ETHNO-PHARMACOLOGY AND NEPHROPROTECTIVE ACTIVITY OF: PINUS STROBUS AND ARAUCARIA HETEROPHYLLA EXTRACTS -ON GENTAMICIN INDUCED NEPHROTOXICITY IN RAT MODELS

Nahid Fatima M.Pharm.*, Dr. Syed Ahmed Hussain M.Pharm ., Ph.D. **,**
Rabia Fatima M.Pharm * , Amatur Rasheed yashfeen M.Pharm *.

Department of pharmacology,
Jawaharlal Nehru Technological University Hyderabad,
Shadan Women"S College Of Pharmacy.
Post Code No.500008, Telengana, India.

***Abstract*: In the following research study, Pinus strobus and Araucaria heterophylla extracts shown their nephroprotective impact on geneticin-induced nephrotoxicity. Phytochemical analysis was also performed and plants were founf to be rih in alkaloids, terpenes, phenolic compounds. Pinus strobus was found to have thunbergol, 3-carene, cembrene, α-pinene, quercetin, xanthone, Vit C (needles). Whereas, araucaria heterophylla was confirmed for the presence of labda-8, dominated by 13-epi-dolabradiene(42.7%), beyerene (22.2%), rimuene (13.7%), rich in bioflavonoid**

In all groups, the effects of nephroprotective activity were contextual. During the examination both moderate tubular dilatation and degenerative alterations and tubular edoema were detected.

Due to large doses (400mg/kg), Groups 5 Pinu srobus and 7 Araucaria heterophylla had greater therapeutic effect. The nephroprotective effect level was deemed adequate both for safety and for efficacy in sample groups (Group 3 - Group 8), because Group 3 is standardized market formulation and Group 8 is modest combination dose of both plant combinations.

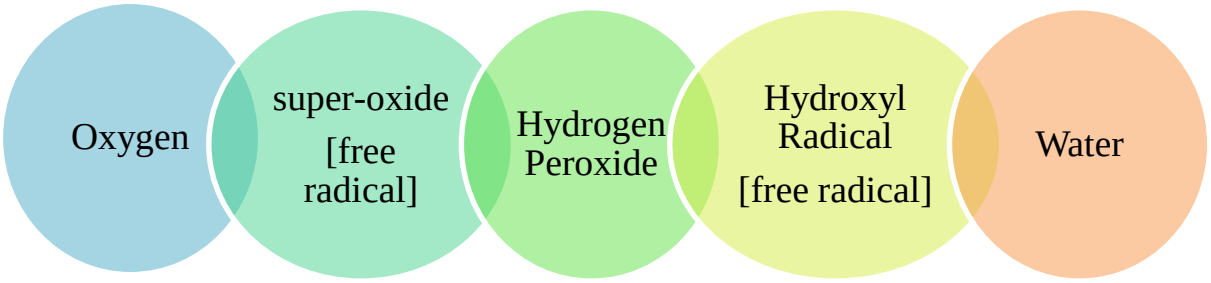
The findings indicate that detailed extracts for the experimentally introduced of Pinus strobus and Araucaria heterophyll may be used to decrease the need for conventional nephroprotective therapies such as nephroprotective drugs to maintain your salt, sodium and water systems. The effectiveness and practicality of this combination to help your kidneys release more salt to your urine have been shown in a pre-clinical study utilizing a lower dose for assessments of Pinus strobus and Araucaria heterophylla extracts.

***Keywords*: Nephroprotectives, *Araucaria heterophyll*, *Pinus strobus*, Gentamicin.**

INTRODUCTION:

The Kidneys play an important role in the maintenance of our endocrine, acid-base balance, blood pressure and erythropoiesis. The main functions of the kidney are formation of urine, maintenance of water and electrolyte balance as well as production of hormones and enzymes. Kidneys have some delicate tasks, especially when they have to deal with unwanted substances.
Nephrotoxicity: Is renal dysfunction that arises as a direct result of exposure to external agents such as drugs and environmental chemicals. [1]

Gentamicin induced toxicity: Aminoglycoside, widely used in treatment of gram-negative bacterial infections .It is not absorbed in the small intestine so is not active when given orally. It is administered by IP, IV, IM or topical route. However, ototoxicity is the major limitations in clinical use.
It generally causes drug induced **dose dependent Nephrotoxicity** in 10 to 20% of therapeutic courses. Characterized by direct **tubular necrosis** through preferential **accumulation** in the renal **proximal convoluted tubules** (50 to 100 times greater than serum) without morphological changes in glomerular structure. The ability of gentamicin to alter mitochondrial respiration has been well documented by both in vitro and in vivo studies [2]. Gentamicin **enhanced generation** of **super-oxide, hydroxyl radical, hydrogen peroxide** in vitro and in vivo [3,4,5] is explained.



There is a constant search for agents that provide nephro-protection against the renal impairment caused by drugs like gentamicin and cisplatin for which allopathy offers no remedial measures. Thus, it is obligated to turn towards alternative systems of medicine as natural products. So, plants containing antioxidant properties are recommended as nephroprotective agents.

Araucaria heterophylla Salisb. Franco (Family Araucariaceae /syn.Araucaria excelsa) commonly known as 'Norfolk Island pine', is a popular columnar tree used as Christmas tree [6] is distributed throughout many countries in southern hemisphere. It is an important medicinal plant. Foliar extract of the plant is reported to possess antioxidant activity [7].

PLANT A INTRODUCTION:

Classification:

Kingdom: Plantae

Division: Pinophyta

Class: Pinopsida

Order: Pinales

Family: Araucariaceae

Genus: ***Araucaria***

Species: heterophylla

Scientific Name: ***Araucaria heterophylla***

Synonyms: Araucaria excelsa.

Common Names: norfolk Island pine, Pino, Star pine, Polynesian pine, Triangle tree, Living Christmas tree.

Description: Norfolk Island pine is an evergreen tree ; rarely exceeds 1-2m (3-6 feet) indoors, where it grows very slowly – no more than 15cm (6 inch) a year. Its branches are tiny needles clustered together like a narrow, elongate, pyramidal crown arranged in tiers of horizontal spreading branches. New growth – which is normally produced in spring – is a bright fresh green and this color

is held until the autumn, when it darkens. The branches are heavy but they do not need to be given any extra support, since the main stem turns woody in time.

A beautiful tree widely planted as an ornamental and avenue tree throughout the subtropics and tropics.

Location: ***Araucaria heterophylla*** is native to Norfolk Island, a sub-tropical island in the Pacific Ocean between Australia, New Zealand and New Caledonia; the Araucariaceae are a very ancient family of conifers originally existing almost world wild but became extinct in the northern hemisphere.

Norfolk Island pine is grown throughout India. Its mainly grown in states of Karnataka, Bangalore, Maharashtra, Kashmir.

Phytochemical Constituents: Preliminary phytochemical screening of aqueous extract of leaves of A. heterophylla yielded alkaloids, flavanoids, sterols, cardiac glycosides, saponins, tannins, phenols, and terpenoids. [28] As per literature it is found to contain chemical constituents like - phenolic content, anti-oxidants [29], labda-8, dominated by 13-epi-dolabradiene(42.7%), beyerene (22.2%), rimuene (13.7%), rich in bioflavonoid. [30]

Pharmacological Activity:

In ancient ayurvedic system of medication, it has been used for treating toothaches, extracting teeth. [31]. Modern literature additionally revealed the medicinal use of this plant for-antibacterial [32] anti-inflammatory and antipyretic activity [33], anti-cancer and antioxidant [34] activities.

World Health Organization created a central data to record all medicinal plants used globally and have listed quite twenty thousand species till date.

PLANT B INTRODUCTION:

Classification:

Kingdom: Plantae

Division: Pinophyta

Class: Pinopsida

Order: Pinales

Family: Pinaceae

Subgenus: P.subg.Strobus

Species: strobus

Scientific Name: ***Pinus strobus***

Synonyms: Pinus laricio var. prostrata Beissn

Common Names: Eastern white pine , Weymouth pine, Soft pine, American white pine.

Description: White pine is a large tree. It is perennial and grows in a cold climate up to 57 meters. Best used for cough and cold. The outstanding characteristics of the genus are mostly erect, dwarf branches, twigs with long shoots and scaled leaves. The leaves are either primary, solitary, scale-like, spirally arranged, Spreading to ascending, 6-10 cm x 0.7-1 mm, and usually deciduous. The unisexual flowers (cones, strobile) appear in the spring on the same tree. Conifers often produce twin trees resulting from the presence of more than one embryo in a single ovule. Traditionally the genus Pinus is subdivided into two main groups:

1) The soft pine, or white pines, and
2) The hard or yellow pines.

Location: Of the ninety or more species of pines, thirty are native to North America, distributed from north of Mexico, eastern, northern and the western states, Himalayan regions in India.

Phytochemical Constituents: Various parts of plant including needles are rich in various biologically active compounds such as thunbergol, 3-carene, cembrene, α-pinene, quercetin, xanthone, Vit C (needles), *many* different acids in needles, essential oils (including terpenes, monoterpenes, sesqueterpenes), resin, starch [35]

Pharmacological Activity:

Specific for **respiratory** and **bronchial complaints**. A tea of the needles, or a decoction of the needles with thin twigs included (the strongest preparation), is helpful to promote expectoration and **removal and thinning of mucous from the lungs**. [36] Used for **coughs, colds, bronchitis, laryngitis,** Wounds, Sores ,Burns, Boils, T.B, Influenza . Also used to treat **scurvy** and as **antiseptic.**

MATERIAL AND METHODS

COLLECTION AND AUTHENTICATION OF PLANT MATERIAL:

Araucaria heterophylla and *Pinus strobus* was collected from zoological garden in its raw form and authenticated by:

College Of Agriculture, Rajendranagar , Hyderabad.

Department Of Horticulture.

The Professor Jayashankar Telangana State Agricultural University.

EXTRACTION OF PLANT MATERIAL :

The leaves of *Araucaria heterophylla* and *Pinus strobus* was collected from zoological garden ,it was cleaned from dust and shade dried for 15 days.

The dried material was powdered in coarse form and weighted. About five hundred grams of powdered extract was collected. The powder extract of both plant materials will be subjected to extraction with 99% pure ethanolic solution in (1:2 ratio) using maceration technique.

MACERATION TECHNIQUE:

Maceration is an extraction process that consists of maintaining contact between the plant material and a liquid (solvent) for a desired period of time

Yield extract (%) = Dry weight of extract recovered after extraction (g) / Intial weight of powder (g) *100

The yield extract used to identify chemical constituents by phytochemical screening techniques.

Maceration of *Araucaria heterophylla* leaves:

The leaves collected was cleaned and dried under the shade for about 15 days , then it was grind to get coarse powder. Maceration was carried out at room temperature. It consisted of immersing the powdered plant material in 99% of pure ethanol in a ratio of 1:2 [100gms of powdered plant material and 200 ml of ethanol] was stored inside the air tight container with frequent stirring.

The whole set up was kept for 3 days. After 3 days the greenish mixture of plant material and ethanol were filtered using muslin cloth, be coming filtrate was collected into a bowl. Further, the mixture was kept 2 to 3 days for evaporation of ethanolic content from extract.

Then the same procedure was repeated in the batches using 100gms of dry powder in 200ml of ethanol. Total 5 batches of extraction was undertaken.

The total final extract were collected and weighted. Resultant extract were stored in air-tight container in refrigerator at less than equal to 7 °C

Maceration of *Pinus strobus* leaves:

The leaves were collected, cleaned and dried under the shade for about 15 days followed by grinding to get coarse powder. Maceration was conducted at room temperature. It consisted of immersing the powdered plant material in 99% of pure ethanol in a ratio of 1:2 [100gms of powdered plant material and 200 ml of ethanol] stored inside the air tight container with frequent stirring

The whole set up was kept for 3 days. After 3 days the greenish mixture of plant material and ethanol were filter using muslin cloth, the collected filtrate was transferred into a bowl. Further, the mixture was kept 2 to 3 days for evaporation of ethanolic content from extract.

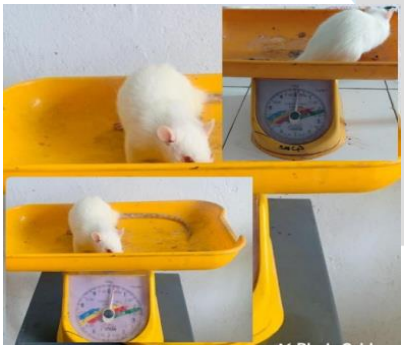
Then the same procedure was repeated in the batches using 100gms of dry powder in 200ml of ethanol. Total 5 batches of extraction was undertaken and the total final extract were collected and weighted. Resultant extract were stored in air-tight container in refrigerator at less than equal to 7 °C.

EXPERIMENTAL ANIMALS:

Healthy adult Albino Wistar rats weighing about (150g-200g) were used for carrying out experimental studies. The selected animals was maintained under standard laboratory conditions, housed in well aerated wire mesh Cage with optimum 12 hours light/dark cycle under required controlled temperature. Free access to diet and water was provided .The animals were allowed to acclimatize to the laboratory environment at least 1 week prior to commencement of experiment.

The studies were carried out at Shadan Institute Of Medical Sciences, Peerancheru, Hyderabad, in accordance with the committee guidelines as of supervision of experiments on animals.

The experimental studies were conducted as per the norms of "Institutional Animal Ethics Committee" Which governs the control and supervision of experimental animals through CPCSEA guidelines, Govt. Of India.



GROUPING OF ANIMALS

Albino Wistar rats (150g-200 g) was divided into 8 groups (n=8)

Group 1 (Control): Control rats received distilled water 1ml (p.o.) for 8 days.

Group 2 (Negative control) : Rats were treated with gentamicin (100 mg/kg) (i.p.) for 8 days [54]

Group 3 (Standard) : Rats were treated with gentamicin (100mg/kg) (i.p) and standard drug Allopurinol (300 mg/day p.o) for 8 days [55]

Group 4 (PS 200) : Rats received gentamicin (100 mg/kg) (i.p.) + Ethanolic extract of Pinus strobus 200 mg/kg (p.o) for 8 days

Group 5 (PS 400) : Rats received gentamicin (100 mg/kg) (i.p.) + Ethanolic extract of Pinus strobus 400 mg/kg (p.o) for 8 days

Group 6 (AH 200) : Rats received gentamicin (100 mg/kg) (i.p.) + Ethanolic extract of Araucaria heterophylla 200 mg/kg(p.o) for 8 days

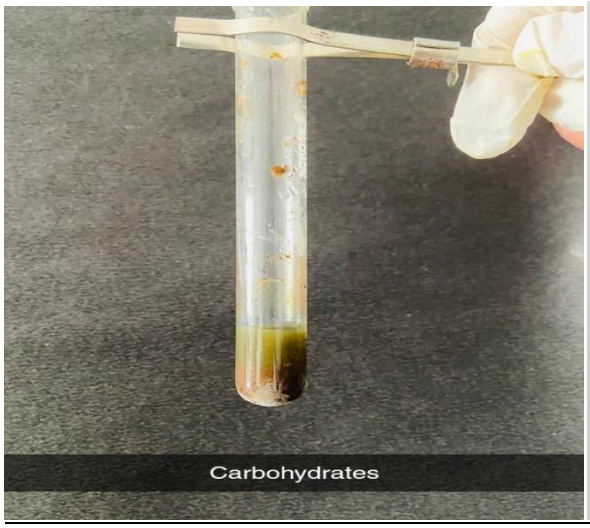
Group 7 (AH 400) : Rats received gentamicin (100 mg/kg) (i.p.) + Ethanolic extract of Araucaria heterophylla 400 mg/kg (p.o) for 8 days

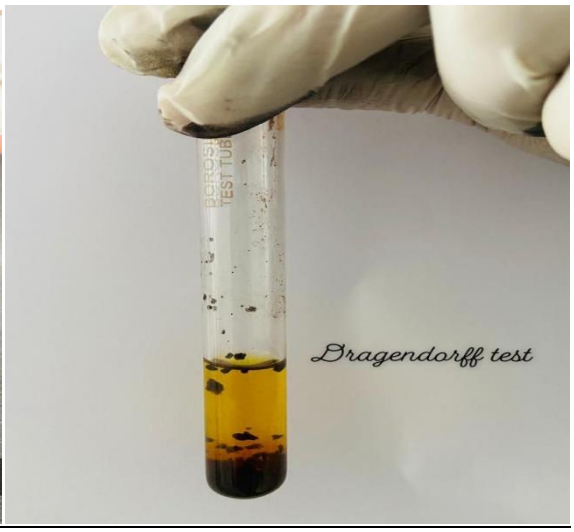
Group 8 (Combination of PS (200) + AH (200)) : Rats were treated with gentamicin (100mg/kg) (i.p) and combination of Araucaria heterophylla 200 mg/kg (p.o) + Pinus strobus 200 mg/kg (p.o) for 8 days

RESULTS:

PHYTOCHEMICAL SCREENING RESULTS:

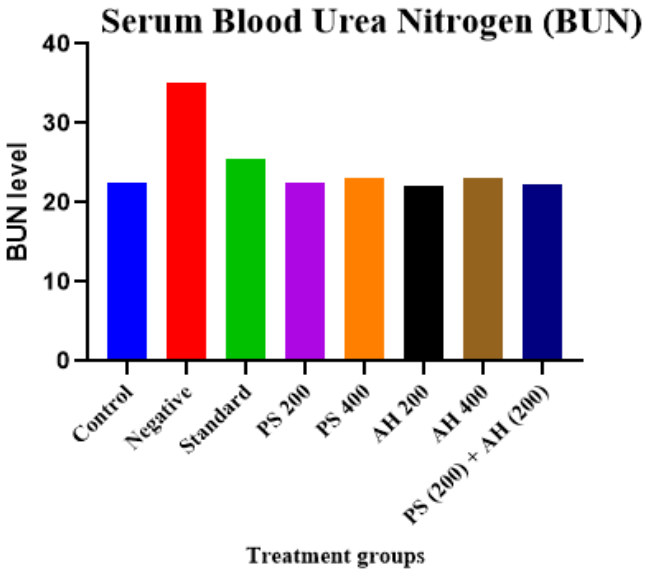
Chemical constituent	Test	PS Extract	AHExtract
Tannins	Ferric chloride test	+	+
	Lead acetate test	+	+
	Acetic acid sol.	+	+
	Dil. Iodine sol.	+	+
Alkaloids	Mayer's test	+	+
	Dragendroff's test	+	+
	Hager's test	+	+
	Wagner's test	+	+
Glycoside			
A. Cardiac glycosides	Baljet's test	+	+
	Legal's test	-	-
	Keller-killiani test	+	+
	Liebermann's test	-	-
B. Steroids	Salkowski test	-	-
	Liebermann-burchard test	-	-
	Liebermann's test	-	-
C.Saponins	Foam test	-	-
D. Flavonoids	Schinoda test	-	-
	Lead acetate test	-	-
	NaOH test	-	-
E. Anthraquinones	Borntrager's test	+	+
	Modified-borntrager's test	+	+
Carbohydrates	Molisch test	+	+
	Fehling's test	+	+
	Benedict's test	+	+
Proteins	Biuret's test	+	+
	Millon's test	+	+





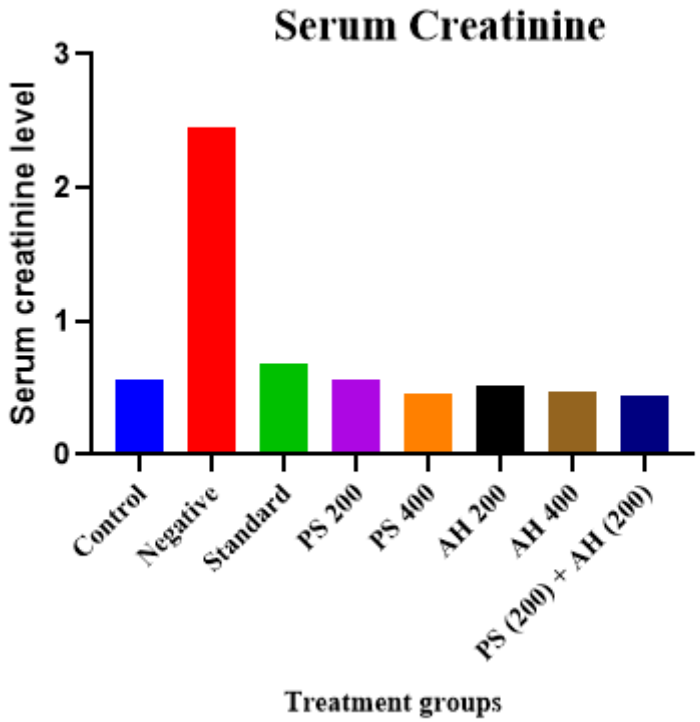
Serum Blood Urea Nitrogen (BUN)

Grouping of animals	**Serum blood urea nitrogen (BUN)**
Group 1 (Control)	22.47±0.003
Group 2 (Negative)	35.16±0.09
Group 3 (Standard)	25.47±0.002
Group 4 (PS 200)	22.58±0.005
Group 5 (PS 400)	23.16±0.008
Group 6 (AH 200)	22.18±0.004
Group 7 (AH 400)	23.14±0.007
Group 8 (PS (200) + AH (200))	22.26±0.002
SD	4.414
SEM	1.560



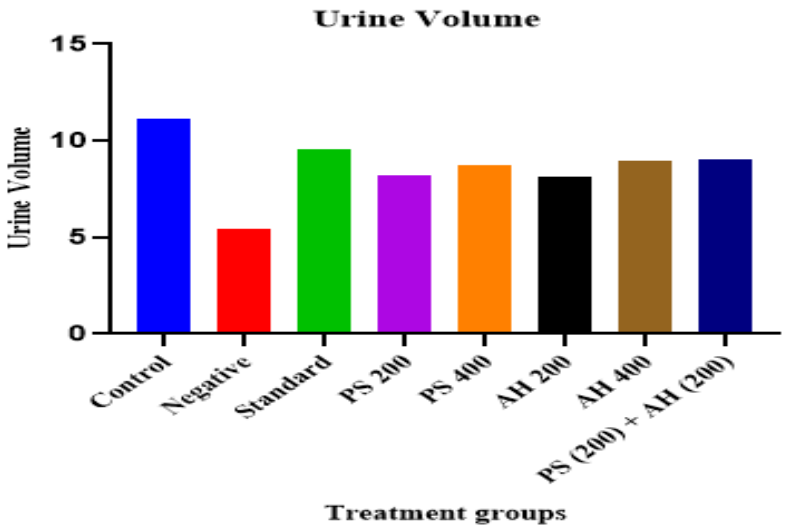
Assessment of Serum creatinine level

Grouping of animals	Serum creatinine
Group 1 (Control)	0.57±0.003
Group 2 (Negative)	2.45±0.09
Group 3 (Standard)	0.68±0.002
Group 4 (PS 200)	0.57±0.004
Group 5 (PS 400)	0.46±0.008
Group 6 (AH 200)	0.51±0.004
Group 7 (AH 400)	0.47±0.007
Group 8 (PS (200) + AH 200)	0.44±0.002
SD	0.683
SEM	0.241



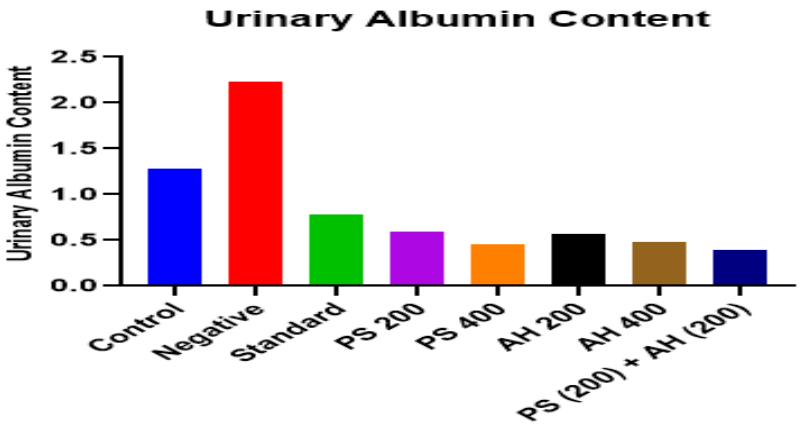
Assessment of urine volume

Grouping of animals	Urine Volume
Group 1 (Control)	11.16±0.003
Group 2 (Negative)	5.46±0.08
Group 3 (Standard)	9.56±0.002
Group 4 (PS 200)	8.24±0.006
Group 5 (PS 400)	8.73±0.008
Group 6 (AH 200)	8.12±0.004
Group 7 (AH 400)	8.97±0.007
Group 8 (PS (200) + AH (200))	9.05±0.002
SD	1.604
SEM	0.567



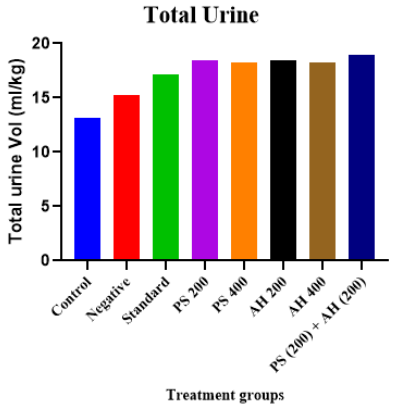
Abnormal Urinary Albumin Content Test

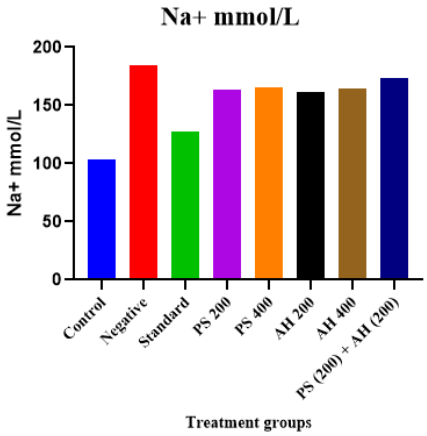
Groups	Urinary Albumin Content
Group 1 (Control)	1.28±0.003
Group 2 (Negative)	2.23±0.08
Group 3 (Standard)	0.78±0.002
Group 4 (PS 200)	0.59±0.007
Group 5 (PS 400)	0.45±0.005
Group 6 (AH 200)	0.57±0.008
Group 7 (AH 400)	0.48±0.003
Group 8 (PS (200) + AH (200))	0.39±0.002
SD	0.626
SEM	0.221

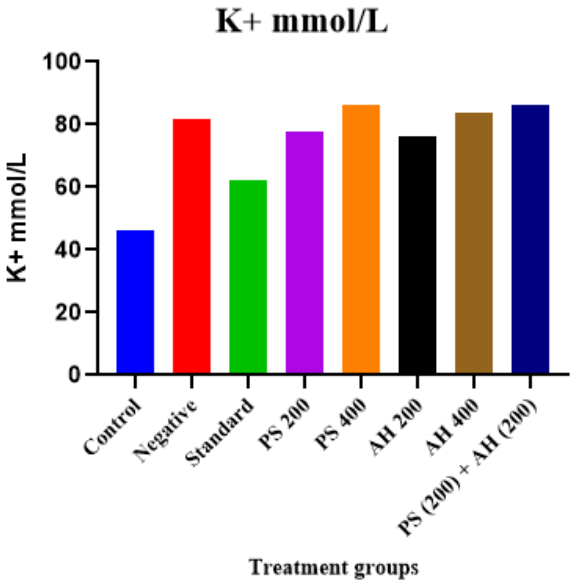


LIPSCHITZ TEST

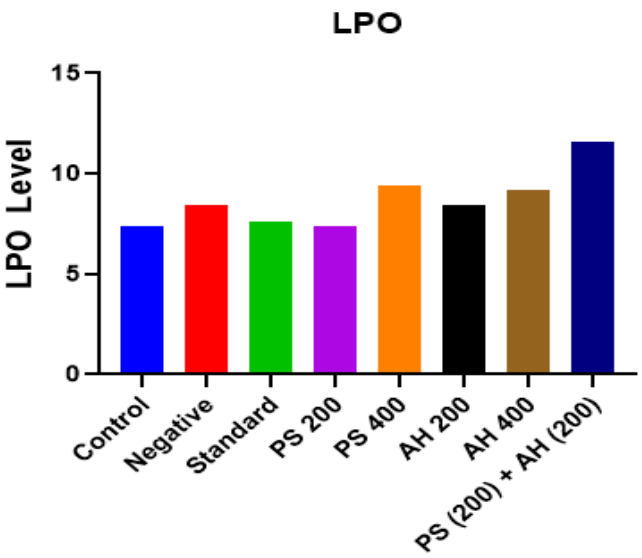
Groups	Total urine Vol (ml/kg BW/5 hrs)	Na+ mmol/L	K+ mmol/L	Cl− mmol/L
Group 1 (Control)	13.16 ±0.003	103.12 ±0.004	46.13 ±0.006	74.12 ±0.005
Group 2 (Negative)	15.23 ±0.09	184.28 ±0.07	81.78 ±0.08	123.12 ±0.06
Group 3 (Standard)	17.18 ±0.008	127.35 ±0.007	62.37 ±0.008	93.87 ±0.006
Group 4 (PS 200)	18.45 ±0.008	163.26 ±0.007	77.58 ±0.005	103.25 ±0.006
Group 5 (PS 400)	18.25 ±0.006	165.46 ±0.005	86.29 ±0.003	114.17 ±0.004
Group 6 (AH 200)	18.47 ±0.007	161.35 ±0.008	76.26 ±0.009	102.19 ±0.008
Group 7 (AH 400)	18.28 ±0.003	164.18 ±0.004	83.68 ±0.005	112.32 ±0.006
Group 8 (PS (200) + AH (200))	18.95 ±0.003	173.29 ±0.004	86.37 ±0.006	116.38 ±0.007
SD	2.024	26.590	14.037	15.509
SEM	0.715	9.401	4.962	5.483







Lipid Peroxide Test



HISTOPATHOLOGICAL STUDIES

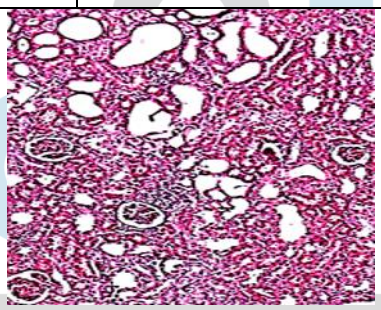
The authors examine the effects of different herbal extracts on nephroprotectivity, electrolyte structure, and histopathological features in rats treated with the extracts.

Purpose of Histopathological studies: Pathological alterations such as reduced and twisted glomeruli, dilated tubules, edema exudate, moderate necrosis, and invasion of inflammatory cells may be seen in the kidneys during exposure to doses possessing medicinal activity, according to histopathological reports. Even then, because of the large intergroup conflict differences, the histo-chemical improvements in the kidneys should occur or it might not exceed effect size.

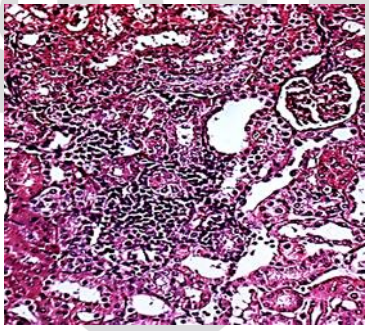
Group 1 (Control): The configuration of the urinary system, tissue was maintained. Inflammation lymphoid cells and epithelial granulomas were generally spread in the tissue. Periportal and perivarian colonization has been observed in mononuclear inflammatory cytokines aggregate particles.

Natural renal cortex and glomerulus in the kidney region is seen.

Groups	**LPO**
Group 1 (Control)	7.4±0.003
Group 2 (Negative)	8.4±0.09
Group 3 (Standard)	7.6±0.002
Group 4 (PS 200)	7.4±0.008
Group 5 (PS 400)	9.4±0.003
Group 6 (AH 200)	8.4±0.007
Group 7 (AH 400)	9.2±0.004
Group 8 (PS (200) + AH (200))	11.6±0.003
SD	1.409
SEM	0.498

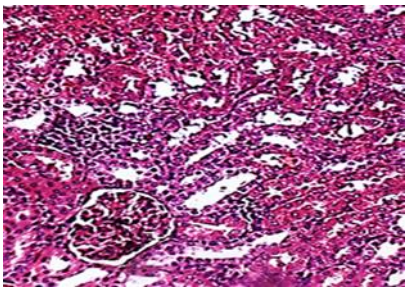


Group 2(Negative): The parenchyma of the adrenal cortex was extracted in half. In the centre of normal nephrotocytes, they are degenerating. In areas of necrosis, the parenchyma had cumulative inflammatory deposition. Infused proinflammatory cytokines invaded lymphocytes, neutrophils, and histocytes on the periphery. There are less and twisted glomeruli observed.

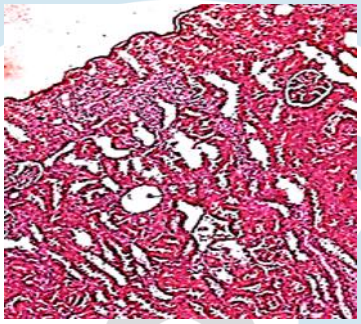


Group 3(Standard): The configuration of the glomerulus tissue was held. Cytokines, macrophages, and histocyte aggregates were present in the tissue. The nephrotocytes were shown to be degenerating or proliferating in focal areas. Mononuclear inflammatory cells observed lymphocytes and histocytes as peripheral and perivarian infiltrations.

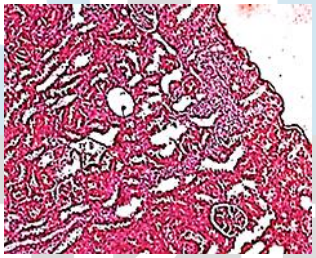
Infiltration of leukocytes, edema granulation tissue, and necrotic sigils is seen



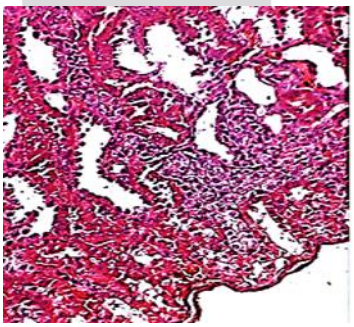
Group 4(PS 200): The configuration of the renal tubules tissue was held. In the center of normal nephrotocytes, a number of nephrotocytes were replenished. Mononuclear swelling cell aggregates could be seen in the tissue. Medullary and perivular penetration of fragmented mononuclear inflammatory individual cells lymphatic cytokines and histocytes is shown. In certain areas, bile duct multiplication has been noted.



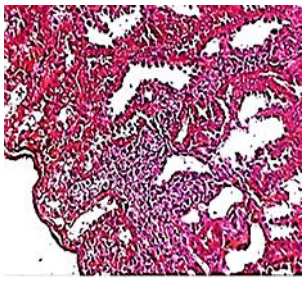
Group 5(PS 400): Mononuclear swelling cell aggregates could be seen in the tissue. Medullary and perivular penetration of fragmented mononuclear inflammatory individual cells lymphatic cytokines and histocytes is shown. In certain areas, bile duct multiplication has been noted.



Group 6(AH 200): The configuration of the nephron parenchyma has been protected. The nephrotocytes were found to be expanded sinusoids and congested. Within the epitheloid there are few parenchymas. Scattered mononucleic incendiary cells have been appeared to invade periportally and perivasively. Inflammation happened within the major veins.

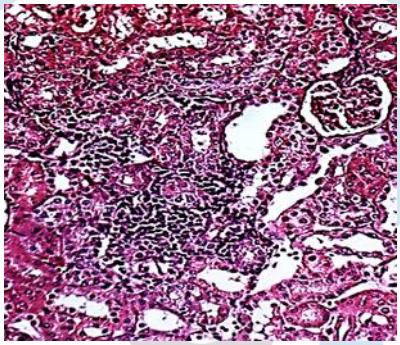


Group 7(AH 400): Scattered mononucleic incendiary cells have been appeared to invade periportally and perivasively. Inflammation happened within the major veins.



Group 8(PS (200) + AH (200)):

Direct tubular dilatation and degeneration, as well as tubular irritation, were included within the biopsy. Within the cortical zone of the kidney, in any case, multi central tubular recovery has been watched in fundamental and secondary tubules. Multi central recovered tubules emerged within the cortical range of the kidney within the endoscopic curettings. Incendiary cells invade the glomeruli and tubules, causing them to get to be twisted.



DISCUSSION

The Nephroprotective effect of ***Pinus strobus*** and ***Araucaria heterophylla*** extracts on Gentamicin-induced nephrotoxicity is demonstrated in the following research project.

The results of Nephroprotectives were contextual in both categories. Moderate tubular dilatation and degenerative changes, as well as tubular swelling, were both observed during the investigation.

For Groups 5 and 7 showed more therapeutic activity due to high dosages. This results are extremely relevant.

The Nephroprotective impact levels in sample groups (Group 3 - Group 8) were considered sufficient for both safety and efficacy as group 3 is standard market formulation and group 8 is moderate dosage of both plant combination.

CONCLUSION

The foregoing results show that detailed extracts of ***Pinus strobus*** and ***Araucaria heterophylla*** for the experimentally induced may be utilized to reduce the requirement for typical nephroprotective treatments such as nephroprotective medicines to retain your system of salt, sodium and water. The efficacy and feasibility of employing this combination as a prospective agent to aid your kidneys release more salt into your urine was shown in a pre clinical investigation using smaller dosage for evaluations of ***Pinus strobus*** and ***Araucaria heterophylla*** extracts.

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