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RESEARCH ARTICLE

Evaluation of Diuretic Activity of Alcoholic Extract of Cinnamomum Zeylanicum in Swiss Albino Rats

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ABSTRACT

The present study was undertaken to evaluate the diuretic activity of alcoholic extract of Cinnamomum zeylanicum in swiss albino rats. The animals were divided into five groups with six rats in each group. First group of six rats were taken as control, and they received 0.9% normal saline 25ml/kg body weight orally. Second group of six rats were treated with standard drug hydrochlorothiazide 2.5mg/kg orally. Third, fourth and fifth groups were taken as test groups and they received the alcoholic extract of cinnamomum zeylanicum at the doses of 50,100 and 200mg/kg respectively. The diuretic effect of the extracts was evaluated by measuring urine volume, pH, sodium, potassium and chloride contents. Urinary volume was increased significantly with alcoholic extract of cinnamomum zeylanicum and this volume was maximum with 200mg/kg ($P<0.001$) in comparison with the control group. Cinnamomum zeylanicum showed maximum Na+ excretion with a dose of 100mg/kg ($P<0.001$) which is comparable with the standard drug hydrochlorothiazide. Potassium ion excretion was elevated significantly with increasing doses of the test drug and this K+ loss was more when compared with standard and control groups. Similarly chloride ion excretion was also elevated significantly with test group when compared with the control and standard groups. We conclude that alcoholic extract of cinnamomum zeylanicum showed significant saluretic effect but natriuretic and carbonic anhydration inhibition properties were found absent. Potassium loss was more with cinnamomum zeylanicum when compared with the standard drug which may cause significant electrolyte disturbances.

KEYWORDS

Cinnamomum zeylanicum, Diuretic Activity, Urinary Volume

INTRODUCTION

Herbal plants as medicines are the mainstay of health care in several developing as well as under developing countries. Medicinal plants are one of the most important sources of some unknown chemical substances with potential therapeutic effects. The World Health Organization has estimated that over 75% of the world's population still relies on these herbal for food and cure of some ailments.[1]

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This kind of traditional medicine practice is widespread in Pakistan, Sri Lanka, China, Japan and Thailand.

The Plant

Cinnamomum zeylanicum Blume (Family Lauraceae) which is popularly known as cinnamon is classified in the botanical division Magnoliophyta, class Magnoliopsida.[2]

Generally in India, Cinnamomum zeylanicum is cultivated in south India.[3] The genus cinnamomum has 250 species and many of them are aromatic and flavouring.[4]

Different parts of the plants have different medicinal properties. Cinnamon (inner bark of shoots) is used as flu-preventive, indigestion and flatulence control and the bark is used in mouth washes.[5] Cinnamomum zeylanicum is also used for the treatment of some gastrointestinal conditions such as mild spastic conditions, fullness, and loss of appetite. It is also used to treat menstrual pains associated with amenorrhoea, dysmenorrhoea and abdominal pain with diarrhoea. Cinnamon is also used to treat impotency, toothache, inflammation of the eye, rheumatism, neuralgia, leukorrhoea, vaginitis, wounds, and diabetes.[6] Cinnamonium zeylanicum also have various pharmacological activities like antioxidant, anti inflammatory, antibacterial, antidiabetic, antifungal, insecticidal, nematicidal, antipyretic, analgesic and antimicrobial activities.[7] Since the diuretic effect of Cinnamomum zeylanicum has never been experimentally confirmed, the main aim of the present study was to evaluate the diuretic activity of alcoholic extract of Cinnamomum zeylanicum in swiss albino rats.

EXPERIMENTAL SECTION

Collection of the Plant

Cinnamomum zeylanicum was obtained from a garden near Khammam. The identification and authentication of the plant was donate the department of Botany, Government degree college, Khammam.

Extraction Procedure

Cinnamomum zeylanicum was dried in shade and coarsely powdered. The preparation of alcoholic extract of Cinnamomum zeylanicum was done in the Department of Pharmacology, Mamata Medical College, Khammam using continuous Soxhlet Extraction or Soxhelation.

Animals

Adult male Swiss albino rats, weighing between 175-225gm were used in the study. The animals were given free access to food and water. The experiment was approved by the Institutional Animal Ethics Committee (IAEC), Mamata medical college, Khammam. The animals were stabilized for 1 week; they were maintained under standard conditions at room temperature, 60 ± 5% relative humidity and 12 hr light and dark cycles. They were given a standard pellet diet and water ad-libitum. The animals were given free access to food and water. The guidelines for the investigation of experiments in conscious animals were followed in all tests.

Drugs Used

Tab. Hydrochlorothiazide 25mg, manufactured by Sun Pharmaceuticals is used as the standard drug in the study.

Toxicity Study in Swiss Albino Rats

The alcoholic extract of cinnamomum zeylanicum was tested for its acute toxicity in albino rats. Acute oral toxicity was performed as per OECD- 423 guide lines.[8] To determine this the extract was administered orally in an ascending order and in widely spaced doses that is 0.25g/ kg, 0.5g/kg, 0.75g/kg and 1g/kg to different groups of albino rats. Two albino rats were used in each group; the control albino rats received normal saline). The animals were observed periodically for forty eight hours. The parameters used for the observation were sedation, loss of righting reflex, respiratory rate, hyperactivity and convulsions. No toxic effects and mortality were observed at the end of study. The optimization of the effective dose was calculated by taking $1/10^{th}$ of the maximum dose, that is 100mg/kg and the other two doses which were taken were half and double of the one tenth dose, which is 50mg/kg and 200mg/kg respectively. These doses were compared with the control group which received normal saline 25ml/kg body weight and with the standard group which received hydrochlorothiazide 2.5mg/kg body weight for the evaluation of the diuretic activity.

Experimental Design

The diuretic activity in albino rats was studied by modified Lipchitz test.[9] Adult male Swiss albino rats weighing between 175-225gm was used. The room temperature was maintained between 27-29degC. The animals had free access to the water but they were deprived of food 18 hours prior to the experiment. All the animals were hydrated

with 25ml/kg of 0.9% normal saline orally. The animals were divided into five groups with six rats in each group. In all the animals drug was administered after emptying the urinary bladder. First group of six rats were taken as control, and they received 0.9% normal saline 25ml/kg body weight orally. After treatment, the animals were then transferred to the metabolic cages; three animals per cage and the time was noted. Second group of six rats were treated with normal saline 25ml/kg and standard hydrochlorothiazide 2.5mg/kg orally. The treated animals are then transferred to the metabolic cages housing three animals per cage and time is noted. The third group of six rats was taken as test group and the alcoholic extract of cinnamonium zeylanicum which was obtained in liquid form was given orally along with normal saline at the dose of 50mg/kg, keeping the volume administered constant. After the treatment, animals were transferred to metabolic cages housing, three animals per cage. The fourth group of six rats were given 100mg/kg of test dose orally along with normal saline, keeping the volume administered constant. The fifth groups of six rats were given 200mg/kg of test dose orally along with normal saline, keeping the volume administered constant. The urine was collected in beakers for a period of five hours in all three groups. The animals were deprived of food and water during the experiment. At the end of five hours, the bladder of each rat was emptied by pulling the base of the tail to collect the residual urine. Urinary volume and urinary pH was noted and samples were taken for estimation of urinary electrolytes for sodium, potassium and chloride using spectrophotometer. The rats were transferred to the metabolic cages and urine was collected after twenty four hours for urinary analysis including Natriuretic and carbonic anhydrase inhibition activity.

Measurement of Urinary Volume and Electrolytes

The urine collected from the animals was estimated for its volume and its pH was measured by a digital pH meter. The pH readings were noted for the control, standard (hydrochlorothiazide) and different doses of test animals. The urinary electrolytes were estimated by a digital spectrophotometer (Mfd by Electronics India, Model 301) and an electrolyte kit manufactured by M/S Excel Diagnostics, Pvt. Ltd, and Hyderabad.

Saluretic, Natriuretic and Carbonic Anhydrase Inhibition

The sum of Na+ and Cl- excretion was calculated as a parameter of Saluretic activity. The ratio Na+/ K+ was calculated for Natriuretic activity. The ratio Cl- /Na+ + K+ (ion quotient) was calculated to estimate carbonic anhydrase inhibition[7].

Statistical Analysis

The results are expressed as mean values ± S.D (standard Deviation) Statistical comparison was carried out by analysis of variance (ANOVA). The difference between the means of treated groups and the non-treated control group was evaluated by the Dunnette's Multiple Comparisons Test. The results were considered statistically significant when P was < 0.05.

RESULTS AND DISCUSSION

All the values were expressed as Mean ± SEM (Standard error of mean). The differences were compared using one way analysis of variance (ANOVA) followed by Dunnet's t test. The p values <0.05 were considered significant.

Evaluation

The sum of Na^+ and Cl^- excretion is calculated as Parameter for Saluretic activity. The ratio Na^+/K^+ is calculated for Natriuretic activity. Values greater than 2.0 indicate a favorable Natriuretic effect. Ratios greater than 10.0 indicate a potassium-sparing effect. The ratio $Cl^-/Na^+ + K^+$ (ion quotient) is calculated to estimate carbonic anhydrase inhibition. Carbonic anhydrase inhibition can be excluded at ratios between 1.0 and 0.8. With decreasing ratios slight to strong carbonic anhydrase inhibition can be assumed.

Urinary Volume (Table 1)

The urinary volume (UV) at the end of 5hr collection in Group-I (control group) was 7.3 ± 0.2ml/kg. In Group-II (Standard group) treated

with 2.5 mg/kg of hydrochlorothiazide, there was a significant increase in the Urinary volume i.e. 19.13 ± 0.73ml/kg. In the test groups, there was a significant increase in the urinary volume as compared to the control group which is maximum with 200mg/kg of alcoholic extract of cinnamomum zeylanicum and was found to be 17± 1 ml/kg. (Figure 1)

***Urinary pH* (Table 1)**

The urinary pH of Group-I (control group) and Group-II (Standard group) was found to be 7.4 ± 0.12 and 7.12±0.12 respectively. The urinary pH of the test group which received 200mg/kg of alcoholic extract of cinnamomum zeylanicum was found to be 7.23 ± 0.23 respectively. The changes in pH in the test groups were not significant when they were compared with that of the control and the standard.

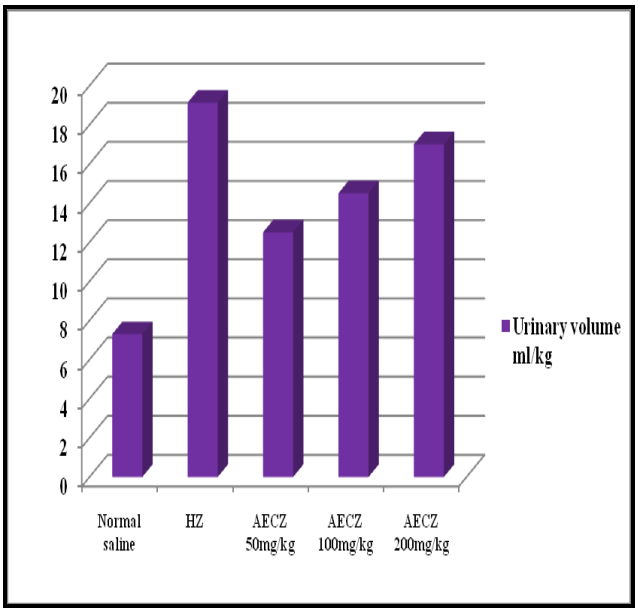

Figure 1: Effects of Alcoholic Extract of Cinnamomum zeylanicum on Urinary volume

Table 1: Effect of Cinnamomum zeylanicum extracts on Urine volume, pH, Sodium, Potassium and Chloride excretion in albino rats

Groups (n=6)	pH	Urinary volume ml/kg	Urinary sodium excretion meq/L	Urinary potassium excretion meq/L	Urinary chloride excretion meq/L
Group-I Control NS 25ml/kg,	7.4 ± 0.12	7.3 ± 0.2	77.74 ± 1.2	21 ± 1	262.2 ± 0.5
Group-II Standard HZ 2.5mg/kg	7.12±0.12	19.13 ± 0.73	133.45± 6.10	23.01 ± 0.15	116.7 ± 6.52
Group-III (Test-I) AECZ 50mg/kg	7.21 ± 0.21	12.5± 0.5**	103.3±1.15**	81.15 ± 1.35**	401.7 ± 1.54**
Group-IV(Test-II) AECZ 100mg/kg	7.20 ± 0.20	14.5 ± 0.5**	117.8.±1.05**	71.5± 1.5**	424.8 ±4.55**
Group-V(Test-III) AECZ 200mg/kg	7.23 ± 0.23	17± 1**	105 ± 5**	73.05 ± 0.24**	452.9 ± 1.94**

All the values are expressed as Mean±SEM. AECZ -- Alcoholic extract of Cinnamomum zeylanicum, HZ—Hydrochlorothiazide. *significant P<0.05, **Highly significant P<0.001

Urinary Sodium **(Table 1)**

Urinary Sodium (Na^+) during the period of the 5hr collection in the Group-I (control group) was 77.74 ± 1.2meq/L. In Group II (standard group) which was treated with 2.5 mg/kg of hydrochlorothiazide, there was a significant increase in the Na+ excretion i.e. 133.45± 6.10meq/L (P<0.001). In the test groups, significant increase in Na+ excretion was observed gradually with increasing doses and maximum values are seen with 100mg/kg of alcoholic extract of cinnamonium zeylanicum i.e. 117.8.±1.05meq/L (P<0.001).

Urinary Potassium **(Table 1)**

Urinary Potassium (K^+) during the period of the 5hr collection in the Group-I (control group) was 21 ± 1meq/L. In Group II (standard group) which was treated with 2.5 mg/kg of hydrochlorothiazide, there was a significant increase in the K^+ excretion i.e. 23.01 ± 0.15meq/L (P<0.001). In the test groups, significant K^+ loss was observed and maximum values are seen with 50 mg/kg of alcoholic extract of cinnamonium zeylanicum i.e. 81.1 ± 1.35meq/L (P<0.001).

Urinary Chloride **(Table 1)**

Urinary Chloride (Cl^-) during the period of the 5hr collection in the Group-I (control group) was 116.7 ± 6.5meq/L. In Group II (standard group) which was treated with 2.5 mg/kg of hydrochlorothiazide, there was a significant increase in the Cl^- excretion i.e. 262.2 ± 0.5meq/L (P<0.001). In the test groups, dose dependent increase in Cl^- excretion was observed and maximum values are seen with 200mg/kg of alcoholic extract of cinnamonium zeylanicum i.e. 452.9 ± 1.94meq/L (P<0.001).

Effects on Natriuretic, Saluretic and Carbonic Anhydrase Inhibition at the end of 5 hrs (Table 2)

The alcoholic extract of cinnamonium zeylanicum showed dose dependent saluretic activity. Natriuretic and carbonic anhydrase inhibition properties were found absent with all doses of cinnamonium zeylanicum.

Table 2: 5 hrs Saluretic, Natriuretic and Carbonic anhydrase inhibition

Groups (n=6)	Saluretic Effect	Natriuretic Effect	Carbonic Anhydrase inhibition
Group-I Control NS 25ml/kg,	194.4	3.7	1.18
Group-II Standard HZ 2.5mg/kg	395.6	5.77	1.67
Group-III (Test-I) AECZ 50mg/kg	505	1.27	2.17
Group-IV(Test-II) AECZ 100mg/kg	542.6	1.64	2.24
Group-V(Test-III) AECZ 200mg/kg	557.9	1.43	2.54

AECZ -- Alcoholic extract of Cinnamomum zeylanicum, HZ—Hydrochlorothiazide.

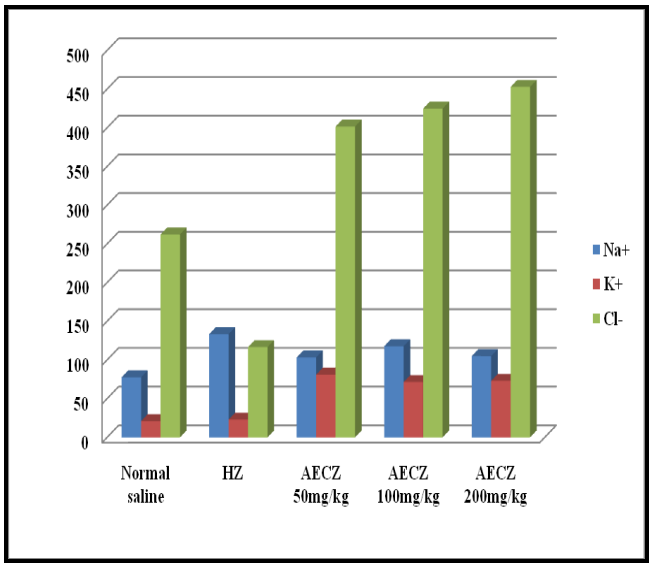

Figure 1: Effects of Alcoholic Extract of Cinnamomum zeylanicum on Na^+, K^+ and Cl^- excretion

DISCUSSION

Diuretics are drugs capable of increasing urinary volume, so these drugs can be used for the treatment of diseases associated with retention of fluids. Many herbal medicines act as diuretics by exerting direct action on electrolyte balance of minerals. Diuretic activity is useful in a number of conditions like hypertension, hypercalciuria, and cirrhosis of liver. Diuretics also relieve pulmonary congestion and peripheral edema.[10] Since diuretics are employed clinically in the treatment of edema, it would be highly important to demonstrate their effectiveness in the presence of electrolyte and water.[11] Diuretics from natural sources include caffeine in coffee, tea, and cola, which inhibit Na^+ reabsorption and alcohol in beer, wine and mixed drinks, which inhibit secretion of Antidiuretic hormone.[12,13] The most common adverse effects of these drugs include fatigue, impotence and weakness.

There are a number of herbal diuretics out of which most important ones are Foeniculum vulgare, Fraximus excelsior, Hibiscus sabdariffa and Spegulariapurpurea.[14] Cinnamomum zeylanicum has its origin from the island of Srilanka (formerly called Ceylon) and it is also cultivated in south india.[15] The production of cinnamon is mostly limited to the wettest low land areas of South east Asia and cultivated upto an altitude of 500 meters above mean sea level having the mean temperature 27^0C and annual rain fall 2000-2400mm. It prefers sandy soil enriched with organic matter.[16] The genus cinnamomum has 250 species and many of them are aromatic and flavoring .[17] Differents parts of the cinnamonium plant have different medicinal properties. Cinnamon (inner bark of shoots) is used as flu-preventive, indigestion and flatulence control and the bark is used in mouth washes.[5] Cinnamonium zeylanicum also have various pharmacological activities like antioxidant, anti inflammatory, antibacterial, antidiabetic, antifungal, inseticidal, nematicidal, antipyretic, analgesic and antimicrobial activities.[7] Since the diuretic effect of Cinnamomum zeylanicum has never been experimentally confirmed, the main aim of the present study was to evaluate the diuretic activity of alcoholic extract of Cinnamomum zeylanicum in swiss albino rats.

In the present study, diuretic activity of cinnamomum zeylanicum is compared with the standard drug like hydrochlorthiazide. The common parameters used for the comparison are urinary volume, urinary Na^+, K^+ and Cl^- respectively. We have observed that there was a dependent increase in the urinary volume with alcoholic extract of cinnamomum zeylanicum and this volume was maximum with 200mg/kg ($P<0.001$) which is almost equal with the standard drug hydrochlorthiazide. Cinnamomum zeylanicum showed maximum Na+ excretion with a dose of 100mg/kg ($P<0.001$) which is comparable with the standard drug but it was decreased with the dose of 200mg/kg. Potassium ion excretion was elevated significantly with increasing doses of the test drug and this K+ loss was more when compared with standard and control groups. Similarly chloride ion excretion was also elevated significantly with test group when compared with the control and standard groups. The alcoholic extract of cinnamonium zeylanicum showed dose dependent saluretic activity. Natriuretic and carbonic anhydrase inhibition properties were found absent with all doses of cinnamonium zeylanicum. The presence of phytoconstituents like terpenoids, saponins, flavonoids has been reported previously to be

responsible for the diuretic activity in plants.[18,19] The best diuretic effects could be associated to the flavonoid content, also it promote high levels of Na+ and K+ in urine. There are correspondence between the volume of urine and the concentration of Na+, this aspect is logical because the mechanism of action of diuretic drugs is to decrease the tubular reabsorption of this ion, it produces the dragging of the osmotic equivalent of water, other explanation that can support this, is the high ion concentrations in this medicinal plants.[20,21] However, the contribution of polyphenolic compounds to diuretic effect cannot be ruled out. Further studies like isolation and characterization of diuretic principle is needed to understand and confirm the exact mechanism of action.

CONCLUSION

The results obtained in this study revealed that alcoholic extract of cinnamomum zeylanicum showed significant saluretic effect but natriuretic and carbonic anhydration inhibition properties was absent. Also we found that potassium loss in the urine was more with cinnamomum zeylanicum when compared with the standard drug which may cause significant electrolyte disturbances. So, further studies are needed to support the exact mechanism of potassium loss with alcoholic extract of cinnamomum zeylanicum.

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