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Possible effect of *Sphenocentrum jollyanum*, *Baphia nitida* and seeds of *Pinus koraiensis* and sildenafil on sodium, potassium, urea and creatinine levels of albino rats

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Abstract
This study was carried out to evaluate the possible effect of *Sphenocentrum jollyanum*, *Baphia nitida* and seeds of *Pinus koraiensis* and Sildenafil on sodium, potassium, urea and creatinine levels of albino rats. A total of 25 adult rats (220-280g) were bought and randomly divided into 5 groups of 5 rats per group, using water as the control group while four of the 5 groups were administered (oral) with ethanolic extract of Sildenafil, seeds of *Pinus koraiensis*, *Sphenocentrum jollyanum* and *Baphia nitida*. At the end of the experimental period (30days) the blood samples were collected for kidney function tests. The study revealed that the mean urea levels in the test groups administered Sildenafil and the various plant extracts were lower ($p>0.05$) compared to the control. In the mean levels of creatinine (30seconds and 150 seconds respectively) the study revealed that there was no significant difference ($p>0.05$) in the test groups compared to that of the control. However, only sildenafil group was lower than the control in the 30 seconds creatinine level and Creatinine levels were significantly higher ($p<0.05$) in the *Spenocentrum jollyanum* group compared to the control and *Pinus koraiensis group* in the 150 seconds creatinine level. Also, the mean sodium and potassium levels in the test groups showed no significant difference ($p>0.05$) as compared to the control. However sildenafil administered was highest in both sodium and potassium levels. From the experiment, it was observed that the ethanolic extract of Sildenafil, seeds of *Pinus koraiensis*, *Sphenocentrum jollyanum* and *Baphia nitida*, did not show any adverse effect on the kidney function parameters studied over a period of 30days.

Keywords: *Sphenocentrum jollyanum*, *Baphia nitida*, *Pinus koraiensis*, electrolytes, urea, creatinine

Introduction
The term medicinal plants include various types of plants used in herbalism and some of these plants have medicinal activities. These medicinal plants are considered as rich resources of ingredients which can be used in drug development and synthesis. Also, these plants play a critical role in the development of human cultures around the whole world.
Medicinal plants have been identified and used throughout human history. Plants make many chemical compounds that are for biological functions, including defence against insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total [1]. Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work.

***Sphenocentrum jollyanum* (Menispermaceae)**

Fig 1: *Sphenocentrum jollyanum* plant [2]

This is a perennial plant that grows naturally along the west coast sub region of Africa with expanse from Cameroon across Nigeria to Sierra Leone. *Sphenocentrum jollyanum* is an evergreen shrub growing around 1.5 metres tall and has been in traditional medicine practice for centuries with its leaves, roots and latex all in use [3].

***Baphia nitida* (camwood, also barwood)**

Fig 2: *Baphia nitida plant* [4]

Also known as African sandalwood, is a shrubby, leguminous, hard-wooded tree from central West Africa. This wood is of a very fine colour, and is used in woodturning for making knife handles and similar articles. The tree's bark and heartwood are commonly used to make a brilliant but non-permanent red dye, which is soluble in alkali [5].

***Pinus koraiensis* (Korean pine)**

Fig 3: *Pinus koraiensis Seed* [6].

This is a member of the white pine group. It grows to 30-50' tall in cultivation, but may reach 100' or more in its native habitat. When young, this tree typically grows in a narrow pyramidal form with ascending branching. With age, it relaxes into a loose pyramidal shape with a rounded crown and branching that is almost horizontal [7].

It has therefore become necessary to investigate the effect of ethanol root extract of *Sphenocentrum jollyanum Baphianitida, Pinus koraiensi s*and sildenafil on kidney parameters such as creatinine and urea parameters.

Sildenafil

Fig 4: Sildenafil drug [8]

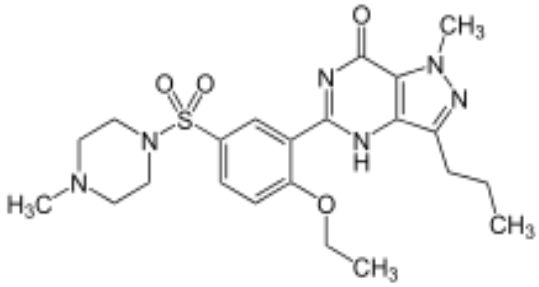


Fig 5: Chemical structure of Sildenafil

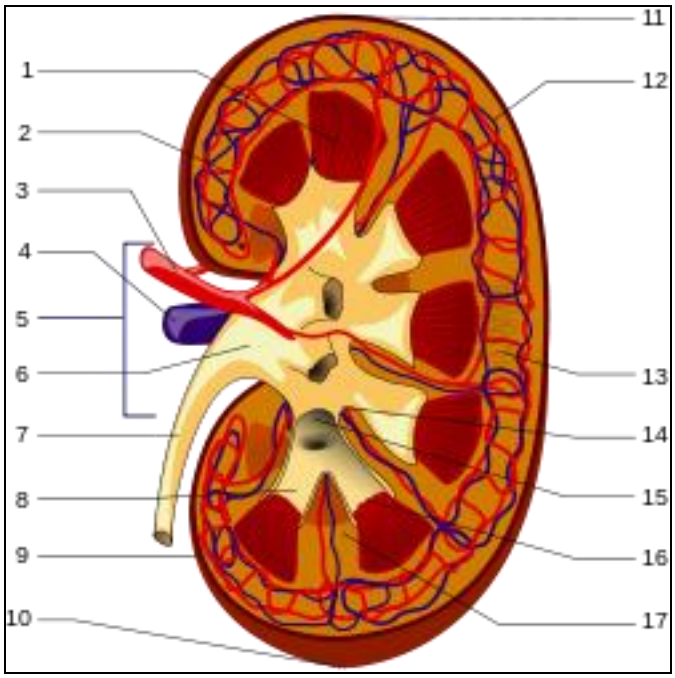
Description of sildenafil

Sildanefil with the common trade name as Viagra is a medication used to treat erectile dysfunction and pulmonary arterial hypertension. Its effectiveness for treating sexual dysfunction in women has not been demonstrated. Common side effects include headaches and heartburn, as well as flushed skin. Caution is advised in those who have cardiovascular disease. Rare but serious side effects include prolonged erections, which can lead to damage to the penis, and sudden-onset hearing loss. Sildenafil should not be taken by people who take nitrates such as nitroglycerin, as this may result in a severe and potentially fatal drop in blood pressure.

The Kidney

The kidneys are bean-shaped organs that serve several essential regulatory roles in vertebrates. Their main function is to regulate the balance of electrolytes in the blood, along with maintaining pH homeostasis. They also remove excess organic molecules from the blood, and it is by this action that their best known function is performed by the removal of waste products of metabolism.

Structure of the kidney



1. Renal pyramid, 2. Interlobular artery, 3. Renal artery, 4. Renal vein, 5. Renal hilum, 6. Renal pelvis, 7. Ureter, 8. Minor calyx, 9. Renal capsule, 10. Inferior renal capsule, 11. Superior renal capsule, 12. Interlobular vein, 13. Nephron, 14. Minor calyx, 15. Major calyx, 16. Renal papilla, 17. Renal column

Fig 6: diagram showing the kidney

Materials and Methods

Plant Materials

The dried roots of *Sphenocentrum jollyanum* and *Baphia nitida* were purchased from Aba, Abia state. The seeds of *Pinus koraiensis* and 100mg tablets of sildenafil were purchased from Port Harcourt, Rivers state.

Materials /Equipment used

Spectrophotometer, Dissecting set, Centrifuge, Water bath, Electrolyte analyser, Electronic weighing balance, Surgical gloves, Sterile syringe, Filter paper, Cages, Test tubes, Spatula, Micropipette

Chemical Reagents

Urea kit (Randox), Creatinine kit (Randox), Chloroform (Sigma-Aldrich), Ethanol, Distilled water, Normal saline

Methods

Animal Sample

The animal types used for this research work were healthy red eyed albino rats with an average weight of 161g. They were bought from the animal house of the Federal University of Technology, Owerri. A total of 25 rats were used.

Housing

Experimental animals were housed in cages in the animal house of the Department of Biochemistry, Madonna University, Elele campus. The house was properly located with good sanitary conditions as to reduce contamination. All experimental animals were housed in well ventilated rooms and kept on a 12h light/12h dark cycle. The cages were wire-screened with facilities for faecal collection, water and feeding troughs. Before the experimental study, the rats acclimatized for a period of one week to have proper adaptation to their "new" environment.

Feeding

A brand of commercial vital feed grower's pellet produced by grand cereals feed mills co. Ltd (pelletized) at km 17, zawan roundabout, Jos, Plateau state, was fed to the rats.

Preparation of extract

Sphenocentrum jollyanum and *Baphia nitida were* pulverized with a hammer-mill to obtain a coarse powder. 1000 g of the powder of *Sphenocentrum jollyanum* and 500 g of the powder of *Baphia nitida* was extracted with 99/100% ethanol for 72 h respectively. Using a rotary evaporator, the ethanolic filtrate was concentrated to obtain a crude extract which was then air dried at room temperature (28°C) for 36 hours.

The seeds of *Pinus koraiensis* and tablets of sildenafil were grinded with a mortar and pestle.

Administration of extract

The extract prepared above was provided to the rats for a period of 30 days.

Experimental Design

Male albino rats were used for this research. Before the commencement of the experiment, the rats were acclimatized for a week. The rats were weighed and placed in 5groups which contained 5 rats each and were treated accordingly. Group 1-5 shows the different ways in which treatments were given;

Group 1: The Control was only fed with feed and water.

Group 2: They were administered 200mg/kg body weight of the extract of *Sphenocentrum jollyanum.*

Group 3: They were administered 200mg/kg body weight of the extract of *Baphia nitida.*

Group 4: They were administered 80mg/kg body weight of the grinded tablets of sildenafil.

Group 5: They were administered 600mg/kg body weight of the grinded seeds of *Pinus koraiensi.*

Collection of blood samples

The rats were anaesthetized using chloroform vapour. They were dissected and the blood was collected through ocular puncture. The blood samples were collected and centrifuged which was used for different assay methods.

Assay Method

The assay methods used in this study was sodium, potassium, Urea and Creatinine assay. These assays were used to measure the kidney function test.

Assay Procedures

Urea Assay

This assay is used for the *in-vitro* determination of urea in serum, plasma and urine.

Method: Urease- Berthlot's method

Reagent Composition
1. R1a: Urease
2. R1b: Sodium nitropruside
3. R2: Phenol concentrate
4. R3: Hypochlorite concentrate
5. CAL standard

Principle
Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction. Ammonia reacts with color reagents to form indophenols.

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{Hypochlorite} + \text{Phenol} \longrightarrow \text{Indophenols (Blue compound)}$$

Sample Material: Serum, heparised or EDTA plasma, or dilute urine can be used. In this study, the serum was used.

Procedure

Table 1: Urea test procedure

Pipette into the cuvette:			
	Blank	Standard	Test
Sample	-	-	10µl
Standard (CAL)	-	10µl	-
Distilled H_2O	10µl	-	-
Reagent 1	100µl	100µl	100µl
Mix and incubate at 37^0c for 10 minutes			
Reagent 2	2.50ml	2.50ml	2.50ml
Reagent 3	2.50ml	2.50ml	2.50ml

Mix and incubate at 37^0c for 15 minutes. Zero with the blank and read the absorbance of the samples at 546nm.

Calculations

$$\text{Urea concentration} = \frac{\text{Abs sample} \times \text{Standard conc. (mmol/l)}}{\text{Abs standard}}$$

Creatinine Assay
Colorimetric Method
Creatinine forms in alkaline solution, an orange-red complex with picric acid. The absorbance of this complex is proportional to the creatinine concentration in the sample.

Reagent Composition
1. Picric acid
2. Sodium hydroxide
3. Creatinine standard solution

Principle
Creatinine in the serum in alkaline solution reacts with picric acid to form a colored complex. The amount of complex formed is directly proportional to the creatinine concentration.

$$\text{Creatinine} + \text{Picric acid} \longrightarrow \text{Creatinine-picrate complex}$$

Stability of working reagent preparation
Mix equal volumes of solution picric acid and sodium hydroxide ie reagent 1 and reagent 2.
The standard is ready to use.

Sample Material
Serum, heparized plasma or urine (avoid hemolysis).

Procedure

Table 2: Creatinine Test Procedure

Pipette into cuvette		
	Standard	Sample
Working reagent	1.0 ml	1.0 ml
Standard solution	0.1 ml	-
Sample	-	0.1 ml

Mix and after 30 seconds, read the absorbance, A1 of the standard and sample. Exactly 2 minutes later, read the absorbance, A2 of standard and sample at 492nm.

Manual Calculation
$A2 - A1 = \Delta A_{\text{standard}}$ or ΔA_{sample}.

Concentration of creatinine in serum or plasma

$$\frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times \text{Standard conc. (µmol/l)} = \text{µmol/l}$$

Concentration of creatinine in urine

$$\frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times \text{Standard conc. (µmol/l)} = \text{µmol/l}$$

Sodium and Potassium assay method
Electrolyte analyser which is the ISE (Ion Selective Electrode) was used.

Principle
The measured voltage is proportional to the Logarithm of the concentration, and the sensitivity of the electrode is expressed as the electrode Slope - in millivolts per decade of concentration. Thus the electrodes can be calibrated by measuring the voltage in solutions containing, for example, 10ppm and 100ppm of the target ion, and the Slope will be the slope of the (straight) calibration line drawn on a graph of mV versus Log concentration.

Procedures
Blood was collected into plain sample bottle and assay for sodium and Potassium using ISE (Ion selective Electrode).

Results
Effect of sildenafil and plant extract administration on urea levels in albino rats
As shown in Figure 3.1 below, the mean urea levels were higher in the control rats (6.38 ± 0.91 mmol/L) compared to the group administered Sildenafil (4.49 ± 0.41 mmol/L), *Baphia nitida* (Abosi) (4.93 ± 0.37 mmol/L), *Spenocentrum jollyanum* (Ikeagwu) (4.18 ± 0.33 mmol/L) and *Pinus koraiensis* (4.70 ± 0.37 mmol/L). However, the mean

difference were not statistically significant (p>0.05).

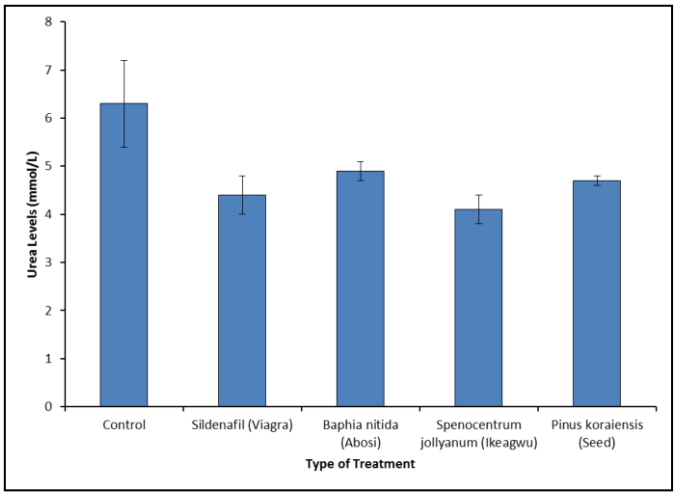


Fig 7: Effect of sildenafil and plant extract administration on urea levels in albino rats

Effect of sildenafil and plant extract administration on creatinine (30 sec) levels in albino rats

Figure 3.2 below shows that, there was no significant difference (p>0.05) in the mean levels of creatinine between the control rats (2.82 ± 0.02 mg/dL) compared to the group administered Sildenafil (2.52 ± 0.37 mg/dL), *Baphia nitida* (Abosi) (2.85 ± 0.37 mg/dL) *Spenocentrum jollyanum* (Ikeagwu) (2.95 ± 0.37 mg/dL) and *Pinus koraiensis* (2.62 ± 0.38 mg/dL).

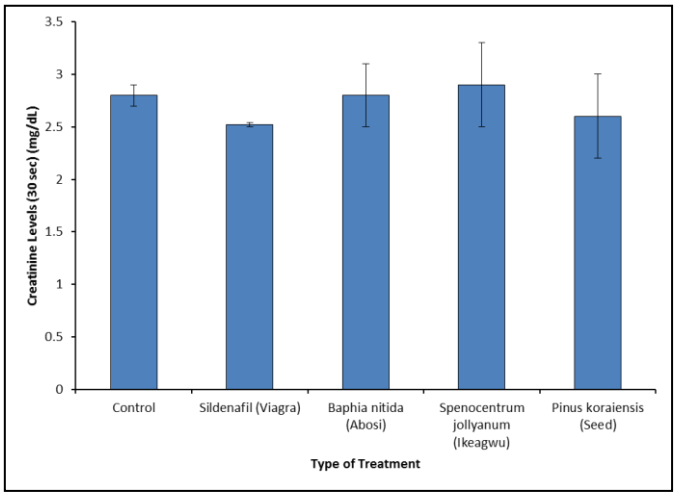


Fig 8: Effect of sildenafil and plant extract administration on creatinine levels in albino rats

Effect of sildenafil and plant extract administration on creatinine (150 sec) levels in albino rats

As shown in Figure 3.2 below that, there was no significant difference (p>0.05) in the mean levels of creatinine between the control rats (1.76 ± 0.16 mg/dL) compared to the group administered Sildenafil (1.46 ± 0.18 mg/dL), and *Baphia nitida* (Abosi) (1.49 ± 0.11 mg/dL). Creatinine levels were significantly higher (p<0.05) in the *Spenocentrum jollyanum* (Ikeagwu) treated rats (2.03 ± 0.35 mg/dL) compared to the *Pinus koraiensis* treated rats (1.19 ± 0.08 mg/dL).

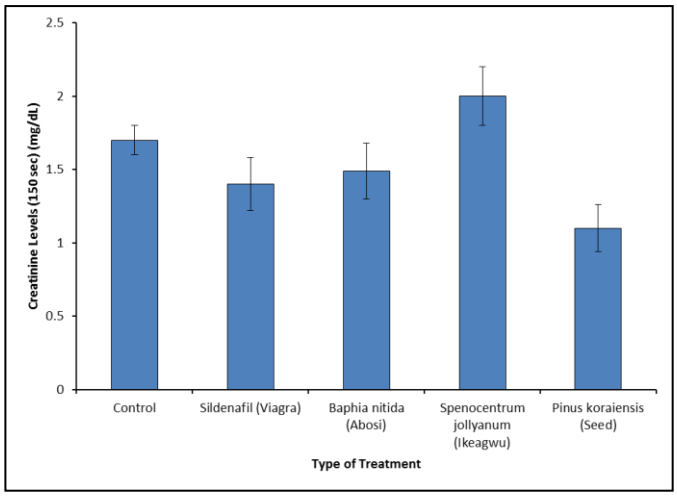


Fig 9: Effect of sildenafil and plant extract administration on creatinine levels in albino rats

Effect of sildenafil and aphrodisiac plant extract administration on sodium levels in albino rats

As shown in Figure 3.1 below, there was no significant difference (p>0.05) in the sodium levels of the rats administered different treatments. Sodium levels in the group administered Sildenafil was higher (144.49 ± 7.41 mmol/L), compared to the control rats (144.00 ± 4.91 mmol/L), and rats administered the aphrodisiac plants *Baphia nitida* (Abosi) (143.93 ± 2.37 mmol/L), *Spenocentrum jollyanum* (Ikeagwu) (142.18 ± 5.33 mmol/L) and *Pinus koraiensis* (143.70 ± 5.37 mmol/L), however, the mean difference were not statistically significant (p>0.05).

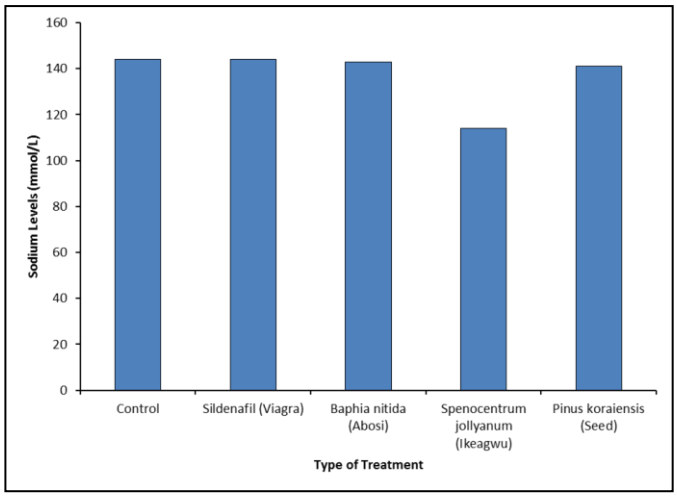


Fig 10: Effect of sildenafil and aphrodisiac plant extract administration on sodium levels in albino rats

Effect of sildenafil and aphrodisiac plant extract administration on potassium levels in albino rats

Figure 3.2 below shows that, there was no significant difference (p>0.05) in the potassium levels of the rats administered different treatments. Potassium levels in the group administered Sildenafil was higher (6.02 ± 0.41 mmol/L), compared to the control rats (4.64 ± 0.81 mmol/L), *Baphia nitida* (Abosi) treated (5.66 ± 0.07 mmol/L),

Spenocentrum jollyanum (Ikeagwu) treated (4.96 ± 0.93 mmol/L) and *Pinus koraiensis* treated (6.00 ± 0.17 mmol/L), however, the mean difference were not statistically significant ($p>0.05$).

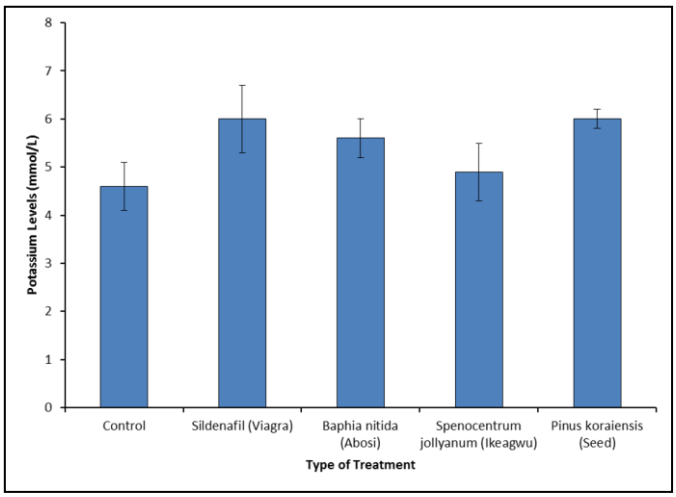


Fig 11: Effect of sildenafil and aphrodisiac plant extract administration on potassium levels in albino rats

Discussion

Sphenocentrum jollyanum, *Baphia nitida*, seeds of *Pinus koraiensis* and Sildenafil are aphrodisiac plants found to have significance in traditional medicine [9]. The ethanolic extract of *Sphenocentrum jollyanum and Baphia nitida* are strongly believed to have antioxidant and anti-inflammatory property [10, 11, 12, 13], likewise the seeds of *Pinus koraiensis* and sildenafil drug also [14, 15]. This study was carried out to evaluate the possible effect of *Sphenocentrum jollyanum*, *Baphia nitida*, seeds of *Pinus koraiensis* and Sildenafil on electrolyte, urea and creatinine levels of albino rats.

From the results of the urea and creatinine tests done, it was revealed that there was no significant difference generally in the levels of urea and creatinine in the test groups as compared to the control. This was in tandem with the work of [16] who reported similar results on his experiment. These plant root extracts have been shown to have medicinal activities as shown above. A possible reason for the insignificant difference in the urea and creatinine levels between the treated groups and the control is the presence of phytochemicals. Studies like [16, 12, 14, 15] all help buttress the point.

Similarly there was generally no significant difference in the levels of sodium and potassium in the test groups as compared to the control. It was just observed that sildenafil was higher than the control in both test groups. This was also in agreement with the work of [16]. The presence of phytochemicals were also responsible for the seeming insignificant difference between the test groups and the control groups.

Conclusion

It can be suggested that the administration of sildenafil, *Pinus koraiensis* and ethanolic extracts of *Sphenocentrum jollyanum* and *Baphia nitida* had no significant effect on the electrolytes, urea and creatinine levels in albino rats, however, caution must always be exercised when ingesting any of these plants.

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