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In Vivo Antioxidant and Diuretic Activity of Anogeissus *Latifolia Roxb* (Dhava) In Wistar Rat

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KEYWORDS
Herbal plant, Diuretic, Pharmacology, Antioxidant

ABSTRACT:
The present study was undertaken to investigate the diuretic activity and acute toxicity profile of the hydroalcoholic extract of the leaves of Anogeissus latifolia (2000Milligram/ kilogram or 3000 Milligram/ kilogram orally).in saline-loaded Wister rat. Wister of either sex was divided into 4 groups (six animals in each group). The control group received normal saline, while the standard group received furosemide (10 mg/kg i.p). Group III to Group VI received the test substances at dose levels of 2000, and 3000 mg/kg orally, respectively. At the end of the fifth hour, urine was collected, and total volume of urine excreted by each animal was recorded. Concentrations of urinary Na^+ and K^+ Cl^- were determined, and the Na^+/K^+ Cl^- ratio was calculated to make comparison among the groups. The acute toxicity of the most active fraction was also evaluated. The findings demonstrated that extract of Anogeissus latifolia leaves showed significant diuretic (P<0.01), natriuretic (P<0.01), and kaliuretic (P<0.01) effects. At the dose of 3000mg/kg, was show highly diuretic property comparison to2000mg/kg. It also showed a good natriuretic activity.

1. Introduction

There are times when conventional medicine is overlooked when it comes to the provision of medical services all over the world, despite the fact that it is very important. Especially in the treatment and management of long-term health disorders, its application in health promotion, illness prevention, and therapy is well-established and acknowledged. Its use in these areas is particularly noteworthy.1. There is evidence that people have relied on plants as diuretics from the dawn of time.A medication that stimulates the production of more pee by the body is known as a diuretic. In conditions where the body is experiencing an excess of fluid, such as in edematous disorders such as cirrhosis, nephritic syndrome, and heart failure, they are employed as a treatment option.3) The management of fluid overload, which can manifest itself in the form of pulmonary edoema, ascites, or swollen ankles, is made possible by the use of diuretics as part of

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treatment programmes. They contribute to the maintenance of fluid balance and the circulation of fluid out of the interstitium, which results in a significant reduction of symptoms and an improvement in the quality of life of patients in relation to their health. There are a number of adverse effects that are associated with the majority of the diuretics that are currently available, such as thiazides and loop diuretics. These adverse effects include acid-base imbalance, metabolic abnormalities (hyperglycemia and hyperlipidemia), acute hypovolemia, and electrolyte abnormalities (hypokalemia, hyperuricemia, and hyponatremia).5. Therefore, it is of the biggest importance to locate a diuretic that does not have these unfavourable and, in some cases, fatal side effects. Anogeissus latifolia, which is widely used in the indigenous Indian medicinal system, is thought to include leucocyanidins and tannoid principles, such as ellagic acid and its derivatives. Both of these classes of compounds are believed to be present in the plant. The purpose of this study was to explore the antioxidant activity of A. latifolia extract, taking into mind the chemical composition of the extracted plant as well as its widespread application. There were a number of antioxidant activities that were studied in relation to the extract. These activities included those that included hydrogen donation, nitric oxide, superoxide scavenging, hydrogen peroxide breakdown, and lipid peroxidation. Evaluation of the integrated antioxidant capacity was carried out by the use of the chemiluminescence technique. Another field of research that was conducted on the extract was the lipid peroxidation assay, which utilised the thiobarbituric acid-reactive substances (TBARS) method with rat liver homogenate. Based on the findings, it may be concluded that the extract of A. latifolia operates as an efficient antioxidant. Moreover, in order to ascertain the most likely reason for the intense activity, the percentage of gallic acid was computed and found to be 0.95 percent. This was done in order to reach a conclusion. There is a possibility that this is one of the reasons why the plant has such powerful antioxidant action.

Collection & authentication of Plants

Anogeissus latifolia leaves were obtained from a farm in Lucknow that was located nearby. ICAR, situated at the Pusa Campus in New Delhi. We preserved the leaves by air-drying them at a temperature of twenty degrees Celsius plus or minus twenty degrees Celsius. In order to preserve the dried plant material until it was required, it was stored in a dark container.

Extraction of plant material

Following the removal of five grammes of dried plant material with either water or ethanol, the material was pulverised into particles that ranged in size from two to five microns of size. For ten minutes, we used a temperature of 100 degrees Celsius plus or minus one degree Celsius, five grammes of dried herbal tea, and two hundred millilitres of pure water. A glass flask with a plastic lid that could be screwed on was used to hold each individual aqueous extract. With regard to the extraction of phenolic and flavonoid compounds from plants, it was discovered that longer infusion durations and higher solvent temperatures were more successful.

Analysis of Phytochemical Srudy

Phytochemical components of medicinal plants were screened and detected using extracts and powder specimens using standard procedures.

Test for Alkaloids

After stirring in a few drops of diluted hydrochloride solution with a tiny amount of dried extract, the mixture was filtered. There are indications of the presence of alkaloids in the filtrate material.

a.) **Mayer's test**: When treated with Mayer reagents, alkaloids produce a cream-colored precipitate.

b.) **Dragendroff' s test**: a.) When treated to the Dragendroff test, alkaloids produce an orange-yellow precipitate.

c.) Wager's test: When combined with other reagents, alkaloids produce a reddish-brown precipitate.

d.) **Hager;s reagent:** The Hager test will show a yellow colour precipitate if alkaloids are present.

Test for Carbohydrates

The content of carbohydrates was investigated after a tiny quantity of extract was dissolved in distiller's water, filtered, and then subjected to the investigation.

a.) **Molisch' s test:** After passing a few drops of Molisch's reagent and one millilitre of concentrated sulfuric acid slowly down the slides

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of test tubes, a red-violet colour ring was observed on the treated filter.

b.) **Fehling's Test:** When an equal volume of each of the Fehling reagents is combined together, a hue that looks like brick is produced.

Test of Glycosides

a. Borntranger's test: A minute quantity of extract was hydrolyzed with diluted hydrochloric acid on a water bath for a couple of hours. The presence of glycosides can be determined by the appearance of a hue that is reddish brown at the intersection of the two layers.

b. Keller – Killiani test: After allowing the mixture to cool, a few drops of ferric chloride were added, and then a little amount of extract was mixed with one millilitre of glacial acetic acid. After that, the contents were transferred to a test tube that had 1 ml of concentrated H2SO4 attached to the side of the test tube. After being allowed to stand, the presence of glycosides may be determined by a reddish-brown coating that becomes blue green after being exposed to light.

Test for Saponins:

a. **Foam test:** The extract was mixed with 20 millilitres of distilled water before being agitated for 15 minutes in a graduated cyclinder. The presence of saponins was demonstrated by the formation of foams that were about one centimetre thick.

Test for Phenolic Compounds and tannins

A little amount of the extract was mixed in an isolated container with aq. Find the answer and do the test for phenolic compounds.

a. **Add 10% W/v lead acetate solution:** Observed for white color precipitates.

Test for Amino acid and protein:

Following the dissolution of a tiny amount of extract in a few millilitres of distilled water, the following reagents were applied to the mixture.

Millon's reagents: The colour of the colour red It can be seen from the precipitate that proteins and amino acids are present.

a. Ninhydrin reagents: a. The presence of protein and amino acid may be determined by the colour purple that is present.

Test for Flavonoids:

Shinoda Test: A little amount of extract was dissolved in ethanol, and then a piece of metallic magnesium or zinc was added to the mixture. This was followed by the addition of two drops of concentrated hydrochloric acid, and the resulting hue was noted to be pink.

Ammonia Test: A minute quantity of extract was mixed with ethanol and shaken. A strip of filter paper was dipped in an alcoholic solution, the strip was then made ammoniated, and the resulting colour was monitored as it changed from white to orange.

Experimentation's Design

We chose animals that weighed between 180 and 220 grammes, with a weight range of 20-30 grammes. The AIBPS Institutional Animal Ethical Committee has approved studies that will examine the diuretic effects of HAEMC in animals. Research using animals can begin after they have been acclimated for fifteen days. Throughout the trial, the rats were housed in one of six metabolic cages, with temperatures ranging from 22 to 20 degrees Celsius. Using modified hydro-ethanolic extracts, we will study the possible diuretic effects of Anogeissus latifolia leaf. Male wiener rats normally weigh around 180–200 grammes. The four groups were each comprised of six people. All of the animals in Group I were given distilled water to use as a standard. Group II served as a control and received oral administration of furosemide at a dosage of 10 mg/kg. Both Group IV and Group III were given the hydroalcoholic extract of Anogeissus lactiflora. All four of the experimental animals will be housed in metabolic cages. The severity of the illness being investigated determines how often urine samples should be taken for testing, which typically range from five to twenty-four hours. The pH, salt, potassium, and chlorine levels in your urine should all be checked.

Parameters of hematology

Neutrophils (N), lymphocytes (L), macrophages (M), eosinophils (E), mean corpuscular volume (MCV), concentration of monocytes (MH), and a mean CV/MCV ratio (N) were all determined from the blood samples that were tested (MCHC).

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Blood samples are tested for biochemical properties.

Blood samples were spun at 4000 revolutions per minute for 10 minutes to extract ALT, AST, Urea, Creatinine, and glucose, which were then used to make the serum for these assays.

Histopathology

Upon completion of the study, a board-certified pathologist carefully examined each rat under a microscope. The rats' internal organs were measured, dissected, and checked for anomalies. Prior to histological analysis, all specimens were immersed in a formaldehyde buffered solution with a 10% concentration. We used a microscope to examine the tissues under a closer magnification after embedding them in paraffin and staining them with haematoxylin and eosin.

A study based on numbers

The experimental results were expressed using the mean SEM with a sample size of six. GraphPad Prism was used to conduct one-way analysis of variance and Dunnett's 't' tests.

2. RESULT & DISCUSSION

Phytochemical Analysis

There is a chance that alkaloids can be identified by looking for turbidity and/or the production of precipitates. Because of the steroid, the colour of certain samples changed from violet to blue or green. The presence of terpenoids caused an interface that was reddish brown in colour. The colour measurement technique developed by Toshio Shinado reveals that flavonoids appear red. Tannins were identified by colour changes in the test tube.

Table No: 1 Phytochemical Study

Phytochemicals	A. *Latifolia.*
1. Alkaloids. Test	---
2. Steroids. Test	++++
3. Terpenoids. Test	++++
4. Flavonoids. Test	++++
5. Tannins. Test	++++

++: Presence of the compound --: Absence of compound

Acute toxicity study

The administration of Anogeissus latifolia extract to rats through oral administration at doses ranging from 2000 mg/kg to 3000 mg/kg did not result in any deaths or toxicity during the course of the study. In spite of this, the haematological, biochemical, and histological indicators of the animals did not exhibit any abnormalities when the Anogeissus latifolia extract was considered. The dosage of diuretics that is suggested is between 2,000 and 3,000 milligrammes per kilogramme of body weight. It is important to keep a close eye on any animal groups throughout the course of a single day for any indications of the aforementioned conditions, such as excessive urination, hair loss, convulsions, or movement, in order to determine whether or not any of the aforementioned characteristics are present. All of the research groups indicated that there were no harmful effects connected to diabetes or the pancreas when the extract dose was administered for a period of fifteen days. After 15 days of therapy, neither of the groups acquired diabetes or pancreatic dysfunction as a result of the medicine, according to the laboratory tests conducted on animals. Additionally, the daily body weight of each group of mice was examined, which was in line with the findings of a previous study on mice. The mice who were given extract doses did not exhibit any signs of obesity or raised cholesterol levels, as was revealed in the previous section. According to the findings of the animal experiments, the doses of the extract did not cause any hair loss, tumours, or problems with the immune system. On the other hand, the doses of the

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extract did not result in any troubles with urination, and the animal groups that were examined did not suffer any problems with their urinary systems.

Haematological

Platelets, neutrophils, lymphocytes, monocytes, hematocrit, and red blood cell counts were unaffected. After seven days of treatment, none of the aforementioned signs altered. Compared to the 0th, the 15th had less swings. Since the medicine was administered without side effects, the intervals of 0, 7, and 15 days are equivalent.

Biochemical Evaulation

All three of these substances were within normal ranges when compared to ASAT and ALAT levels before the medication was administered (Table 3). After the first week of treatment, there was no change in glucose, urea, or creatinine levels. Compared to the zero day, the biochemical changes on day fifteen were not as significant. When looking at comparisons between 0, 7, and 15 days above the criterion, the control group showed no negative impacts. Antioxidant characteristics of the extract were demonstrated by the increase in ASAT (U/I) and ALAT (U/I) levels.

Histopathology test

No cellular alterations were noted in the kidney, liver, heart, and spleen were administered doses of 3000 mg/kg and 2000 mg/kg, respectively.

Table No-2: Acute toxicity assessment of Anogeissus latifolia Hydro Alcoholic Extract on rats

	Day of 0			Day of 7th		Day of 15th	
Parameter	Normal rang	2000mg/kg	3000mg/kg	2000mg/kg	3000mg/kg	2000mg/kg	3000mg/kg
RBC count	8.89 ± 0.63	6.7 ± 0.70	7.79 ± 0.81	7.56 ± 0.45	8.24 ± 0.54	8.18 ± 0.7	8.48 ± 0.87
Haemoglobin (Hg)	13.58 ± 0.63	13.55 ± 0.36	14.26 ± 0.21	13.07 ± 0.84	13.25 ± 0.64	15.8 ± 0.65	15.71 ± 0.65
Hematocrits	49.75 ± 0.69	41.08 ± 0.58	41.93 ± 0.55	41.75 ± 0.67	42.65 ± 0.92	43.65 ± 0.94	43.26 ± 0.98
Platelets count	723 ± 53	584.00 ± 81	595.00 ± 77	641 ± 53	587 ± 95	589 ± 82	587 ± 92
WBC count	11.54. ± 3.1	11.62 ± 0.35	12.13 ± 0.78	12.06 ± 0.10	12.75 ± 0.80	11.83 ± 0.98	12.85 ± 0.55
Neutrophils count	21.75 ± 2.89	21.8 ± 0.22	21.62 ± 0.98	20.12 ± 0.3	21.28 ± 0.86	22.17 ± 0.30	21.34 ± 1.18
Lymphocytes count	60.51 ± 3.66	71.98 ± 2.16	71.46 ± 2.11	71.81 ± 2.67	70.03 ± 4.07	69.1 ± 3.71	68.91 ± 3.83
Monocytes count	3.03 ± 1.17	3.01 ± 0.18	2.98 ± 0.10	2.15 ± 0.19	3.00 ± 0.28	2.01 ± 0.4	2.13 ± 0.23
Eosinophils count	2.7 ± 1.43	2.7 ± 0.47	2.7 ± 0.26	1.83 ± 0.36	2.73 ± 0.45	1.76 ± 0.22	1.65 ± 0.44
MCV count	41.47 ± 2.25	52.8 ± 0.89	50.7 ± 0.74	52.02 ± 1.55	52.37 ± 0.96	52.92 ± 1.88	53.5 ± 0.84
MCH count	19.34 ± 1.69	18.75 ± 0.73	17.15 ± 0.55	18.87 ± 0.76	18.97 ± 0.92	19.46 ± 0.98	19.33 ± 1.72
MCHC count	35.17 ± 1.47	35.2 ± 0.46	35.3 ± 0.26	35.03 ± 0.67	35.46 ± 0.98	35.2 ± 0.50	35.01 ± 0.97

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Fig No. 1: Heamatological parameters of Rats

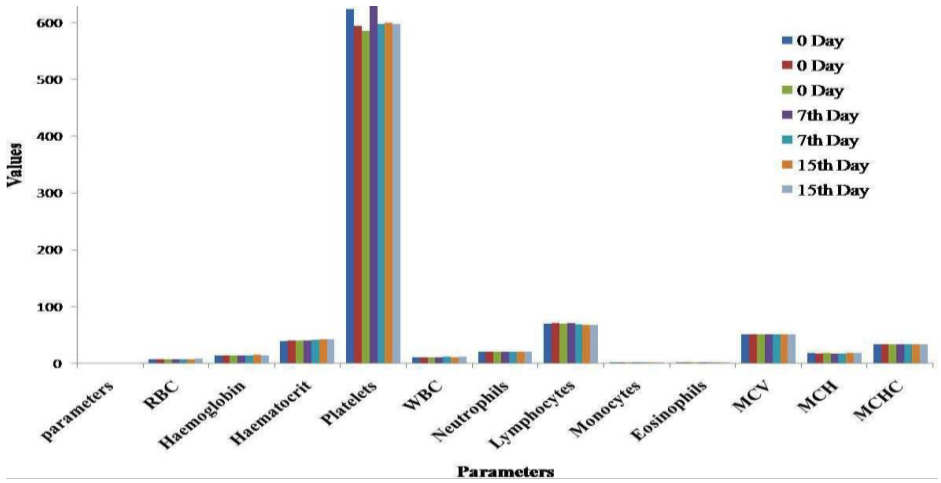
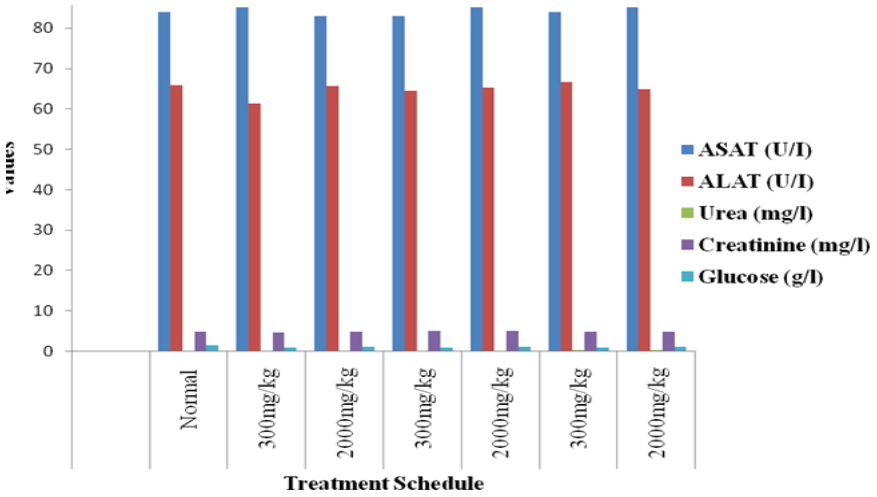


Table. No: 3: A acute toxicity study of Hydro Alcoholic Extraction of *Anogeissus latifolia*

Schedule of treatment							
Days	0		7th			15th	
Parameters	Normal Range	3000.mg/kg	2000.mg/kg	3000.mg/kg	200.mg/kg	3000mg/kg	2000.mg/kg
1. ASAT. (U/I)	85 ± 1.1	74 ± 2.1	85 ± 1.9	64 ± 2.1	75 ± 2.0	85 ± 2.0	76± 2.4
2. ALAT. (U/I)	66.85 ± 5.3	12.36 ± 5.5	65.56 ± 3.75	75.55 ± 4.17	66.16 ± 4.28	67.55 ± 4.21	54.93 ± 2.12
3. Urea. (mg/l)	0.36 ± 0.15	1.36 ± 0.14	1.18 ± 0.13	1.35 ± 0.19	0.36 ± 0.17	0.44 ± 0.14	1.45 ± 1.16
1. Creatinine. (mg/l)	6 ± 0.60	3.83 ± 0.52	3.86 ± 0.17	6.13 ± 0.38	5.21 ± 0.30	4.98 ± 0.50	4.02 ± 2.17
5. Glucose. (g/l)	1.65 ± 0.12	1.12 ± 0.18	2.25 ± 0.9	2.18 ± 0.5	2.21 ± 0.40	2.17 ± 0.46	2.02 ± 1.45

Figure No. 2: Biochemical values of rat



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HISTOPHATHOLOGICAL STUDY

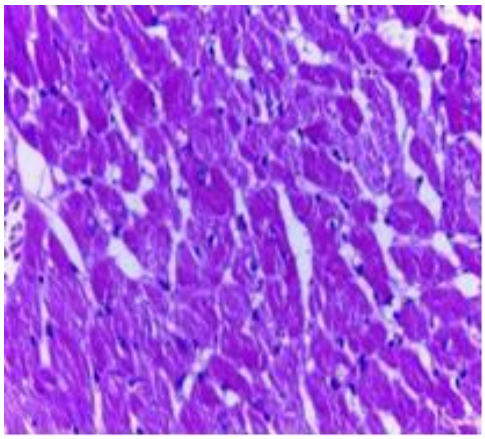

Fig 3 a: Control group heart

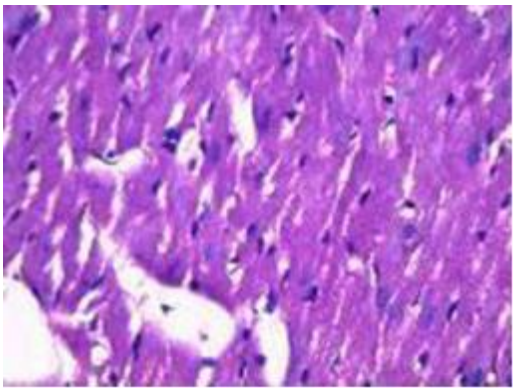

Fig 3a-: Control group liver

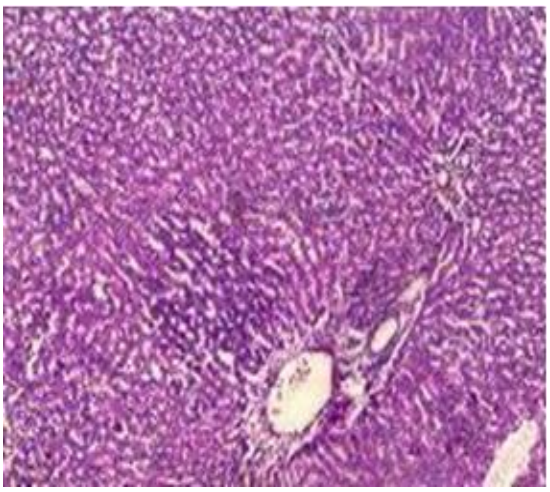

Fig 3b: Control group lungs

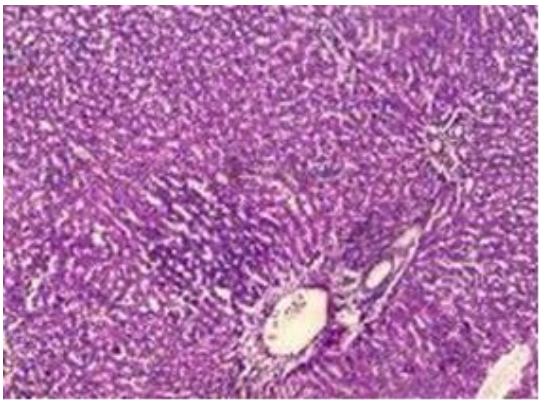

Fig 3 c : Control group Spleen

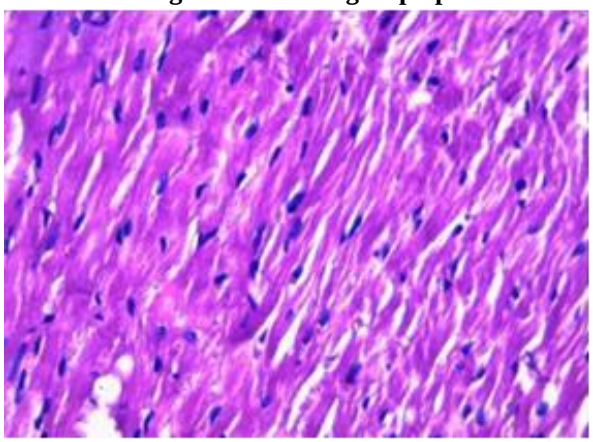

Fig3 d:- Control group kidney

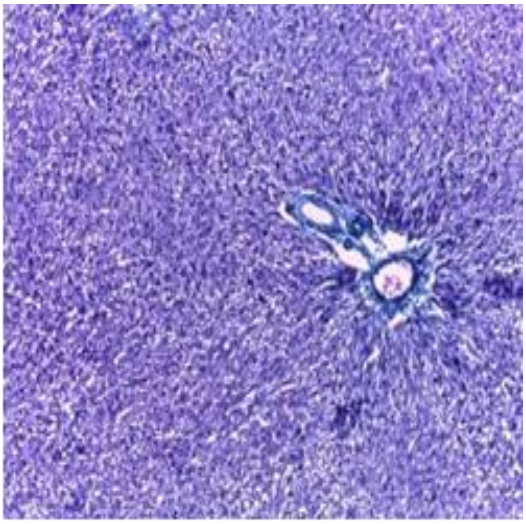

Fig 3 e:- Standard group Heart

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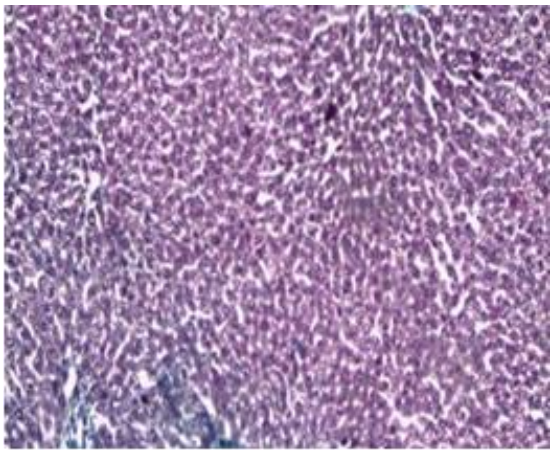

Fig 3 f: Standard group liver

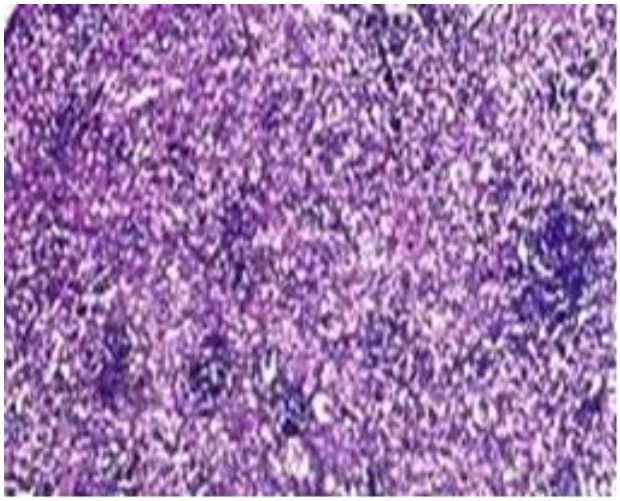

Fig 3 g: Standard group liver Spleen

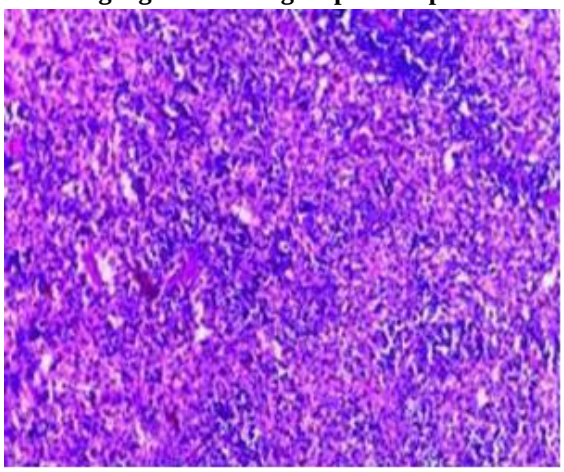

Fig 3 h: Standard group kidney

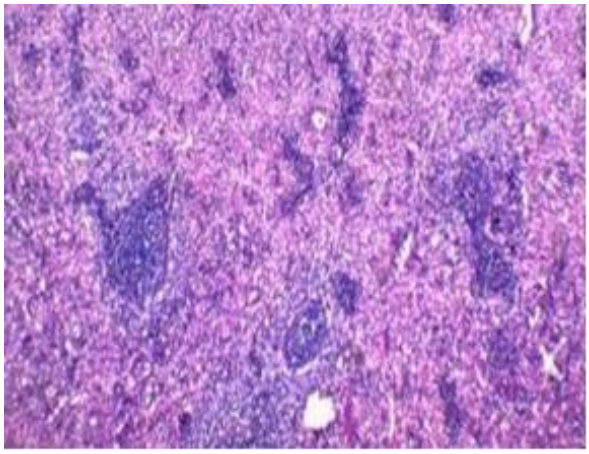

Fig 3 i: Standard group Lungs

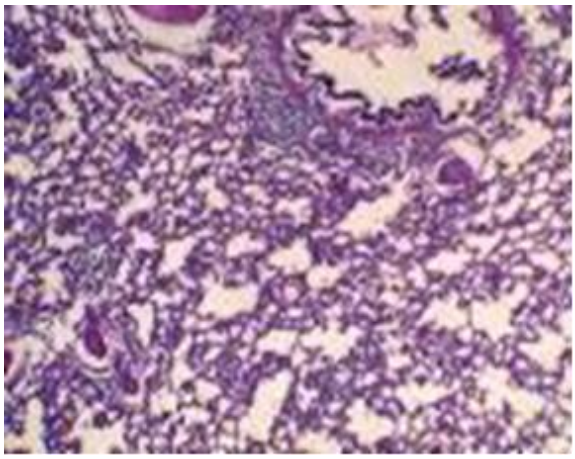

Fig 3 j:-3000mg./Kg Lung

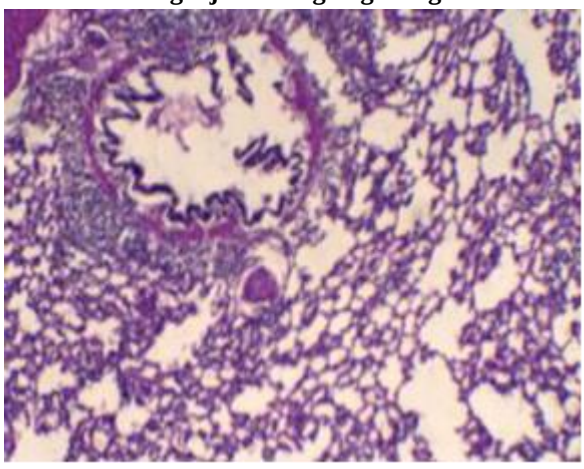

Fig 3 k:-2000 mg/Kg. Lung

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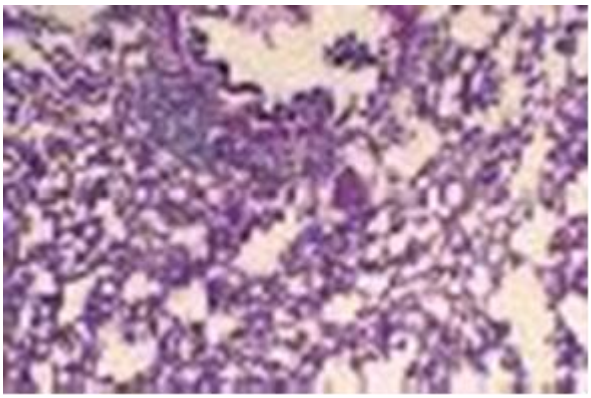

Fig 3 l: 2000mg/Kg kidne Diuretics action

Here is a table that ranks the extracts based on their quality. The relationship between urine volume (ml/100g/8h) and urinary salt and potassium concentrations (mequiv/100g/8h) is well-established. Table 1 shows that the amount of urine you excrete increased by 54% due to HCTZ. Hydration of Anogeissus latifolia increased urine production. The hydroalcoholic extract of Anogeissus latifolia showed an 18% (P 0.01) and 41% (P 0.001) increase at 2000 and 3000 mg/kg body weight, respectively.

Table No:- 4: Hydro Alcoholic Extraction of Anogeissus latifolia Acc to Lipschiz Test Method for Diuretic Effect

Group.	Total urine Volume (ml/kg BW/5hrs)	Na^+ m. mol/L	K^+ m. mol/L	Cl^- m. mol/L
Control Group. (10 ml/kg BW)	13.24±0.32	107.40±0.75	48.18±0.32	78.00±0.23
Standard group (Frusemide 10 mg/kg BW)	21.01±0.03***	189.12+0.64***	82.12±0.44***	129.00±0.24***
A. latifolia hydro alcoholic extract Group (2000 mg/kg BW	27.35±0.51*	168.12±0.83*	81.35±0.45**	124.05±0.16***
A. latifolia hydro alcoholic extract Group (3000 mg/kg BW	29.35±0.51***	175.12±0.83***	91.35±0.45****	134.05±0.16****

All told, six different species of animals were considered for this study. Each one had their standard error of evaluation (SEM) determined.** The significance level of the difference between the experimental and control groups was higher than 0.001. *** Table 4 shows that HAEAL has a diuretic effect, and Hydroxide, potassium, and chloride (Na+, K+, and Cl-) excretion were all markedly improved by

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the HAEAL extract group as compared to the control group. Urine output was greater in the extract group when compared to furosemide when administered at a dose of 400 mg/kg HAEMC.

3. CONCLUSION

With the exception of medicinal herbs and minerals, all of the ingredients in herbal treatments are organic. Traditional Indian medicine has included the use of medicinal plants since ancient times. Since most Indian doctors write and dispense their own prescriptions, a lot of paperwork and investigation is needed. An important barrier in contemporary medicine is the lack of clinical and scientific evidence as well as a better understanding of the effectiveness and safety of herbal remedies. Herbal medicines were the traditional method of treating edoema. This study made use of Anogeissus latifolia Hydro Alcoholic Extract. To test the diuretic effect of Anogeissus laxiflora leaf extract, animals will have their ions and electrolytes extracted. Anogeissus latifolia will be tested for acute toxicity to the human body. Anogeissus latifolia has diuretic qualities that have been demonstrated to be medicinally useful in animal experiments; hence, this herb could be utilized in human clinical trials.

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