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DIURETIC POTENTIAL OF *HALOXYLON RECURVUM* IN INDOMETHACIN INDUCED OLIGURIA

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ABSTRACT: Indometacin has a range of side-effects including oliguria, so the aim was to evaluate the diuretic potential of the medicinal plant, *Haloxylon recurvum* against this problem in an oliguric model. For this purpose, an aqueous-methanolic extract of *H. recurvum* was prepared by maceration and the phytochemical constituents were identified qualitatively. Wistar rats were administered an oral dose of indomethacin (5mg/kg on days 0, 14 and 28 of a protocol), either alone or in combination with *H. recurvum* plant extract (250-500mg/kg) or a reference diuretic agent, furosemide (10mg/kg), orally administered daily for 28 days. The results showed that administration of indometacin by itself induced a progressive decrease in body weight, urine output and urine flow rate. In addition, serum creatinine was increased, while urinary creatinine was decreased, whereas both serum urea and BUN ($p < 0.05$) also declined. Simultaneously, serum sodium was elevated and serum potassium was reduced compared to controls, reflecting a decreased saluretic effect and an overall electrolyte imbalance that accompanied the oliguria. Both *H. recurvum* extract and furosemide reversed or normalized all these parameters that were disrupted by indomethacin, and the plant extract at the higher dose, induced a comparable effect to furosemide. The diuretic activity of *H. recurvum* constituents warrants further mechanistic investigation.

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used medicines according to the world health organization (WHO) list of essential medicines. Statistical data has shown that approximately 30 million individuals use these drugs everyday as over the counter medicines in several countries (1). These drugs have effective pharmacological actions as analgesic, anti-inflammatory and antipyretic agents. Nevertheless, findings from not only clinical trials, but also meta-analytical studies have demonstrated that NSAIDs produce adverse effects on gastrointestinal, hepatic, myocardial, renal, cerebral and pulmonary systems that can provoke multiorgan toxicity (2). Indometacin is an NSAID (3) that inhibits cyclooxygenase enzymes (COX-1 and COX-2) thereby blocking the production of prostaglandins (4) such as prostaglandin E2 (PGE2). In regard to this, prostaglandins are necessary for vasodilation that is essential to the maintenance of kidney function, and indometacin increases vascular resistance, blood urea nitrogen (BUN) as well as creatinine levels (5). In addition, it has been reported that indometacin and other NSAIDs have a range of side-effects including oliguria mediated by inhibition of prostaglandins that potentiate the effect of antidiuretic hormone (6).

Medicinal plants are used in a variety of traditional medicines and they occupy a noteworthy place in drug development. *Haloxylon recurvum* is a medicinal plant belonging to the family Chenopodiacea (7) that is indigenous to the Mediterranean region as well as central and southern Asia(8). The plant has diuretic, abortifacient, and emmenagogic properties in traditional medicine and it has also been reported

to possess antilipoxygenase, antibacterial and antifungal activities. (8); (9). This study was designed to evaluate any in-vitro or in vivo propensity of *H. recurvum* against indometacin induced oliguria in rats as a conceivable lead to unveiling a potentially useful oral diuretic agent.

MATERIAL AND METHODS

Collection of plant material

H. Recurvum plants were collected from the Cholistan desert of Bahawalpur, Pakistan. After identification of the whole plant by a botanist from The Islamia University, Bahawalpur, the sample was preserved in the Pharmacology herbarium. The plant was then crushed and ground down to obtain a coarse powder which was then immersed in (70/30 (v/v) methanol to water) for seven days with intermittent stirring at room temperature. A filtrate was obtained by filtration through a muslin and Whatman's filter paper, and subsequently, it was dried in a rotary evaporator under reduced temperature and pressure. Following evaporation, the concentrated solvent was kept in an oven to remove any remaining solvent. A thick paste was finally obtained as a crude plant extract that was weighed and labelled for further use.

Chemicals and Assay kits

Furosemide (Medibest Pharma Pakistan), indometacin (Shanxi Guangsheng Pharmaceutical, China), formalin (Wah Nobel Chemicals, Pakistan), methanol (Sigma Aldrich, Germany), hydrochloric acid (Sigma Aldrich, Germany), chloroform (Merck),ferric chloride (Pakistan Chemical), iodine (Thermo Fisher Scientific) , Benedict's reagent, Hager's reagent, xylazine (Prix Pharmaceuticals, Pakistan), ketamine (Global Pharmaceuticals), creatinine kit (Arena BioScien, Egypt), urea kit (Arena BioScien, Egypt), serum sodium (spectrum), and serum potassium (spectrum) kits and chemicals were purchased for biochemical analysis.

In-vitro testing

Phytochemical analysis of crude extract of *H. recurvum*

The majority of pharmacological activities are derived from phytochemical elements present in the plant. Therefore, a qualitative phytochemical analysis was performed to identify several possible constituents.

Quinones: the plant extract was mixed with concentrated HCl solution and then shaken vigorously. The formation of a subsequent yellow precipitate designated the presence of quinones.

Glycosides by the Keller Killiani test: a solution was prepared by mixing the plant extract with 2.0 ml of distilled water and admixing 0.4ml of glacial acetic acid with traces of $FeCl_3$, followed by careful addition of concentrated H_2SO_4 (0.5 ml) down the walls of the test tube. The presence of cardiac glycosides was denoted if the acetic acid layer of the solution turned blue.

Terpenes: the plant extract (500 mg) was mixed with 2.0 ml of water, then 1.0 ml of chloroform was added, followed by a few drops of concentrated H_2SO_4. The presence of tri-terpenes was detected by the appearance of a golden yellow coloration.

Diterpenes: the plant extract solution was mixed with few drops of copper acetate. The presence of diterpenes was indicated by the production of an emerald green tint.

Tannins by the gelatin test: 0.5 g of the plant extract was mixed with distilled water and the solution was then treated with 5.0 ml of gelatin solution (1%) plus NaCl. The development of a white precipitate indicated the presence of tannins.

Phenols by the ferric chloride test: to the plant extract was added a small quantity of distilled water and 2.0 ml of 5% ferric chloride solution was then admixed. A green, blue or violet colored precipitate showed the presence of polyphenols.

Phenols by the dilute iodine test: to the plant extract (1.0 ml) was added a few drops of dilute iodine solution, and a transient red color gave an indication of a polyphenolic presence.

Phenols by the lead acetate test: the plant extract in water was mixed with a few drops of lead acetate solution, and the appearance of a white precipitate manifested a polyphenol content.

Protein by the ninhydrin test: ninhydrin solution (2.0 ml; 0.2%) was mixed with a small quantity of plant extract and boiled. A resultant violet color of the mixture signified the presence of proteins.

Protein by the biuret test: to 1.0 ml of plant extract was added 1.0 ml of sodium hydroxide solution (40%) and 2 drops of $CuSO_4$ solution (1%), producing an initial blue color. The subsequent formation of a pinkish or purple-violet color indicated the presence of proteins.

Carbohydrates: Benedict's solution (2.0 ml) was added to a small quantity of plant extract in a test tube, which was shaken and boiled. Formation of a reddish-brown precipitate revealed the presence of carbohydrates.

Alkaloids: a few drops of Hager's reagent, was added to 1.0-2.0 ml of filtrate, and the presence of a yellowish colored precipitate at the bottom of test tube detected the presence of alkaloids.

Flavonoids by the Alkaline Reagent test: the plant extract (1000 mg) was boiled with 10 ml of water for 5 minutes and then filtered. After adding a few drops of 20 % NaOH solution to 1.0 ml cooled filtrate, a dark yellow color changed to a colorless solution, indicating the presence of flavonoids.

Saponins: the plant extract (0.2 g) was dissolved in distilled water (5.0 ml) and heated until boiling on a water bath, then the formation of a foam indicated the presence of saponins.

In-vivo studies

Experimental Animals

Normal healthy Wister albino rats weighting 140 ± 30 gm were purchased from the vivarium of the Faculty of Pharmacy, the Islamia University of Bahawalpur, Pakistan. After acclimatization, they were randomly assigned from different home cages to five groups (n=6) by an independent researcher who was "blind" to the treatments. The animals were provided with standard rodent diet and free access to water. In the laboratory, conditions were maintained at a temperature of 25 ± 3.0◦C (humidity > 65%), on an alternating 12h/12h light/dark cycle. The study and protocol were approved by the Pharmacy Animal Ethics Committee, Faculty of Pharmacy, Islamia University of Bahawalpur.

Indometacin induced oliguria animal model

Oliguria was induced using indometacin in the animals(10) and furosemide was utilized as a reference comparator diuretic drug(11).The experimental animals were grouped as follows:

Group 1: received distilled water (orally [p.o.]) with no other treatment (Control).

Group 2: received indometacin (5mg/kg p.o.) on days 0, 14 and 28 of the protocol (oligouria group).

Group 3: received indometacin (5 mg/kg) on protocol days 0, 14 and 28, plus furosemide (10 mg/kg) p.o (combination reference diuretic) for 28 days.

Group 4: received indometacin (5mg/kg p.o. on protocol days 0, 14, and 28) plus extract of *H. recurvum* (250mg/kg p.o.) for 28 days.

Group 5: received indometacin (5mg/kg p.o. on protocol days 0, 14, 28) plus extract of *H. recurvum* (500mg/kg p.o.) for 28 days.

Collection of metabolic data

Body weight, urinary output and urinary flow rate were measured on days 0, 14 and 28 of the protocol with the aid of metabolic cages. The urinary flow rate was calculated from the following formula:

Urine flow rate (µl/min/100g body weight) = Urine output (ml/24h) × 1000 × 100/1440 × animal weight (g)

Collection of blood samples

Blood samples were collected by retro-orbital puncture from all experimental animals on protocol days 0, 14 and 28 under anesthesia using ketamine (500mg/10ml) plus xylazine (23.32mg/ml) in the ratio of 10:1. The blood was collected in EDTA tubes and centrifuged for 15 minutes at 3000rpm. Serum was separated and then kept at -4°C for further biochemical investigation.

Assessment of diuretic activity of crude extract of Haloxylon Recurvum

The diuretic activity of the animal groups was evaluated by the measurement of urine output and urine flow rate, in addition to the biochemical parameters; serum creatinine, urine creatinine, serum urea, serum blood urea nitrogen (BUN), serum sodium and serum potassium. The blood urea nitrogen was calculated from the following formulae.

BUN = 0.466 × concentration of urea in serum

STATISTICAL ANALYSIS

Data values were presented as mean ± SEM (n=6). The Shapiro-Wilk test was applied to confirm normal distribution of the data, then, statistical significance between different experimental groups was evaluated by two-way ANOVA with post hoc Bonferroni's test with the aid of GraphPad Prism (version 8.0). Values of $p < 0.05$ were considered statistically significant (12).

RESULTS

Phytochemical Analysis of *H. Recurvum* aqueous methanolic extract

Phytochemical analysis of *H. Recurvum* extract revealed that the plant contained a range of phytochemicals as shown in Table 1.

Effect of indometacin treatment, and its combination with furosemide or *H. Recurvum* extract on rat body weights over 28 days

Indomethacin treatment caused a progressive loss of rat body weight during the 28-day treatment protocol. In contrast, combined treatment with furosemide reversed the indomethacin body weight loss, and similarly, *H. recurvum* extract had a time-dependent, dose-graded restorative action on indomethacin induced weight loss (Table 2).

Effect of indometacin treatment and its combination with furosemide or *H. Recurvum* on rat urine output over 28 days

Indometacin treatment evoked an established oliguria up to the last day of the protocol, while furosemide reversed this indometacin oliguric action from protocol day14 onwards, such that there was a significant overall diuresis (Fig. 1). The *H. recurvum* extract also reversed indomethacin oliguria from protocol day 14 – 28, to a degree that was ultimately statistically comparable to that of furosemide as shown in Fig. 1.

Effect of indomethacin treatment, and its combination with furosemide or H. recurvum extract on rat urine flow rate over 28 days

Indometacin administration produced a 57.1% decrease in urine flow rate by the end of the treatment protocol, and this was associated with **the** reduction observed on urinary output (i.e oliguria). Dosing with H. recurvum reversed the indometacin decrease in flow rate, augmenting it by 208.7% at the higher dose of the plant extract (500 mg/kg). Analogously, furosemide also elevated the indomethacin impaired flow rate by 335.0% (Table 3).

Effect of indomethacin 28-day treatment, and its combination with furosemide or *H. recurvum* on rat serum and creatinine

Twenty-eight-day oral administration of indomethacin elevated the creatinine concentration in rat serum by a three-fold factor, while combined frusemide treatment reduced it to the control level. Likewise, combined administration of *H. recuvum* extract at both dose levels, reduced the indomethacin-boosted serum creatinine level by a significant degree (50.5%) (Table 4A).

By way of contrast, the urinary creatinine concentration in the indomethacin treated animals was significantly lower than that of controls, and this level was maintained throughout the protocol. Combined treatment of indomethacin with either frusemide or *H. recurvum* extract, yielded creatinine concentrations in urine which were higher than those animals that received indomethacin alone but they were comparable with controls (Table 4B).

Effect of indomethacin 28-day treatment, and its combination with furosemide or *H. recurvum* on serum urea and blood urea nitrogen (BUN) in rats.

Treatment with indometacin produced a steady increase in serum urea concentration during the

time course of the protocol, and by the last day, the elevation of urea level amounted to 143.2% above control. Co-treatment of indomethacin with either frusemide or *H. recurvum* extract reversed the indomethacin rise in serum urea and, in the case of the higher dose of plant extract, there was a restoration of urea level which was not significantly different from the control group (Table 5).

Analogously, indometacin induced a time-related elevation of BUN over the treatment protocol duration, which by day 28 was 97.7% greater than the controls. Both *H. recurvum* extract and frusemide treatment in combination with indometacin normalized BUN to the control level (Table 6).

Effect of indomethacin 28-day treatment, and its combination with furosemide or *H. recurvum* on serum electrolyte levels in rats

Indometacin administration generated a significant increase (16.7%) in serum sodium, and a substantial (36.0%) reduction in serum potassium by the end of the treatment protocol (Table 7 and 8). Both frusemide and *H. recurvum* given in combination with indometacin, restored the disrupted serum electrolyte concentrations to levels equivalent to controls throughout the treatment **protocol (Table 7 and 8).**

DISCUSSION

The initial study aim was to identify the phytochemical components in the aqueous methanolic extract of *H. recurvum*, and subsequently, to evaluate any possible diuretic potential against indomethacin-induced oliguria. Qualitative phytochemical analysis of the plant extract demonstrated that it contained quinones, glycosides, terpenes, diterpenes, tannins, phenols, carbohydrates, flavonoids, saponins and alkaloids. Reports in the literature have revealed that the presence of these phytochemicals in the plant, produces a prospective diuretic effect by increasing blood flow, vasodilation and inhibition of renal tubular reabsorption. In addition, the saponins possess other physiological properties such as hemolytic activity and alteration of membrane permeability over and above modulation of renal sodium excretion(13); (14).

Our data has shown that there is a progressive decrease in body weight, urine output and urine flow rate in animals that were administered indometacin by itself. However, treatment with indometacin in combination with *H. recurvum* extract, dose-relatedly improved oliguric parameters in comparison with indometacin administered independently. The diuretic action of the plant extract was compared with the standard reference drug, furosemide, which is used clinically as a diuretic drug in congestive heart failure, liver cirrhosis and nephrotic syndrome among other conditions. Although frusemide is widely used clinically, it can cause ototoxicity, hypokalemia, hypomagnesemia, metabolic alkalosis and hyperuricemia(15). In our study, oral administration of the plant extract (500 mg/kg) produced an effect on diuresis which was equivalent to that of furosemide.-

Tests on blood and urine represent fundamental methods to monitor renal function since these fluids transport metabolic waste products that are normally excreted by the kidney. Among the biochemical parameters evaluated, serum creatinine was increased, while urinary creatinine was decreased, whereas both serum urea and BUN declined following treatment with indomethacin on its own. All these test outcomes may well be indicative of kidney injury with a resultant reduction in glomerular filtration. They are also consistent with the finding that NSAID-induced nephrotoxicity may be ascribed to a non-specific inhibitory effect on cyclooxygenase thereby reducing prostaglandin synthesis, causing kidney ischemia(16).

It was clear that administration of indomethacin significantly altered electrolyte excretion in the form of sodium and potassium from the body. Thus, serum sodium was significantly raised in comparison with control while serum potassium was decreased, and these upshots reflected a

decreased saluretic activity and an electrolyte imbalance. It has been reported that the prostaglandins (notably PGE_2) are potent regulators of renal hemodynamics, and Na^+ and Cl^- reabsorption in the tubular thick ascending limb as well as the cortical collecting duct. In this context, prostaglandin synthesis is inhibited by indomethacin, leading to decreased excretion of sodium(17).

Both *H. recurvum* extract and frusemide reversed the oliguria and all the currently measured renal parameters provoked by indomethacin treatment. In relation to this, diuretics are employed in oliguric patients(18), though a standardized approach to their use has been advised(19). It is therefore likely that the phytochemical constituents of *H. recurvum* extract produce diuresis and reversal of indomethacin oliguria, so further investigation is warranted.

CONCLUSION

This study substabtiates the ethnopharmocological use of *Haloxylon Recurvum* as a diuretic agent. Thus, results showed that the plant extract possessed a significant and dose dependent diuretic activity which may be attributable to the presence of active phytochemicals. The higher dose of extract produced a renal biochemical profile, which was comparable to furosemide. Hence, further studies are necessary to elucidate possible mechanisms of action, and any active constituents responsible for the diuretic and ani-oliguric activity of the *H. recurvum* plant extract.

Limitations Of The Study

Since this is an initial study designed to establish if there is any diuretic propensity of *H. recurvum* plant extract, no specific analysis of the constituents against renal damage has currently been performed. However, the investigation has now positively confirmed that the plant extract is diuretic, so a logical step might involve an evaluation of the constituents ostensibly in the proportions occurring in the extract.

Conflicts Of Interest/Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

Iqra Asif: Data curation; formal analysis; investigation; methodology, Fiaz-ud-Din Ahmad: project administration; supervision, Ammara Asif: Software, writing original draft, Robert D. E. Sewell: formal analysis, writing, review and editing.

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Table 1: **The phytoconstituents phytochemical screening of plant extract. The final column shows the presence or absence of constituent**
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Group	Phytoconstituent	Identification test name	Result Positive = present Negative = absent
1	Quinones	Conc. HCl test	Positive
2	Glycosides	Keller Killiani test	Positive
3	Terpenes	Chloroform test	Positive
4	Diterpenes	Copper acetate test	Positive
5	Tannins	Gelatin test	Positive
6	Phenols	Ferric chloride test, Dilute iodine test and Lead acetate test	Positive
7	Protein	Ninhydrin test and Biuret test	Negative
8	Carbohydrates	Benedict test	Positive
9	Alkaloids	Hager's test	Positive
10	Flavonoids	Alkaline reagent test	Positive
11	Saponins	Foam test	Positive

Table 2: Effects of aqueous methanolic extract of *H. recurvum* or furosemide on body weights of rats treated with indometacin.

Body Weight (g)		Observational Protocol Days		
Group	Treatment	Day 0	Day 14	Day 28
1	**Control**	171 ± 2.80	173 ± 2.61	173 ± 2.61
2	**Indometacin (5 mg/kg)**	139 ± 1.28*	137 ± 1.10*	135 ± 0.76*
3	**Indomethacin (5 mg/kg) + Furosemide (10 mg/kg) (reference diuretic)**	166 ± 5.2$^{\#}$	171 ± 4.00$^{\#}$	174 ± 4.37$^{\#}$
4	**Indometacin (5 mg/kg) + *H. recurvum* extract (250 mg/kg)**	149 ± 3.3*	154 ± 3.19$^{*\#@}$	157 ± 2.3$^{\#}$
5	**Indometacin (5 mg/kg) + *H. recurvum* extract (500 mg/kg)**	**162 ± 3.94$^{\#}$**	**166 ± 4.11$^{\#}$**	**171 ± 4.01$^{*\#}$**

Values are shown as mean ± SEM. One-way ANOVA was performed with post hoc Bonferroni test for statistical analysis. $^{*}p < 0.05$ versus control; $^{\#}p < 0.05$ versus indomethacin alone; $^{@}p < 0.05$ versus indometacin + furosemide reference diuretic.

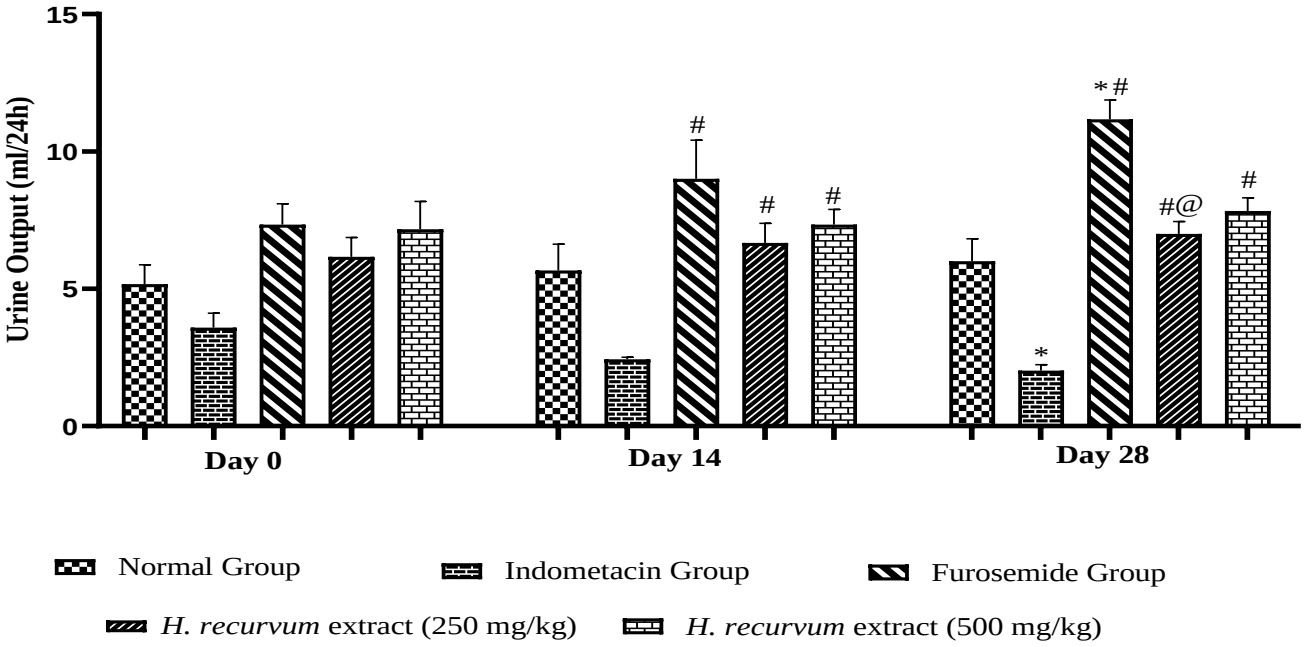

Figure 1: Effect of aqueous methanolic extract of *H. recurvum* on urine output in rats treated with indometacin. Values are shown as mean ± SEM. One-way ANOVA was performed with post hoc Bonferroni test for statistical analysis. $^{*}p < 0.05$ versus control; $^{\#}p < 0.05$ versus indomethacin alone; $^{@}p < 0.05$ versus Indomethacin + furosemide reference diuretic.

Table 3: Effect of aqueous methanolic extract of *H. recurvum* or furosemide on urine flow rate of rats treated with indometacin.

Urine Flow Rate (µl/min)		Observational Protocol Days		
Group	Treatment	Day 0	Day 14	Day 28
1	**Control**	**2.09 ± 0.16**	**2.21 ± 1.80**	**2.40 ± 1.33**
2	**Indometacin (5 mg/kg)**	**1.78 ± 0.90**	**1.23 ± 0.02**	**1.03 ± 0.19***
3	**Indomethacin (5 mg/kg) + Furosemide (10 mg/kg) reference diuretic**	**3.05 ± 0.07**	**3.66 ± 1.60$^{\#}$**	**4.48 ± 0.07$^{\#}$**
4	**Indometacin (5 mg/kg) + *H. recurvum* extract (250 mg/kg)**	**2.80 ± 0.68**	**2.99 ± 0.01$^{\#}$**	**3.09 ± 0.10$^{\#}$**
5	**Indometacin (5 mg/kg) + *H. recurvum* extract (500 mg/kg)**	**3.03 ± 1.08**	**3.06 ± 0.70$^{\#}$**	**3.18 ± 0.46$^{\#}$**

Values are shown as mean ± SEM. One-way ANOVA was performed with post hoc Bonferroni test for statistical analysis. $^{*}p < 0.05$ versus control; $^{\#}p < 0.05$ versus indomethacin alone.

Table 4: Effects of aqueous methanolic extract of *H. recurvum* or frusemide on (A) serum creatinine and (B) urinary creatinine in rats treated with indomethacin.

(A) Serum Creatinine (mg/dl)		Observational Protocol Days		
Group	Treatment	Day 0	Day 14	Day 28
1	**Control**	**0.31 ± 0.03**	**0.30 ± 0.02**	**0.32± 0.01**
2	**Indomethacin (5 mg/kg)**	**0.7 ± 0.06**	**0.96 ± 0.12***	**1.03 ± 0.06***
3	**Indomethacin (5 mg/kg) + Furosemide (10 mg/kg) reference diuretic**	**0.45 ± 0.11**	**0.40± 0.03#**	**0.35 ± 0.05#**
4	**Indometacin (5 mg/kg) + *H. recurvum* extract (250 mg/kg)**	**0.68 ± 0.09**	**0.66 ± 0.51**	**0.55 ± 0.08#**
5	**Indometacin (5 mg/kg) + *H. recurvum* extract (500 mg/kg)**	**0.63 ± 0.09**	**0.51 ± 0.12**	**0.51 ± 0.07#**

Values are shown as mean ± SEM. One-way ANOVA was performed with post hoc Bonferroni test for statistical analysis. *$p < 0.05$ versus control; #$p < 0.05$ versus indomethacin alone.

(B) Urinary Creatinine (mg/dl)		Observational Protocol Days		
Group	Treatment	Day 0	Day 14	Day 28
1	**Control**	**4.03 ± 0.08**	**4.1 ± 0.08**	**4.08 ± 0.1**
2	**Indometacin (5 mg/kg)**	**3.9 ± 0.02**	**2.68 ± 0.1***	**2.5 ± 0.16***
3	**Indometacin (5 mg/kg) + Furosemide (10 mg/kg) (reference diuretic)**	**3.56 ± 0.20**	**3.76 ± 0.25#**	**3.93 ± 0.27#**
4	**Indometacin (5 mg/kg) + *H. recurvum* extract (250mg/kg)**	**3.06 ± 0.10***	**3.3 ± 0.14**	**3.6 ± 0.25#**
5	**Indometacin (5 mg/kg) + *H. recurvum* extract (500mg/kg)**	**3.1 ± 0.05**	**3.4 ± 0.06**	**3.76 ± 0.28#**

Table 5: Effects of aqueous methanolic extract of *H. recurvum* or frusemide on serum urea levels in rats treated with indometacin.

Serum Urea (mg/dl)		Observational Protocol Days		
Group	Treatment	Day 0	Day 14	Day 28
1	**Control**	**19.16 ± 0.74**	**21.66 ± 0.7**	**22.00 ± 0.63**
2	**Indometacin (5 mg/kg)**	**43.16 ± 1.70***	**49.16 ± 1.93***	**53.5 ± 0.76***
3	**Indometacin (5 mg/kg) +**	**31.33 ± 0.42*#**	**26.5 ± 1.54#**	**23.16 ± 1.66#**

	Furosemide (10 mg/kg) (reference diuretic)			
4	**Indometacin (5 mg/kg) + *H. recurvum* extract (250mg/kg)**	**39.16 ± 1.13$^{*@}$**	**35.16 ± 1.42$^{*@}$**	**29.0 ± 1.43$^{*\#}$**
5	**Indometacin (5 mg/kg) + *H. recurvum* extract (500mg/kg)**	**36.5 ± 0.76$^{*\#}$**	**31.66 ± 1.11***	**27.33 ± 1.93$^{\#}$**

Values are shown as mean ± SEM. One-way ANOVA was performed with post hoc Bonferroni test for statistical analysis. $^{*}p < 0.05$ versus control; $^{\#}p < 0.05$ versus indomethacin alone; $^{@}p < 0.05$ versus indomethacin + frusemide.

Table 6: Effects of aqueous methanolic extract of *H. recurvum* or frusemide on serum BUN in rats treated with indometacin.

Blood Urea Nitrogen (BUN)		**Observational Protocol Days**		
Group	Treatment	Day 0	Day 14	Day 28
1	**Control**	**8.93 ± 0.85**	**10.09 ± 0.81**	**10.25 ± 0.72**
2	**Indometacin (5 mg/kg)**	**20.11± 1.94***	**22.91 ± 2.21***	**24.93 ± 0.87***
3	**Indometacin (5 mg/kg) + Furosemide (10 mg/kg) (reference diuretic)**	**14.60 ± 0.48$^{*\#}$**	**12.34 ± 1.76$^{\#}$**	**10.79 ± 1.89$^{\#}$**
4	**Indometacin (5 mg/kg) + *H. recurvum* extract (250mg/kg)**	**18.25 ± 1.29$^{*\#@}$**	**16.38 ± 1.62$^{*\#@}$**	**13.51 ± 1.64$^{*\#}$**
5	**Indometacin (5 mg/kg) + *H. recurvum* extract (500mg/kg)**	**17.00 ± 0.87$^{*\#}$**	**14.75 ± 1.27$^{\#}$**	**12.73 ± 2.1$^{\#}$**

Values are shown as mean ± SEM. One-way ANOVA was performed with post hoc Bonferroni test for statistical analysis. $^{*}p < 0.05$ versus control; $^{\#}p < 0.05$ versus indometacin alone; $^{@}p < 0.05$ versus indometacin + frusemide.

Table 7: Effects of aqueous methanolic extract of *H. recurvum* or frusemide on serum sodium in rats treated with indometacin.

Serum Sodium (mEq/L)		Observational Protocol Days		
Group	Treatment	Day 0	Day 14	Day 28
1	**Control**	**129.5 ± 0.42**	**130 ± 0.71**	**131.5 ± 0.42**
2	**Indometacin (5 mg/kg)**	**146.5 ± 1.52***	**150.8 ± 1.13***	**153.5 ± 0.99***
3	**Indometacin (5 mg/kg) + Furosemide (10 mg/kg) (reference diuretic)**	**135.5 ± 2.50**	**132.0 ± 6.32#**	**130.3 ± 3.41#**
4	**Indometacin (5 mg/kg) + *H. recurvum* extract (250mg/kg)**	**142.0 ± 2.30**	**139.1 ± 2.79**	**137.0 ± 2.06#**
5	**Indometacin (5 mg/kg) + *H. recurvum* extract (500mg/kg)**	**139.0 ± 3.08**	**136.3 ± 2.09**	**134.16 ± 1.07#**

Values are shown as mean ± SEM. One-way ANOVA was performed with post hoc Bonferroni test for statistical analysis. *$p < 0.05$ versus control; #$p < 0.05$ versus indomethacin alone.

Table 8: Effects of aqueous methanolic extract of *H. recurvum* or frusemide on serum potassium in rats treated with indometacin.

Serum Potassium (mEq/L)		Observational Protocol Days		
Group	Treatment	Day 0	Day 14	Day 28
1	**Control**	4.50 ± 0.11	4.53 ± 0.14	4.55 ± 0.26
2	**Indometacin (5 mg/kg)**	3.81 ± 0.15	3.01 ± 0.1*	2.91 ± 0.19*
3	**Indometacin (5 mg/kg) + Furosemide (10 mg/kg) (reference diuretic)**	3.65 ± 0.31	3.80 ± 0.16	4.01 ± 0.16
4	**Indometacin (5 mg/kg) + *H. recurvum* extract (250 mg/kg)**	3.26 ± 0.21	3.41 ± 0.3	3.65 ± 0.30
5	**Indometacin (5 mg/kg) + *H. recurvum* extract (500 mg/kg)**	3.48 ± 0.25	3.61 ± 0.4	3.80 ± 0.27

Values are shown as mean ± SEM. One-way ANOVA was performed with post hoc Bonferroni test for statistical analysis. *$p < 0.05$ versus control