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Assessment of nephroprotective activity of *Pippalyadi Agad* on Diclofenac Sodium induced nephrotoxicity in Albino Mice: An experimental study

Research Article

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Abstract

Background: Nephrotoxicity due to Diclofenac Sodium can be correlated with this concept of *Dushi Visha* and nephroprotective drugs mentioned in Ayurveda texts may play an important role in this area. *Pippalyadi Agad* is the herbo-mineral medication stated by Yogaratnakara for treatment of *Dushi Visha.* Some of the drugs in this formulation have shown efficacy in correcting nephrotoxicity. Objective: To study the nephroprotective activity of *Pippalyadi Agad* on diclofenac sodium induced nephrotoxicity in albino mice. Methods: The experimental study was carried out in albino mice as per CPCSEA guidelines after getting approval from the Institutional Animal Ethics Committee (IAEC). 30 albino mice, each weighing about 20-30 gm, were procured from Bharat serum and vaccines. The mice were housed in the institutional animal house in standard conditions. 30 albino mice were equally divided into five groups having six animals in each group as Trial Drug (low dose), Trial Drug (medium dose), Standard Control, Disease Control and Vehicle Control. For assessment of nephrotoxicity, the animals were monitored on Day 0, Day 15 and Day 45 by carrying out blood investigations such as BUN, Serum Creatinine, Body weight and CBC. The Data were subjected to appropriate statistical tests to derive results. Results: *Pippalyadi Agad* treatment at low and medium doses significantly lowered BUN and Serum Creatinine in comparison with Disease and Standard Control at Day 45. *Pippalyadi Agad* treatment at low and medium doses significantly ($p < 0.001$ and $p < 0.001$) increased body weights of the animals in comparison with Disease and Standard Control at Day 45. No significant difference in CBC parameters was observed in any of the Groups except the Disease Control Group on Day 15 and Day 45. Conclusion: *Pippalyadi Agad* has shown nephroprotective activity based on biochemical changes observed in Serum Creatinine, BUN and CBC.

Key Words: *Pippalyadi Agad,* Nephrotoxicity, Diclofenac sodium*, Dushi Visha.*

Introduction

The kidneys play an important role in human physiology, maintaining fluid homeostasis, regulating blood pressure, erythrocyte production and bone density, regulating hormonal balance, and filtering and removing nitrogenous and other waste products. (1) The kidney gets affected by various etiological factors which cause structural as well as functional damage to kidneys termed as Nephrotoxicity. Nephrotoxicity can be defined as the adverse effect of some substances like chemicals & medications on renal function. Several therapeutic agents can adversely affect the kidney resulting in acute renal failure, chronic interstitial nephritis and nephritic syndrome. (2)

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Gradually this problem of nephrotoxicity is increasing due to increase in use of number of potent therapeutic drugs like amino glycoside, antibiotics, chemotherapeutic agents and NSAIDS. (3) Diclofenac Sodium is an analgesic, anti-inflammatory and antipyretic compound used in a variety of painful conditions like osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, renal colic, in dentistry and to reduce postoperative pain. (4,5) Prolonged use of these drugs sometimes produces moderate to marked degree of Nephrotoxicity.

Kidney is an important target site for untoward effect of diclofenac sodium in humans as well as in animals. (6, 7) It causes Acute interstitial nephritis, altered intra-glomerular haemodynamics, chronic interstitial nephritis, glomerulo-nephritis. In Ayurveda, poisons are classified as *Sthavar Visha* (Inanimate poison), *Jangam Visha* (Animate poison) & *Kritrima Visha* (Artificial poison). (8 - 11)

Dushi Visha is a part of *Sthavara, Jangama* or *Krutrima Visha* which cannot be eliminated from the body completely but it is destroyed or denatured due to previous use of anti-poisonous remedies dried by fire, air,

or exposure to sunlight & lose its original properties is known as *Dushi Visha*. Due to its low potency, it does not kill the human instantly but as it is encapsulated by *Kapha Dosha,* it remains accumulated in the body for several years. Prolonged use of any drug leading to chronic toxicity is also a type of *Dushi Visha*.

So, here Nephrotoxicity due to Diclofenac Sodium can be correlated with this concept of *Dushi Visha*. Looking at this, nephroprotective drugs may play an important role in this area. *Pippalyadi Agad* is the herbo-mineral medication stated by Yogaratnakara for treatment of *Dushi Visha.* Some of the drugs in this formulation have shown efficacy in nephrotoxicity. (12 – 14)

Considering this drug induced nephrotoxicity as a type of *Dushi Visha*, use of *Pippalyadi Agad* can be incorporated here. The main objective of the present study was to study the nephroprotective activity of *Pippalyadi Agad* on diclofenac sodium induced nephrotoxicity in albino mice.

Materials and Methods

Materials

Drugs and chemicals

Pippalyadi Agad

Raw material for study drug *Pippalyadi Agad* i.e., *Pippali, Dhanyaka, Jatamansi, Lodhra, Sukshma Ela, Bruhad Ela, Maricha, Balakam, Suwarchika & Suvarna Gairika* were purchased from Dadar Pharmacy, Mumbai. Authentication of the raw Herbal drugs and the prepared *Pippalyadi agad* were done in Botany Department of Shri Vile Parle Kelavani Mandal's Mithibai College of Arts, Chauhan Institute of Science & Amrutben Jivanlal College of Commerce & Economics. Authentication of Mineral drugs was conducted at Chemo Test Laboratory, Navi Mumbai.

Preparation and standardization of *Pippalyadi Agad*:

Pippalyadi Agad was prepared in the Pharmacy of Department of Rasashastra & Bhaishajya Kalpana at D.Y. Patil deemed to be University, School of Ayurveda, Nerul Navi Mumbai. Raw materials were finely powdered and mixed in equal proportion. Standardization of these raw materials and the prepared study drug was done at Alarsin Pharmaceuticals, Mumbai. Standardization of Mineral drugs was conducted at Chemo Test Laboratory, Navi Mumbai.

Induction of nephrotoxicity was done by Diclofenac sodium (Dynapar Injection 75mg Troikaa Pharmaceuticals Ltd). Standard Control: Silymarin, a known hepato-nephroprotective drug(Silybon – 140mg tablet Micro Labs). Honey(Dabur 100ml bottle) was used as a vehicle control for the study.

Animals

Total 30 albino mice, were procured from Bharat serum and vaccines. The body weight of each mouse was about 20-30 gm. The mice were housed in institutes animal house at room temperature 24^{o}C ± 1^{o}C, relative humidity of 65% ± 10% and 12 hours light and dark cycle. The animals were fed with standard pelleted diet. This experimentation was carried out according to CPCSEA guidelines.

Experimental study

Approval was obtained from the Research Advisory Committee (RAC) of the D. Y. Patil Deemed to be University School of Ayurveda, Nerul, Navi Mumbai. The study was carried out in albino mice as per Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA) guidelines after getting approval from the Institutional Animal Ethics Committee (IAEC). [Project No: BVC/ IAEC/07/2019 dated 05/02/2019]

Place of study

Central Animal House Facility, Bombay Veterinary College, Parel, Mumbai 400 012.

Method of Preparation of study drug Solution:

Pippalyadi Agad formulation was prepared by mixing it with honey. It is administered orally to the animals by gastric intubation using a force-feeding needle. One group was made up of total 6 mice. Hence, for each trial group one packet of study drug was prepared (low dose: 19.5 mg/day and medium dose: 49.5 mg/day). One packet of study drug was mixed with approximately 1.5 ml of honey (considering loss while administration of drug with the vehicle) and 0.2 ml mixture was given to each mouse among 6 of one group. 30 packets of each dose (low & medium dose) were prepared i. e., total 60 packets for 30 days. For low dose - 19.5 mg powder was mixed with 1.5 ml honey and 0.2 ml (3.25 mg) preparation was given to each mouse. For medium dose- 49.5 mg powder is mixed with 1.5 ml honey and 0.2 ml (8.25 mg) preparation was given to each mouse. The Animal dose was calculated by extrapolating the therapeutic dose of humans to mice by referring the table reported by Paget and Barnes 1964. (15)

The Procedure for induction of Nephrotoxicity

Injection Diclofenac sodium 0.2 mg was given by subcutaneous route to each mouse for five days.

Preparation of Silymarin solution

140mg tablet was powdered and dissolved in 35ml of distilled water to prepare a solution of 4% w/v. Then it is administered orally to the animals by gastric intubation using a force-feeding needle.

Administration of Honey

0.2 ml honey was administered by oral route to the animals by gastric intubation using an insulin syringe (for vehicle control group).

Study Design

The experiment was carried out at Bombay Veterinary College, Parel, Mumbai. For the experiment Adult male Albino mice weighing about 20-30gm each were used. The mice were kept in properly numbered large polypropylene cages with stainless steel top grill having facilities for pelleted feed. The animals were maintained in 12 hours light and dark cycle at 28^{o}C ± 2^{o}C in a well-ventilated animal house under natural conditions in large polypropylene cages and they were

acclimatized to laboratory conditions for 7 to 10 days prior to the start of the experiment. The animals were fed with standard pelleted diet. All animal experiments were performed according to ethical guidelines suggested by the institutional animal ethics committee (IAEC). The protocol was approved in IAEC. Corncob was used as bedding material and changed twice a week 30 albino mice were equally divided into five groups having six animals in each group.

Table No 1 - Study Design

Group-I (Trial drug) Low dose	**Group-II (Trial drug)** Medium dose	**Group-III (Standard control)**	**Group-IV (Disease control)**	**Group-V (Vehicle control)**
6 Male Albino mice	6 Male Albino mice	6 Male Albino mice	6 Male Albino mice	6 Male Albino mice
Diclofenac Sodium 15 mg/kg/ day, SC, for 5 days	Diclofenac Sodium 15 mg/kg/ day, SC, for 5 days	Diclofenac Sodium 15 mg/kg/ day, SC, for 5 days	Diclofenac Sodium 15 mg/kg/ day, SC, for 5 days	Honey 10 µl/g body weight
Pippalyadi Agad Minimum dose 3.25 mg/kg/ day 15^{th} -45^{th} days	*Pippalyadi Agad* Medium dose 8.25 mg/kg/ day 15^{th} -45^{th} day	Silymarin 200mg/kg orally 15^{th} -45^{th} days	Diclofenac Sodium 1^{st} – 5^{th} day	Honey 15^{th} -45^{th} days

Parameters of Assessment

For assessment of nephrotoxicity, the above animals were monitored on Day 0, Day 15 and Day 45 by carrying out blood investigations. At the end of treatment, blood samples were collected from retro-orbital plexus by pricking micro capillary tube No.100mm (Borosilicate glass with both ends open) and were allowed to clot at room temperature for 30 minutes and centrifuged at 3000 rpm for 10 minutes to obtain clear serum and aliquots were used for the respective analytical determination. Following investigations were carried out: Blood urea nitrogen (BUN), Serum Creatinine, Body weight and Complete Blood Count (CBC). Blood sample was collected on Day 0, Day 15 and Day 45.

Plan for statistical analysis of data

The study data generated and collected was put to statistical analysis to reach to the results and conclusions. The data obtained in the studies were subjected to tests of significance. GraphPad InStat software was used for statistical analysis of data. Kolmogrov–Smirnov test was applied to test the normality of data. For within the groups' comparison (intra – group comparison), Repeated measures ANOVA test (when data passed normality test) was applied whereas for between the groups' comparison (inter–group comparison) One Way ANOVA test (when data passed normality test) was applied. P value < 0.05 was considered significant.

Results

For assessment of nephrotoxicity, the animals were monitored on Day 0, Day 15 and Day 45by carrying out blood investigations. The results are presented as following:

BUN (Blood Urea Nitrogen)

In Group 1, the mean BUN values at Day 0, Day 15 and Day 45 were 20.33 ± 5.47 mg/dl, 35.67 ± 1.51 mg/dl, 32.33 ± 2.16 mg/dl respectively. The difference in mean BUN values within the groups was statistically significant ($p < 0.0001$). In Group 2, the mean BUN values at Day 0, Day 15 and Day 45 were 21.83 ± 3.25 mg/dl, 39.33 ± 2.42 mg/dl, 29.17 ± 1.47 mg/dl respectively. The difference in mean BUN values within the groups was statistically significant ($p < 0.0001$). In Group 3, the mean BUN values at Day 0, Day 15 and Day 45 were 19.00 ± 5.18 mg/dl, 41.00 ± 4.29 mg/dl, 27.67 ± 2.34 mg/dl respectively. The difference in mean BUN values within the groups was statistically significant ($p < 0.0001$). In Group 4, the mean BUN values at Day 0, Day 15 and Day 45 were 23.67 ± 2.73 mg/dl, 38.00 ± 5.62 mg/dl, 43.50 ± 2.35 mg/dl respectively. The difference in mean BUN values within the groups was statistically significant ($p < 0.0001$). In Group 5, the mean BUN values at Day 0, Day 15 and Day 45 were 25.67 ± 7.01 mg/dl, 43.67 ± 2.42 mg/dl, 27.17 ± 2.23 mg/dl respectively. The difference in mean BUN values within the groups was statistically significant ($p < 0.0001$).

The difference in mean BUN values at Day 15 among 5 groups was statistically significant ($p = 0.0089$). The difference in mean BUN values at Day 45 among 5 groups was statistically significant too ($p < 0.0001$).

Serum Creatinine

In Group 1, the mean Sr. Creatinine values at Day 0, Day 15 and Day 45 were 0.64 ± 0.04 mg/dl, 0.91 ± 0.04 mg/dl, 0.86 ± 0.04 mg/dl respectively. The difference in mean Sr. Creatinine values within the groups was statistically significant ($p < 0.0001$). In Group 2, the mean Sr. Creatinine values at Day 0, Day 15 and Day 45 were 0.65 ± 0.04 mg/dl, 0.97 ± 0.08 mg/dl, 0.74 ± 0.02 mg/dl respectively. The difference in mean Sr. Creatinine values within the groups was statistically significant ($p < 0.0001$). In Group 3, the mean Sr. Creatinine values at Day 0, Day 15 and Day 45 were 0.76 ± 0.04 mg/dl, 0.88 ± 0.03 mg/dl, 0.76 ± 0.08 mg/dl respectively. The difference in mean Sr. Creatinine values within the groups was statistically significant ($p = 0.0074$). In Group 4, the mean Sr. Creatinine values at Day 0, Day 15 and Day 45 were 0.67 ± 0.07 mg/dl, 1.22 ± 0.32 mg/dl, 1.19 ± 0.07 mg/dl respectively. The difference in mean Sr. Creatinine values within the groups was statistically significant ($p = 0.0010$). In Group 5, the mean Sr. Creatinine values at Day 0, Day 15 and Day 45 were 0.46 ± 0.19 mg/dl, 1.00 ± 0.08 mg/dl, 0.66 ± 0.07 mg/dl respectively. The difference in mean Sr. Creatinine values within the groups was statistically insignificant ($p = 0.0002$).

The difference in Sr. Creatinine values at Day 15 ($p = 0.0059$) and Day 45 ($p < 0.0001$) among 5 groups were statistically significant.

Body Weight

In Group 1, the mean body weight at Day 0, Day 15 and Day 45 were 19.33 ± 1.51 gm, 20.17 ± 1.33 gm, 22.33 ± 1.21 gm respectively. The difference in mean body weights within the groups was statistically significant ($p < 0.0001$). In Group 2, the mean body weight at Day 0, Day 15 and Day 45 were 19.50 ± 1.52 gm, 20.67 ± 1.51 gm, 22.83 ± 1.72 gm respectively. The difference in mean body weights within the groups was statistically significant ($p < 0.0001$). In Group 3, the mean body weight at Day 0, Day 15 and Day 45 were 21.17 ± 1.94 gm, 21.33 ± 1.03 gm, 23.17 ± 1.17 gm respectively. The difference in mean body weights within the groups was statistically insignificant ($p = 0.0235$). In Group 4, the mean body weights at Day 0, Day 15 and Day 45 were 20.67 ± 1.86 gm, 20.33 ± 1.37 gm, 19.00 ± 1.10 gm respectively. The difference in mean body weights within the groups was statistically insignificant ($p = 0.1005$). In Group 4, the mean body weights at Day 0, Day 15 and Day 45 were 21.67 ± 1.75 gm, 21.83 ± 2.04 gm, 23.00 ± 2.00 gm respectively. The difference in mean body weights within the groups was statistically significant ($p = 0.0014$).

The difference in body weights among 5 groups at Day 15 was statistically insignificant ($p = 0.2881$) whereas Day 45 was statistically significant ($p = 0.0002$).

Haemoglobin

On Intra – Group comparison, the statistically significant difference in Mean Haemoglobin levels was observed in Group 1 ($p = 0.0002$), Group 3 ($p < 0.0001$).On inter - Group comparison, the difference in Mean Haemoglobin levels among 5 groups at Day 15 ($p = 0.0002$) and Day 45 ($p = 0.0466$) were statistically significant.

RBCs (Red Blood Cells)

On Intra – Group comparison, the statistically significant difference in Mean RBCs levels was observed in Group 3 ($p = 0.0006$) only.On inter - Group comparison, the difference in Mean RBCs levels among 5 groups at Day 15 ($p = 0.1714$) and Day 45 ($p = 0.5415$) were statistically insignificant.

PCV (Packed Cell Volume)

On Intra – Group comparison, the statistically significant difference in Mean PCV levels was observed in Group 1 ($p = 0.0041$), Group 2 ($p = 0.0032$), and group 5 ($p = 0.0314$). On inter - Group comparison, the difference in Mean PCV levels among 5 groups at Day 0 ($p = 0.0335$) and Day 45 ($p = 0.0472$) were statistically significant.

MCV (Mean Corpuscular Volume)

On Intra – Group comparison, the statistically significant difference in Mean MCV levels was observed in Group 1 ($p = 0.0047$), Group 3 ($p = 0.0617$), and group 5 ($p < 0.0001$). On inter - Group comparison, the difference in Mean MCV levels among 5 groups at Day 15 ($p = 0.0112$) and Day 45 ($p = 0.0472$) were statistically significant.

WBCs (White Blood Cells)

On Intra – Group comparison, the statistically significant difference in Mean WBCs levels was not observed in any of the groups. On inter - Group comparison, the difference in Mean WBCs levels was not observed on Day 15 and Day 45.

Platelets

On Intra – Group comparison, the statistically significant difference in Mean Platelets levels was observed in Group 2 ($p < 0.0001$) and Group 5 ($p = 0.0008$). On inter - Group comparison, the difference in Mean Platelets levels was observed on Day 15 ($p = 0.0328$) and not on Day 45 ($p = 0.5285$).

MCH (Mean Corpuscular Haemoglobin)

On Intra – Group comparison, the statistically significant difference in Mean MCH levels was observed in Group 1 ($p = 0.0212$) and Group 2 ($p = 0.0007$). On inter - Group comparison, the difference in Mean MCH levels was observed on Day 15 ($p = 0.0157$) and Day 45 ($p = 0.0382$).

MCHC (Mean Cell Haemoglobin Concentration)

On Intra – Group comparison, the statistically significant difference in Mean MCHC levels was observed in Group 1 ($p = 0.0326$) and Group 3 ($p = 0.0091$). On inter - Group comparison, the difference in Mean MCHC levels was on Day 15 ($p = 0.1275$) was insignificant and on Day 45, it was significant ($p = 0.0454$).

Neutrophils

On Intra – Group comparison, the statistically significant difference in Mean Neutrophils count was not observed in Group 1 ($p < 0.0001$) and Group 3 ($p = 0.0039$). On inter - Group comparison, the difference in Mean Neutrophils count was on Day 15 ($p = 0.1625$) was insignificant and on Day 45, it was significant ($p = 0.0041$).

Discussion

Nephrotoxicity is one of the most common kidney ailments, means renal impairment or renal failure. It can be acute or chronic. It is evident that Diclofenac sodium have anti-prostaglandin activity and thus it can interfere with the kidney's ability to auto regulate glomerular pressure and decrease GFR. It causes inflammatory changes like glomerulo-nephritis, acute or chronic interstitial nephritis, papillary necrosis etc. (16)

Nephrotoxicity is manifested by signs and symptoms of renal failure, such as hyperkalaemia, hypernatremia, metabolic acidosis, sodium retention, which can lead to hypertension, oedema, proteinuria etc. (17)

In Ayurveda, the concept of *Dushi Visha* reflects chronic cumulative poisoning. Long term Toxic effects of Diclofenac sodium, can be considered as one of types of *Dushi Visha*. When Diclofenac sodium is taken in high doses or used in cases of contraindication and also if taken for long term, it accumulates inside the body and exerts its toxic effects by the above phenomenon of *Dushi Visha*. *Samprapti* of Diclofenac sodium induced nephrotoxicity can be explained in context of *Dushi Visha*.

Nephroprotective agents are the substances which possess protective activity against nephrotoxicity. Co-administration of various medicinal plants possessing nephroprotective activity along with different nephrotoxic agents may attenuate its toxicity. Hence, treatment mentioned by Acharya Yogaratnakara for *Dushi Visha* was incorporated here. *Pippalyadi Agad* is the herbo-mineral medication explained for the treatment of *Dushi Visha* by Yogaratnakara. It contains 10 drugs. Most of these drugs have *Katu Vipaka, Ushana Veerya, Katu, Tikta, Kashaya Rasa*, and *Vishaghna, Krimihara, Kushthaghana, Twagdoshahara, Deepaniya, Shothahara, Mutrala, Mutrajanan* effects. These drugs have curative effects on *Mutrakruchha, Bastishotha, Ashmari, Jwara, Shotha* etc. These clinical manifestations are classical features related to *Mutravahastrotasdushti* i.e., nephrotoxicity.

For experimental study, Swiss albino mice were selected as animal model because there was good background data available and were effective to be good in preclinical researches in previous studies. With the suggestions of experts, male albino mice weighing 20-30 gm were included in the study. The literature reviewed to adopt the methodology for induction of toxicity. Toxic dose of diclofenac sodium is 10mg/kg/ day for 5 days. But with the opinion of experts, it was decided as 15mg/kg body weight. Parenteral route of drug administration was preferred. Successful induction of toxicity was achieved in albino mice on biochemical parameters.

In the study, the study drug was used in two different doses i.e., low dose and medium dose to assess dose related response. Maximum dose was avoided with mortality concern. A potent nephroprotective drug i.e., Silymarin, was used as a standard comparator. The powder of study drug was mixed with honey & it was used as a vehicle control. In present study induction of nephrotoxicity was evident from elevated levels biochemical parameters like BUN, Sr. Creatinine and CBC.

BUN (Blood Urea Nitrogen) and Serum Creatinine are commonly used as markers of renal toxicity. Urea and Creatinine are metabolic waste products that are freely filtered by the glomeruli of the kidneys. When kidneys are not able to remove urea and Creatinine from the blood normally, BUN and Serum Creatinine level raises which ultimately reflects as decline in glomerular filtration rate. Thus, high levels of BUN and Serum Creatinine is a sign of kidney damage. Experiment was conducted on 30 male albino mice which were equally divided into five groups having six animals in each group and renal parameters along with CBC were assessed to observe the drug effect on nephrotoxicity.

Pippalyadi Agad treatment at low and medium doses significantly lowered BUN and Serum Creatinine in comparison with Disease and Standard Control at Day 45. *Pippalyadi Agad* treatment at low and medium doses significantly increased body weights of the animals in comparison with Disease and Standard Control at Day 45. No significant difference in CBC parameters was observed in any of the Groups except the Disease Control Group on Day 15 and Day 45.

Pippalyadi Agad is the combination of *Pippali, Dhanyak, Jatamansi, Lodhra, Ela, Maricha, Tagar, Swarnagairik, Suvarchika*. All ingredients are taken in equal quantity & honey is added to it. Most of these herbal drugs have *Katu Vipaka, UshanaVeerya, Katu, Tikta, Kashaya Rasa*, and *Vishaghna, Krimihara, Kushthaghana, Twagdoshahara, Deepaniya, Shothahara, Mutral, Mutrajanan*, *Vishaghna* effects. These drugs have curative effects on *Mutrakruchha, Bastishotha, Ashmari, Jwara, Shotha* etc. These clinical manifestations are classical features related to *Mutravahastrotasdushti* i.e., nephrotoxicity. Mineral drug like *Swarnagairik* i.e., red oxide of iron increases the efficacy & potency of *Pippalyadi Agad*. *Suwarchika,* i. e, Potassium nitrate is diuretic in action, which is curative in nephrotoxicity. *Yogavahi* property of Honey acts like catalyst which enhances the action of *Pippalyadi Agad*. *Katu, Tikta, Ushana* and *Tikshna* properties are Diaphoretic and Diuretic action of some drug individually in the *Pippalyadi Agad* also helps to eliminate the toxins showing its nephroprotective activity.

Nephroprotective effect of *Pippalyadi Agad* can be evaluated in human beings. For long term preservation of *Pippalyadi Agad*, preparation of suitable method of its formulation for marketing purpose can be further area of research.

Conclusion

Pippalyadi Agad has showed nephroprotective activity based on biochemical changes observed in Serum Creatinine, BUN and CBC.

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