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EFFECT OF AQUEOUS EXTRACTS OF ANTHOCLEISTA GRANDIFLORA ON SERUM TOTAL PROTEIN AND KIDNEY HISTOLOGY OF ALBINO RATS.

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ABSTRACT

Anthocleista grandiflora is commonly used traditionally as herbal medicine and has been documented to have a broad range of therapeutic effects. This study was carried out to evaluate the effects of aqueous leaf extract of *Anthocleista grandiflora* on Serum Total Protein and Kidney Histology of Albino rats. A total of twenty (20) albino rats of both sexes were used for this study. The animals were divided into five (5) groups; A - E, with each group containing four (4) rats. Animals in group A were administered with distilled water and they served as control. Animals in group B – E were administered with different doses of the extract of *Anthocleista grandiflora* at dosage levels of 150mg/kg b.w., 300 mg/kg b.w, 450 mg/kg b.w and 600mg/kg b.w respectively. After twenty-one (21) consecutive days of treatment, all the rats were sacrificed. Blood samples were collected in sample bottles for the evaluation of serum total protein levels while the kidney samples were carefully excised and fixed in 4% formaldehyde for histological investigation. Biochemical results showed that at all the extract dose levels studied, the difference in serum total protein levels were not significant ($P>0.05$) when compared with the control. From histological examinations, animals in group A were observed to have normal kidney cytoarchitectural features while those in groups B-E all showed moderate inflammatory response. It was therefore concluded that the aqueous leaf extracts of *Anthocleista grandiflora* is safe for medicinal application especially at moderate dose levels. The continued use of the extract is therefore not discouraged.

KEYWORDS: *Anthocleista grandiflora, total protein, kidney histology.*

INTRODUCTION

Medicinal plants, medicinal herbs, or simply herbs have been identified and used from prehistoric times. Plants make many chemical compounds for biological functions, including defense against insects, fungi and herbivorous mammals.

Over 12,000 active compounds are known to science. These chemicals work on the human body in exactly the same way as pharmaceutical drugs, so herbal medicines can be beneficial and have harmful side effect just like conventional drugs. However, since a single plant may contain many substances, the effect of taking a plant medicine may be complex.

The earliest historical records of herbs are found from the Sumerian civilization, where hundreds of medicinal plants including opium are listed on clay tablets. The Ebers Papyrus from ancient Egypt describes over 850 plant medicines, while Dioscorides documented over recipes for medicines using over 600 medicinal plants in De materia medica, forming the basis of pharmacopoeids for some 1500 years. Drug research makes use of ethnobotany to search for pharmacologically active substances in nature, and has in this way discovered hundreds of useful compounds, and include the common drugs aspirin, digoxin, quinine, and opium. The compound found in plants are of many kinds, but most are in four major biochemical classes, the alkaloids, glycosides, polyphenols and terpenes.

Plants, including many now used as culinary herbs and spices, have been used as medicines from prehistoric times. Spices have been used partly to counter food spoilage bacteria, especially in hot climates (Tapsell, Hemphill, et al, 2006). Angiosperms (flowering plants) were the original source of most plant medicines (Encyclopaedia Britannica, 1993).

Figure 1: Picture of *Anthocleista grandiflora.*

Figure 2: Flowers of *Anthocleista grandiflora.*

MATERIALS AND METHODS

3.2.1 COLLECTION OF SAMPLES

Fresh leaves of Anthocleista grandiflora was collected from a tree along Nekede-Ihiagwa Road in Owerri.

Preparation Of The Aqueous Extract Of Anthocleista Grandiflora

The fresh leaves were air dried at room temperature for two (2) weeks. The dried leaves were milled into fine powder using a mechanical blender. Three hundred grams of the fine powdered plant leaf was suspended in 300ml of distilled water and the suspension was boiled for 30 minutes. After 24 hours, the suspension was filtered and concentrated to dryness in a rotary evaporator at 45-50$^{0C.}$

Experimental Animal Care And Handling

The animal care and handling was done according to the United States National Institute of Health Principles of Laboratory Animals Care (NIH, 1978). Twenty (20) albino rats of same age, sex, bodyweight was purchased. The animals were maintained under the controlled conditions of temperature, humidity, and light. The animals were housed in standard conditions in large metal cages. The animals were weighed and feed daily.

3.2.4 Administration Of Extract To Experimental Animals

The albino rats were allowed to acclimatize for two (2) weeks prior to administration of the extract. The extract was administered for fourteen (14) consecutive days and distilled water was administered for the control. Twenty albino rats was used in the experiment. The rats

were divided into five (5) groups; A-E of four (4) rats per group. The rats in group A were administered with distilled water to serve as control. The rats in group B – E were administered with the aqueous leaf extract for fourteen (14) consecutive days.

3.2.5 Collection Of Samples

Twenty four (24) hours after the last treatment, the animals were sacrificed after exposing them to chloroform vapor. The blood samples were collected by cardiac puncture and transferred into different sample bottles for evaluation of serum total protein by the Biuret method (Tietz 1983) while the kidneys of the rats were carefully excised and fixed in 4% formaldehyde for histopathological studies according to the method described by Akperie (2004).

RESULTS

TABLE 1: Effect of aqueous leaf extract of *Anthocleista grandiflora* on Serum Total Protein levels of Albino rats.

Group	Treatment Dose (mg/kg).	Total Protein (g/dl).
1 (control)	0.00	10.00 ± 0.93^{c}
2	150	9.83 ± 0.87^{c}
3	300	9.83 ± 0.54^{c}
4	450	10.50 ± 0.62^{c}
5	600	10.50 ± 0.46^{c}

Data represent Mean ± SD (n=4)

C: values with same superscript do not differ significantly.

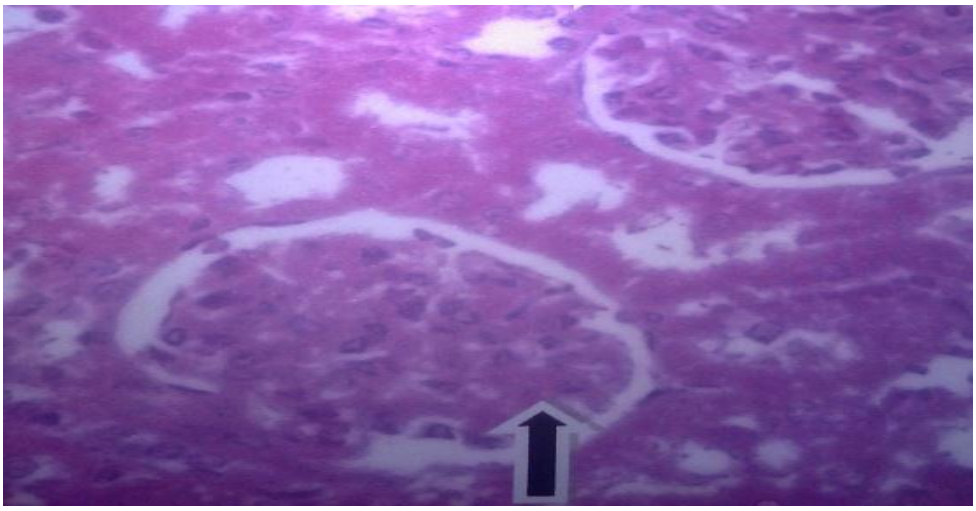

Plate 1: A Photomicrograph of section of kidney structure of rats in group A (control), treated with distilled water showi ng normal morphology of glomerular and capsular architecture with no histopathological lesion seen. (H & E x400).

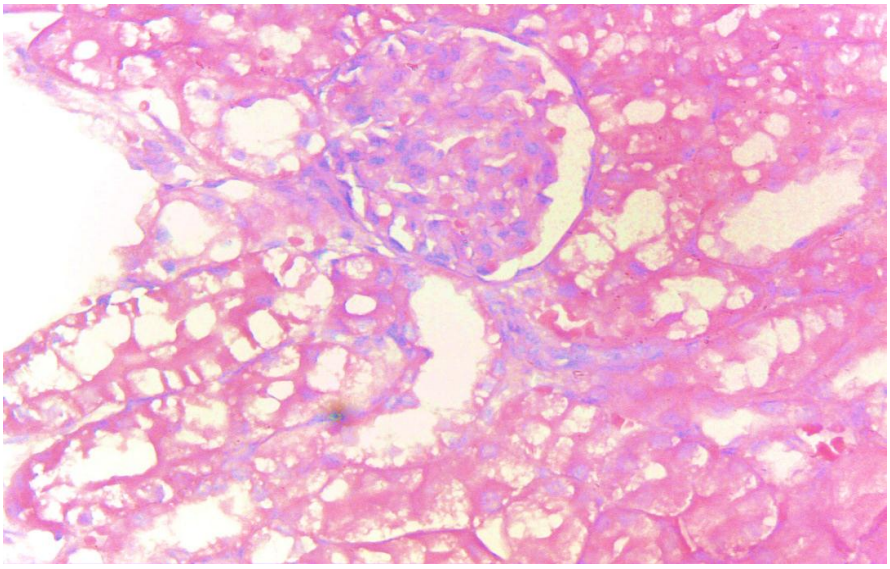

Plate 2: Photomicrograph of section of the kidney of rats in group B, treated with aqueous leaf extract of *Anthocleista grandiflora* at a dose of 150 mg/kg b.w, showing moderate inflammatory change in kidney architecture. No lesion was observed. (H & E x400).

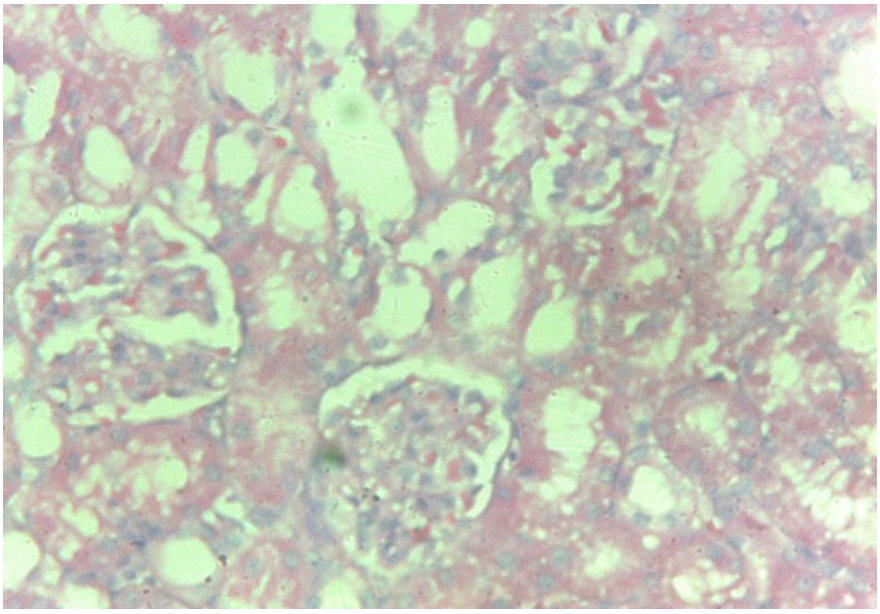

Plate 3: Photomicrograph of section of the kidney of the rats in group C treated with *Anthocleista grandiflora* extract at a dose of 300 mg/kg b.w, showing moderate inflammatory change in kidney architecture. No lesion was observed. (H & E x400).

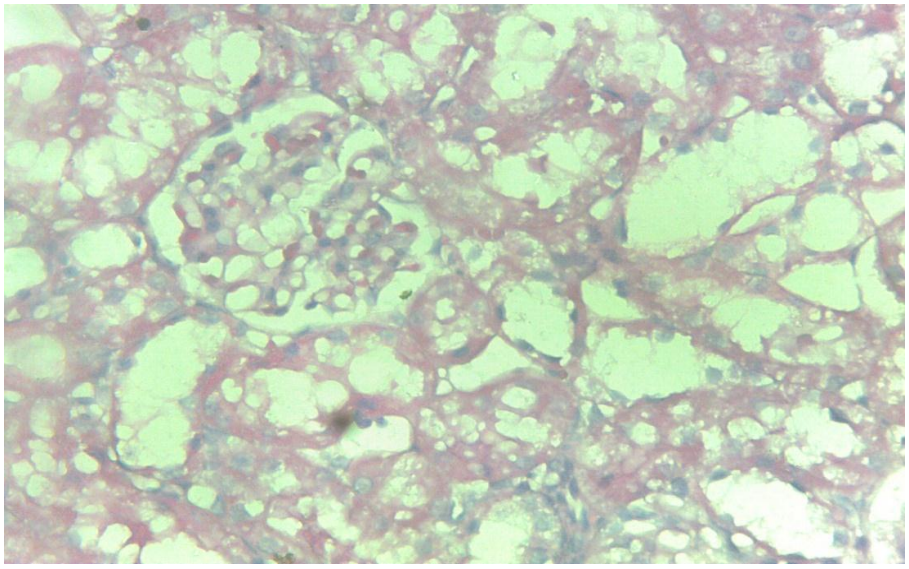

Plate 4: Photomicrograph of section of kidney structure of rats in group D treated with 450 mg/kg b.w dose of the *Anthocleista grandiflora* extract, showing moderate inflammatory change in cytoarchitecture. No lesion was observed. (H & E x400).

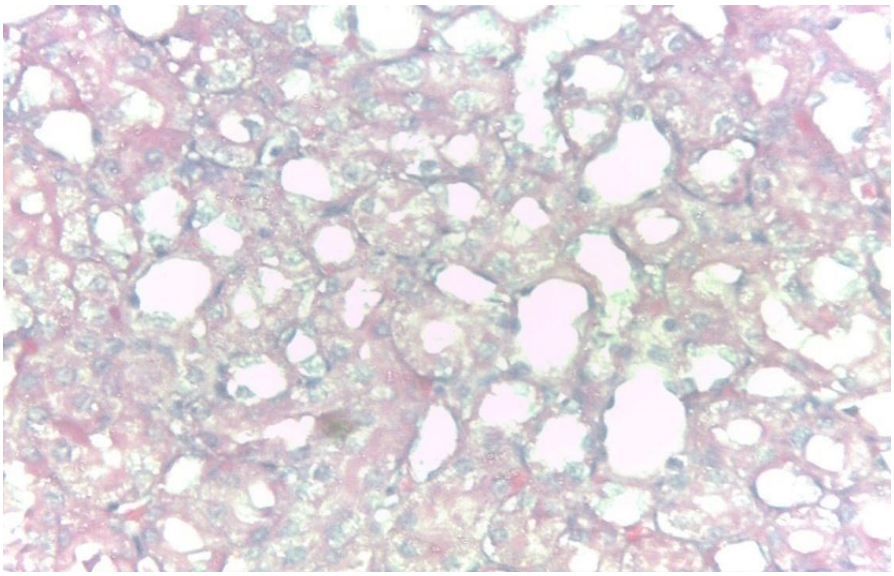

Plate 5: Photomicrograph of section of kidney structure of rats in group E, treated with 600 mg/kg b.w dose of *Anthocleista grandiflora* extract showing moderate inflammatory response. No lesion observed. (H & E x400).

5.1 DISCUSSION

Over the years, man has continuously made use of medicinal plants in quest for complmentary or alternative medication. This has been greatly encouraged due to the fact that medicinal plant has been relatively cheaper compared to orthodox medicines. As has been

pointed out, *Anthocleista grandiflora* is one of such medicinal plants that have gained wide acceptance in the treatment of various ailment in Nigeria folk medicines.

In this study, a conscious effort has been made to evaluate the effect of the medicinal plant on Serum Total Protein and kidney histology of Albino rats, a study that can be extrapolated to its effect on man.

For the animals in the first group treated with distilled water only, Serum Total Protein value was observed to be 10.00± 0.95g/dl.

When the aqueous extract was administered to the group 2 animals at a dose of 15 mg/kg b.w, Serum Total Protein value was observed to be 9.83± 0.87g/dl.

When the extract dose level was raised to 300 mg/kg b.w, Serum Total Protein level was 9.83± 0.54g/dl.

On further increment of extract dose level to 450 mg/kg b.w, Serum Total Protein level was 10.50± 0.62g/dl.

At the maximum dose level of 600 mg/kg b.w, Serum Total Protein level was 10.50± 0.46g/dl.

These very slight (negligible) progressive changes in Serum Total Protein level indicates that the dose effect of the extract on serum total protein is insignificant. Histological studies are carried out with the purpose of having a closer look at the individual cells that constitute the tissues of various organs in the living systems. Histological study of the effect of this extract on the kidneys of albino rats was done with the view of having a detailed look at the cells that have received the impact of the medicinal plant.

From the results obtained, the photomicrograph of the kidney of rats treated with distilled water (control), showed image of kidney with normal morphology of glomerular and capsular architecture. This features are all in line with that of a normal kidney (plate 1).

When the aqueous leaf extract of *Anthocleista grandiflora* was administered to the animals at the 150 mg/kg b.w dose, the kidney features showed moderate inflammatory change in cytoarchitecture. No lesion was however observed (plate 2). This implies that at this dose level the extract was well tolerated.

On the administration of the medicinal extract at 300 mg/kg b.w dose, result showed photomicrograph of kidney with moderate inflammatory change in cytoarchitecture. No lesion was also observed (plate 3). This implies that the extract was well tolerated by the animals at this dose level.

In the fourth group of animals, the aqueous extract was administered at 450 mg/kg b.w dose level. Histological result also showed moderate inflammatory response (plate 4). By implication, the extract was also tolerated by the animals at this dose level.

When the maximum dose level of 600 mg/kg b.w was tested, histological features of the animal's kidney equally showed moderate inflammatory change with no visible lesion observed (plate 5).

In addition to achieving the desired therapeutic result, the goal of using medicinal plant should also include insuring safe use of phyto-products. Thus an extract to be used to take care of any ailment should also be certified safe to all the internal organ and system of the subject.The result of this studies tends to suggest that leaves of *Anthocleista grandiflora* conform to these general goals as was observed at all the dose levels tested. No lesion or necrotic change was observed in all the animals under study.The kidney is a major mammalian organ responsible for the elimination of wastes. As such no animal can be completely healthy when the integrity of any of the kidney cells is compromised. A number of medicinal plant have equally been reported to possess the potency of reversing damages inflicted on internal mammalian organs, for instance Onwusonye et al (2014) also reported that diherbal mixture of *Alstonia boonei* and *Annona senegalensis* were able to ameliorate the histopathological damages inflicted by malaria parasite on the liver of albino rats, while Arhoghro *et al* reported the effect of *Ocimum gratissimum* at enhancing the recovery of albino rats from cisplatin induced kidney damage (Arhoghro *et al.* 2012).

5.2 CONCLUSION

Results of this work has shown that aqueous extract of *Anthocleista grandiflora* brought about mild changes in Serum Total Protein levels of Albino rats up to the maximum dose tested. From the histological investigations, when the extract was used up to the maximum dose level, no outstanding lesion or cellular necrosis was observed in the kidneys of the animals. This implies that the extract was well tolerated in mice up to the maximum dose

level understudy. It could be thus concluded that *Anthocleista grandiflora* is a safe tonic for use in Nigeria folk medicine for the management of the earlier mentioned disease conditions.

5.3 RECOMMENDATION

From the results obtained in this study and the subsequent conclusions drawn, it is hereby recommended that only moderate doses of the aqueous extract should be used to ensure complete safety to the internal organs of the mammalian systems.The active principles responsible for the therapeutic effects of the plant should be isolated and characterized while further studies should also be conducted to elucidate the possible mechanism of action of the phyto product.

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