

EVALUATION OF NEPHROPROTECTIVE ACTIVITY OF ORTHOSIPHON STAMINEUS BENTH EXTRACT USING RAT MODEL

N. Kannappan, Madhukar. A*, Mariymmal, P. Uma Sindhura,
R. Mannavalan

Department of pharmacy, Annamalai University, Annamalai Nagar, Chidambaram,
Post Code No. 608002, Tamil Nadu, India.

*E-mail: madhukar.mpharm@yahoo.com,
kannappan70@yahoo.co.in

ABSTRACT : The work was undertaken to carry out the preliminary phytochemical studies and Nephroprotective activity of *orthosiphon stamineus* family *Laminaceae (Labiatae)*. These studies revealed the presence of flavonoids, tannins, saponins, phenols and terpenoids. The drug is found to be potent diuretic which causes excretion of sodium and potassium. These observations made us to investigate the plant material for its neproprotective activity in rats. Gentamycin is an extensively used aminoglycoside antibiotic. It has been reported to produce nephrotoxicity even at normal therapeutic dose level. The drug was administered intra peritoneally at a dose of 80mg/kg weight for 9 days. Histopathological sections showed marked glomerular, peritubular and blood vessel congestion. These increased levels of serum creatinine, blood urea, urinary protein and extent of renal damage were decreased by the methanolic extract of *Orthosiphon stamineus* at both dose levels that is 100 and 200 mg/kg body weight in rats.

KEYWORDS: Orthosiphon stamineus, Nephrotoxicity Activity, Gentamycin.

INTRODUCTION

The Phytochemical studies and Nephroprotective activity of *Orthosiphon stamineus* Benth extract by using rat modal belongs to family *Laminaceae (Labiatae)*. These studies revealed the presence of flavonoids, tannins, saponins, phenols and terpenoids.

The literature review of *Orthosiphon stamineus* to be reported as so many members using the different types of animal modals. Doan and Nguyen reported as the herbal remedies are widely used alongside modern drugs. They assessed the diuretic effect of four tradition Vietnamese herbal remedies, all claimed to produce an increase of diuresis. And also they reported the diterpenes from *Orthosiphon stamineus* and their nitric oxide inhibitory activity^[1].

Mariam and Asmawi et al. reported as the aqueous extract of *Orthosiphon stamineus* showed the hypoglycemic activity^[2]. Ohashi et al. demonstrated the antihypertensive activities^[3-4] of *Orthosiphon*

stamineus and showed an inhibitory effect on the contractile responses on rat thoracic aorta smooth muscle stimulated with Kcl before hand. Nirnoy and Muangman proved that folio *Orthosiphonis* showed favourable and non favourable effect of stone prevention. Uric acid and uric acid containing stone may be prevented by the increased alkalinity of the urine after drinking *Orthosiphon* tea we feel that *Orthosiphon* may be beneficial in prevention of uric acid formation^[5]. Englert and Harnischfeger reported as an aqueous extract of *Orthosiphon folium*, given orally, enhance considerably ion extraction in rat to a level comparable to that obtained with furosemide^[6].

A. I. Bartanov and E. A. Bartanov reported as the nephrophyte includes *Arctostephylos uva ursi L.*, *Orthosiphon stamineus* Benth, *Poligonum aviculase L.*, *Desmodium canadense L.* extracted a Nephroprotective effect that was conformed by decreasing the creatinine and urea concentration and by dropping proteinuria, increase of GFR^[7] and diuresis compared to controls. Akowuah et al, reported

as the methanolic extracts of *Orthosiphon stamineus* showed to Radical Scavenging activity [8]. Chenclincnamba reported as the screening of Taiwanese crude drugs for antibacterial activity against streptococcus mutans [9]. Devasagayam et al. reported as the extract of *Orthosiphon stamineus* showed free radicals and antioxidants in human health [10]. Farruch and Iqbal ahamadzafer mehmoood reported as the aqueous extract of *Orthosiphon stamineus* showed antioxidant and free radical scavenging properties [11]. Golamreza karimi and Mohammed ramezani reported as the Cisplatin Nephrotoxicity and protection by milk thistle extracts in rats [12]. Lee et al. reported as the Screening of medicinal plant extracts for antioxidant activity [13]. And Lyckander and Malterud also reported as the lipophilic flavonoids from *Orthosiphon stamineus* prevent oxidative inactivation of 15-lipoxygenase [14]. Masuda reported as the Orthosiphon A & B novel diterpenoid inhibitors of TPA induced inflammation [15] from *Orthosiphon stamineus*. Neugarten et al. reported as the role of tubular obstruction in acute renal failure due to gentamicin [16]. The experimental followed under OECD guidelines for the testing of chemical test No. 423. Acute oral toxicity – Acute toxic class method [17]. Vijayakumar and Naidu reported as the Probuocol protects against gentamicin induced Nephrotoxicity in rats [18].

Waiker and shah reported as the Gentamicin enhance production of hydrogen peroxide by renal cortical mitochondria [19]. Yokazawa et al. study on the inhibitory effect of tannins and flavonoids against the 1, 1-diphenyl-2-picrylhydrazyl radical [20].

MATERIALS AND METHOD

1. Botanical Information

Family: *Laminaceae (Labiatae)*

Genus: *Orthosiphon*

Vernacular Names

English: Cat's whiskers

French: The de Java

Tamil: Poonai meesai.

Tongan: Kava I Pusi

2. Experimental Animals

The experimental protocol was approved by the institutional animal ethics committee (IAEC).

Reference No: Central Animal House

Registration number: 160/1999/CPCSEA

Proposal number: 403

Approved date: 16.11.2006

3. Preliminary Phytochemical Screening

The methanolic extract is subjected to preliminary phytochemical screening for their presence or absence of active Phytochemical constituents by the various methods such as alkaloids test, carbohydrates, steroids, proteins, tannins, phenols, flavonoids, gums

and mucilage, glycosides, saponins and test for terpenes.

4. Acute Oral Toxicity study of *Orthosiphon Stamineus*

The acute oral toxicity study was done according to the OECD guideline 423 (Acute toxic class method).

In this method the starting dose of 100mg/kg body weight /p.o of methanolic extract was administered to male rats/ group, respectively and observed for three days. There were no significant changes in body weight before and after termination of the experiment and no signs of toxicity were observed. The experiment was terminated on 14th day. The experiments were repeated ageing with dose level, 200mg/kg p.o of methanolic extract of *Orthosiphon stamineus* for 3 days more. No significant changes were observed from the first set of experiment. LD cut off mg/kg body weight was observed as X (unclassified) and globally harmonized system (GHS) classes also come under X (unclassified). Then the dose level was increased in order of 400, 600, 800, 1000 mg/kg body weight of the animals, on the 14th day toxic signs were observed and LD₅₀ of *Orthosiphon Stamineus* is 600mg/kg.

5. Nephroprotective Activity Study Protocol

Group I- Animals were administered with equivalent volumes of 0.1 ml i.p of normal saline (0.9% w/v NaCl) for 9 days.

Group II – Animals were received 80 mg/kg/day i.p of gentamicin for 9 days to induce nephrosis.

Group III - IV – Animals were received 80 mg/kg/day i.p of gentamicin for 9 days to induce nephrosis and 100 mg/kg, 200 mg/kg *Orthosiphon stamineus* extracts were given respectively to the animals from 10th to 19th day of study.

Group V – Animals were received 200 mg/kg/oral dose of methanolic extract of *Orthosiphon stamineus* throughout the study period.

Biochemical study

The experiment of Neuroleptic activity in the biological studies was done for the significant changes in body weight, blood urea, serum creatinine, total protein and Glutathione levels.

6. Histopathological studies

The Histopathological studies, administration of methanolic extract of the *Orthosiphon stamineus* extract reveal reduced renal injury induced by gentamicin.

Statistical analysis

The Statistical analysis were carried out using one way analysis variation (ANOVA) followed by student 't' test, p values < 0.05 were considered as significant.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The methanolic extract showed the presence of flavonoids, phenols, carbohydrates, steroids, tannins, glycosides, terpins and saponins. Alkaloids, gums and mucilage's are absent.

Acute Oral Toxicity study of *Orthosiphon stamineus Benth*

Administration of alcoholic extract of *Orthosiphon stamineus Benth* orally produced no observable side effects, including death, upto 400 mg/kg body weight in rats even after 14 days of observation.

Nephroprotective Activity

Percentage change in body weight:

Gentamicin treated animals (Group II) showed a significant decrease ($P < 0.000$) in body weight compared to control rats (Group I) (Table-1). There was a significant increase in body weight of animals treated with 100 mg/kg and 200 mg/kg of methanolic extract of *Orthosiphon stamineus Benth* ($p < 0.000$) respectively, when compared with Group II (Fig-1).

Blood Urea:

As compared to Group-I, A significant increase in blood urea level was observed in Group-II

(Table-2) Group-III and Group-IV showed a significant dose depended decrease in blood urea levels as compared to Group-II. Group-V Produced no significant change in blood urea levels (Fig-2).

Serum creatinine

Group-II showed a significant increase in serum creatinine level when compared to Group-I. Group-III and Group-IV produced a significant reversal the raised serum creatinine levels when compared to Group-II. (Table-3).Normal serum creatinine level was observed in rats of Group-V (Fig-3).

Urinary protein

Group-II showed significant increase in urinary protein level when compared to Group-I. Group-III and Group-IV reversed the gentamicin induced increased urinary protein level significantly (Table-4). Group-V shows normal urinary protein level (Fig-4).

Glutathione

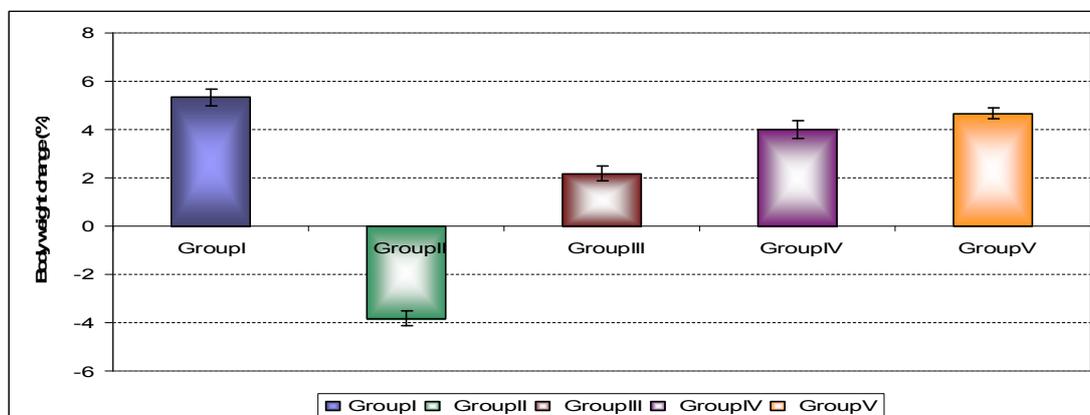
Administration of gentamicin in Group-II animal caused a significant decrease in GSH levels when compared to Group-I (Table-5).As compared to Group-II, Group-III and Group-I reversed the decreased GSH levels significantly (Fig-5).

Body weight change (%). (Table-1)

Group	I	II	III	IV	V
Body weight change (%)	5.33 ±0.33	-3.83 ±0.31a	2.17 ±0.31b	4 ±0.37c	4.67 ±0.21

P values – a < 0.000 vs group I, b< 0.000 vs group II, C <0.000 vs group II

Values are mean± SE of 6 animals in each group

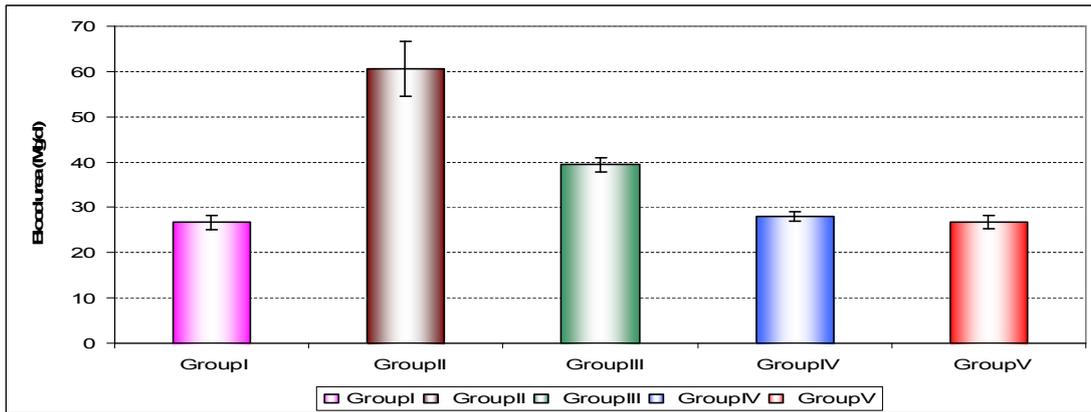


Blood urea (mg/dl). (Table-2)

Group	I	II	III	IV	V
Blood urea mg/dl	26.69 ±1.56	60.55 ±6.02a	39.43 ±1.57b	28.08 ±1.03c	26.77 ±1.44

P values – a < 0.003 vs group I, b< 0.016 vs group II, C <0.002 vs group II

Values are mean± SE of 6 animals in each group

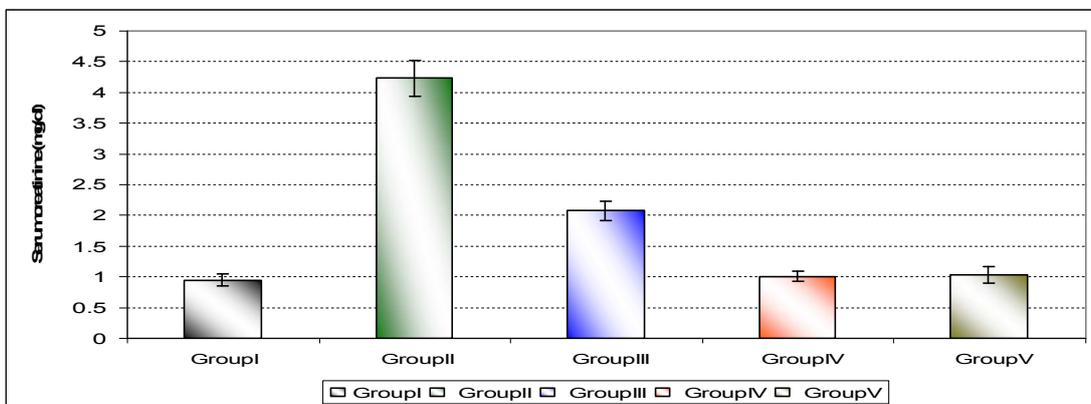


Serum creatinine (Mg/dl). (Table-3)

Group	I	II	III	IV	V
Serum creatinine (Mg/dl)	0.95 ±0.099	4.23 ±0.288a	2.08 ±0.158b	1.01 ±0.083c	1.03 ±0.129

P values – a < 0.000 vs group I, b< 0.003 vs group II, C <0.000 vs group II

Values are mean± SE of 6 animals in each group

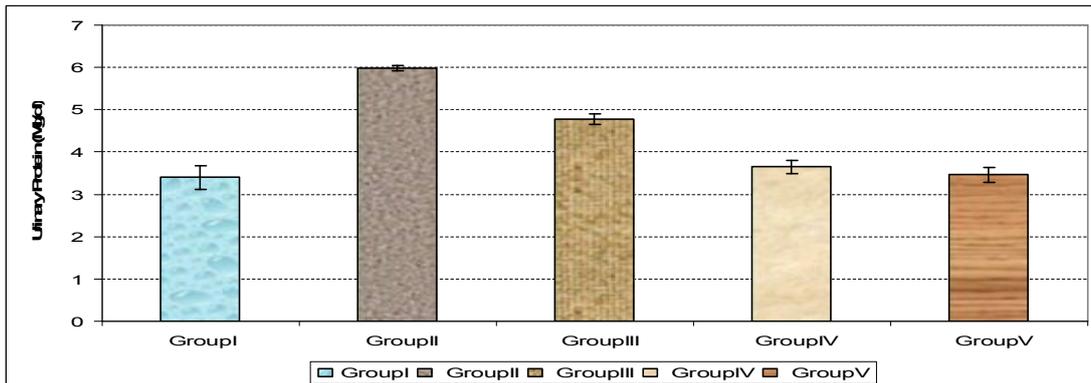


Urinary protein (mg/dl). (Table-4)

Group	I	II	III	IV	V
Urinary protein(mg/dl)	3.40 ±0.277	5.98 ±0.063a	4.77 ±0.124b	3.65 ±0.150c	3.46 ±0.176

P values – a < 0.000 vs group I, b< 0.003 vs group II, C <0.000 vs group II

Values are mean± SE of 6 animals in each group

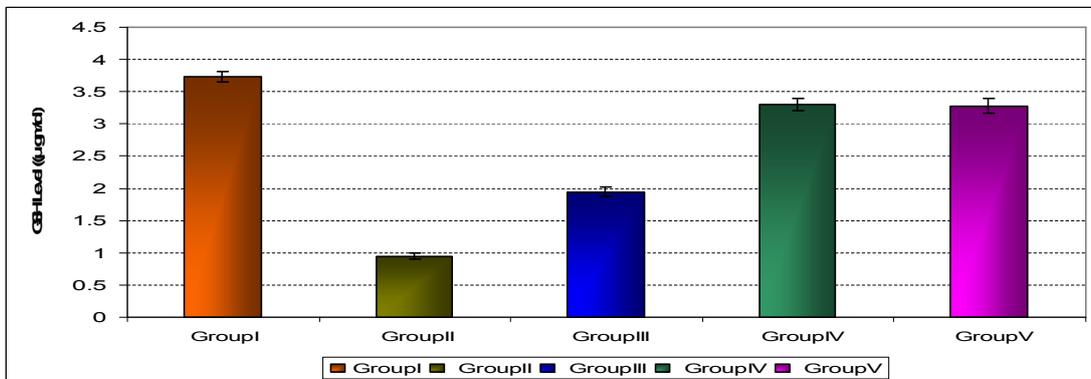


Glutathione (µ gm/dl). (Table-5)

Group	I	II	III	IV	V
GSH level (µgm/dl)	3.735 ±0.078	0.9450 ±0.049a	1.943 ±0.072b	3.30 ±0.090c	3.28 ±0.120

P values – a < 0.000 vs group I, b< 0.003 vs group II, C <0.000 vs group I

Values are mean± SE of 6 animals in each group



HISTOPATHOLOGICAL STUDIES

Effect of alcoholic extract of *Orthosiphon stamineus* in Histopathological features as seen in the kidney in the gentamicin model

Table-6

Histopathological features	Group I	Group II	Group III	Group IV	Group V
Glomerular congestion	-	+	-	-	-
Tubular costs	-	++	-	-	-
Peritubular congestion	-	++	-	-	-
Epithelial desquamation	-	++	+	-	-
Blood vessel congestion	-	++	-	-	-
Inflammatory cells	-	++	+	-	-

REFERENCES

- Doan, D. D, Nguyen, Doan, H. K. Studies on the individual and combined diuretic effects of four Vietnamese traditional herbal remedies. *J. Ethnopharmacol.* 1992, 36(3):225-231.
- Mariam, A., H. Z. Asmawi, et al., Hypoglycemic activity of the aqueous extract of *Orthosiphon stamineus*. *Fitoterpia* 1996, 67(5):465-468.
- Ohashi, K., Bohagari, J. Matsubara, T, Shibuya, H. Indonesian medical plants XXIII. Chemical structures of two new migrated pimarane – type diterpenes neoortho siphols A and B and Suppressive effects on rat thoracic aorta of chemical constituents isolated from the leaves of *Orthosiphon stamineus*. *Bull.* 2000, 48(3):433-435.
- Ohashi, K., Bohagari, J. Matsubara, T, Shibuya, H. Antihypertensive substance in the leaves of Kumis Kuching (*Orthosiphon aristatus*) in java island. *Yakugaku zasshi*, 2000, 120(5):478-482.
- Nirnoy, M., Muangman, V. Effect of *folio Orthosiphon* on urinary stone promoters and inhibitors. *J. Med. Assoc. Thai.* 1991, 74(6):318-321.
- Englert, J., Harnischfeger, G. Diuretic action of *Orthosiphon stamineus* extract in rats. *Planta Med* June 1992, 58(3):237-238.
- A. I. Bartanov, E. A. Bartanov. Nephroprotective effect of nephrophyte in drug nephropathy induced by urographin. *Euromedica hannoeve* 2005, 40-41.
- Akowuah, A. G., Zhari, I., Norhayati, I., Sadikuma. Radical scavenging activity of methanolic extract of *Orthosiphon stamineus*. *Pharm. Biology* 2005, 42(8):629-635.
- Chencplincnamba, TR. Screening of Taiwanese crude drugs for antibacterial activity against streptococcus mutans. *J. Ethnopharmacol*, 1989, 27(3):285-295.
- Devasagayam, TPA, Tilak, J. C, Bollork. Review; Free radical and antioxidants in human health. *Curr stat fut prosp JAPI* 2004, 52, 794-804.
- Farruch, A., Iqbal ahamadzafer mehmood. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medical plants. *Turk. J. Boil.* 2006, 30, 177-183.
- Golamreza karimi, Mohammed ramezani. Cisplatin nephrotoxicity and protection by milk thistle extracts in rats. *Ecam advance access.* July 2005, 26, 1-4.
- Lee, S. E., Hwang, H. J., Hajs. Screening of medical plant extracts for antioxidant activity. *Life Sci.* 2003, 73, 167-179.
- Lyckander, I. M., Malterud, K. E. Lipophilic flavonoids from *Orthosiphon stamineus* prevent oxidative inactivation of 15-lipoxygenase. *Prostaglandins leukol Essent. Fatty acids.* 1996, 54(4):239-246.
- Masuda, T. k., Masuda. Orthosiphol A and B. Novel diterpenoid inhibitors of tpa induced inflammation from *Orthosiphon stamineus*. *Tetrahedron letters.* 1992, 33(8):6787-6792.
- Neugarten, J., Aynedjian, H. S., Bank, N. The role of tubular obstruction in acute renal failure due to gentamicin. *Kidney Int.* 1983, 24(3):330-335.

17. OECD guidelines for the testing of chemical test No. 423. Acute oral toxicity – Acute toxic class method. 1996.
18. Vijayakumar, K., M. V. R. Naidu, Anwar. Probucol protects against gentamicin induced nephrotoxicity in rats. *Ind. J. Pharmacol. Res.* 2000, 32, 108-113.
19. Walker, P. O., Shah, S.V. Gentamicin enhances production of hydrogen peroxide by renal cortical mitochondria. *J. Physiol.* 1987, 253, 495-499.
20. Yokazawa, T., Chen, C. P., Dong, E., Tanaka, T., Nonaka, G. I and Nishioka, I. Study on the inhibitory effect of tannins and flavonoids against the 1, 1-diphenyl-2-picrylhydrazyl radical. *Biochemical pharmacology.* 1998, 56, 213-222.
