potential to alleviate other diseases where steroids may have a role. Other effects reported to the test drug such as diuretic (Wasim et al., 2005) and anti inflammatory (Kang et al., 2011) etc may have a direct bearing on nephrotic syndrome and related conditions and/or act as adjuvant to the principal drug. It is interesting to mention that steroids in addition to their immunosuppressive effect also possess anti-inflammatory effect and may modify the body's immune response to diverse stimuli (Waldman et al., 2007). Thus the combined nephroprotective, anti inflammatory, diuretic and steroid like effect the test drug is attributed with, appears to be in direct commensuration with the physiopathology of nephrotic syndrome. The findings are also suggestive that the diseases with diverse physiopathological appearance can be treated even with a single drug of Unani medicine because they commonly have multiple and related, even synergistic effects. This is one of the many advantages that crude drugs have over the isolated compounds.

Steroidal agents play important part in controlling salt and water balance in the body, and regulating carbohydrate, fat, and protein metabolism. They are responsible for certain metabolic effects although when they are used in immunosuppressive and anti-inflammatory therapy their metabolic and other effects are taken as unwanted side effect (Rhen and Cidlowski, 2005). Therefore, different metabolic effects induced by the steroids are mostly not desirable therapeutically. The present study showed that the test drug has significant hyperproteinaemic and liver glycogen increasing effects. It also produced moderate hyperglycaemia which was significantly less than the findings of standard group, but did not alter the cholesterol level. These findings are more or less in consonance with steroidal activity (Stephenson, 1960). Thus the metabolic effects produced by *Physalis alkekengi* further confirmed that it possessed steroidal effect. Hyperglycaemia and hyperchesterolaemia are not desirable effects of steroids at all whereas hyperproteinemic and glycogenic effects at occasions are used therapeutically. By demonstrating moderate effect on glucose level and not modifying the cholesterol level, the test drug exhibited that it has relatively lesser chances of producing unwanted side effects and is therefore safer than the common

steroidal agents. Although, Tang et al (2008) have reported hypoglycaemic effect in *Physalis alkekengi* but they have studied one of its isolated polysaccharides not the whole drug or its extract, and such a difference in the efficacy of whole drug and its fractionated part is not uncommon.

Thus, the findings of the present study have shown that the fruit of *Physalis alkekengi* possesses marked steroidal and metabolic activity. The steroidal effect may be the basis of its wide therapeutic application in various renal disorders including nephrotic syndrome like condition as described by Unani physicians. It has a definite edge over the pure steroids on account of having minimum chances of producing side effects that are common to the steroidal drugs.

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References

Aawan, M.H., 1993. Kitabul Mufradat Al-Maroof Ba Khawasul Advia Batarz-e-Jadeed, published by Shaikh Ghulam Ali & Sons (Pvt.) Ltd., Lahore, p. 341.

Amin, K.M.Y., Ahmad, S., Khan, N.A., 1994. Antinephrotic syndrome effect of ethno drug *Bisehri Booti* (*Aerva lanata*) – an experimental study of relevant pharmacological actions, Abstract Book, Fourth International Congress on Ethnobiology, NBRI, Lucknow, November 1994, p. 94.

Anonymous, 1996. The Encyclopaedia of Medicinal Plants, published by Dorling Kindersley Ltd., Great Britain, p. 245.

Aziz, M.A., 1948. Mufradat-e-Azizi, published by Sahitya Mandir Press Ltd. Lucknow, p. 47

Chopra, R.N., Nayar, S.L., Chopra, I.C., 1956. Glossary of Indian Medicinal Plants, National Institute of Science Communication and Information Resources, Council of Scientific and Industrial Research, New Delhi, p.191.

Dumas et al., 1971.Clinical Chemistry *Acta*, 31: 87-96

Dymock, W., Warden, C.J.H., Hooper, D. 1891. Pharmacographia India – A History of the Principal Drugs. The Institute of Health and Tibbi Research, Pakistan, Vol. II, p. 303.

Freidrich, E.J., Gehan, E. A., Rall, D.P., Schmidt, L.H. and Skipper, H. E, 1966. Quantitative comparision of toxicity of anti cancer agent in mice, rat, hamster, dog, monkey and man. *Cancer Chemotherapy Report* 50(4): 219-244.

Ghani, M.N. (1920). Khazanat-ul-Advia. Munishi Naval Kishore, Lucknow, Vol. III, p. 240.

Hultman E, 1959. Rapid specific method for determination of aldosaccharides in body fluids, *Nature* 10:183(4654):108-9.

Husain, M. 1872. Bahrul Jawahar. Mohd. Ali Bakhs Khan, Lucknow, p. 242.

Ibn Sina, A. A., 1906. Al Qanoon fit-Tib. Munshi Naval Kishore, Kanpur, Vol. II, p. 127.

Kang, H., Kwon, S.R., Choi, H.Y., 2011. Inhibitory effect of *Physalis alkekengi L. var. franchetii* extract and its chloroform fraction on LPS or LPS/IFN-γ-stimulated inflammatory response in peritoneal macrophages. *J Ethnopharmacol.* 26;135(1):95-101.

Montgomery, R, 1957. Determination of glycogen. *Arch Biochem Biophys* 67(2): 378-86.

Nabi, M.G., 1901. Makhzan Mufradat wa Murakkabat-e-Azam. Narain Das Jangali Mal, Delhi, p. 153.

Nadkarni, A.K., 2000. Indian Materia Medica, vol.1, Bombay Popular Prakashan, Mumbai, p. 950.

Rhen, T. and Cidlowski, J. A., 2005. Antiinflammatory action of glucocorticoids--new mechanisms for old drugs". *N. Engl. J. Med.* 353 (16): 1711–23.

Singh, D., 1974. Unani Darvyagunadarsh, 2nd ed. Ayurvedic and Tibbi Academy, Lucknow, Uttar Pradesh, p. 135.

Stephenson N.R., 1954. Assay of adrenocortical hormones on the thymus of the weanling rat, *Can J Biochem Physiol*. 32(6):689-702.

Stephenson, N. R. 1960. The effect of corticosterol analogues on the thymus gland of the immature rat. *Journal of Pharmacy and Pharmacology* 12 (1), 411-15.

Tong, H., Liang, Z., Wang, G., 2008. Structural characterization and hypoglycemic activity of a polysaccharide isolated from the fruit of *Physalis alkekengi* L. Carbohydr. *Polym.* 71: 316-323.

Trease, G. E. and Evan W.C., 2002. Pharmacognosy, the University Press, Aberdeen, p. 479.

Waldman M, Crew RJ, Valeri A, Busch J, Stokes B, Markowitz G, et al. Adult minimal-change disease: clinical characteristics, treatment, and outcomes. *Clin J Am Soc Nephrol*. May 2007; 2(3):445-53.

Wasim, A., Khan, N.A., Ghufran, A., Shamshad A., 2005. Physicochemical Standardization and Pharmacological Study of Some Nephroprotective Unani Drugs, MD Thesis, Department of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh, India.

Wasim, Ahmad, Khan, N.A., Ghufran, Ahmad, Shamshad Ahmad, 2010, Effect of Kaknaj (*Physalis alkekengi* fruit) on gentamicin model of acute renal impairment in rats, *Hippocratic Journal of Unani Medicine* 5 (3): 107-117.

Wybenga D.R., Pileggi VJ, Dirstine P.H., Di Giorgio, J., 1970. Direct manual determination of serum total cholesterol with a single stable reagent**.** *Clin. Chem.* 16(12): 980-4.

Younan A. S., 1968. Effect of steroids on thymus lymphoid development *in vitro. The Anatomical Record* 161 (2): 187–195.