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DIFFERENTIAL ROLES OF TANNIN – RICH EXTRACT OF CHASMANTHERA DEPENDENS IN MODULATING PIROXICAM INDUCED ELECTROLYTE IMBALANCE IN RATS

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ABSTRACT
The clinical applications of piroxicam are often limited due to its associated renal toxicity. Electrolyte imbalance resulting from piroxicam regimen is often considered a mechanism of its toxic responses in the kidney. *Chasmanthera dependens* is a plant that is well reputed for its remarkable pharmacological properties. However, its effect on drug induced electrolyte imbalance is unknown. Thirty two (32) adult male Wistar rats (weighing 110-150 g) were grouped randomly into four (n=8) as follows; Group 1 (0.5 ml of normal saline {0.9 percent v/v}), group II (20 mg/kg piroxicam), group III (20 mg/kg piroxicam + 200 mg/kg TRECDS), group IV (20 mg/kg piroxicam + 400 mg/kg TRECDS). Piroxicam and TRECDS were administered using oral cannula. Animals were sacrificed after 10 days of treatment using cervical dislocation. The kidney tissues were excised, and homogenized in phosphate buffer. Malondialdehyde (MDA) level, glutathione (GSH) activity, total protein, albumin, Calcium, sodium, potassium, and bicarbonate were analysed using standard methods. Data were analysed using statistical package for social science (version 20.0). Significance was set at $p< 0.05$. Piroxicam administration significantly ($p<0.05$) increased the lipid peroxidation, altered calcium, sodium, potassium, phosphorus and bicarbonate concentrations in the kidney. However, treatment with TRECDS significantly ($p<0.05$) ameliorated lipid peroxidation, curtailed electrolyte imbalance dose dependently, except for sodium and bicarbonate levels. The Tannin – rich extract of *C. dependens* was nephroprotective through remarkable antioxidative and reversal of electrolyte imbalance. Further study is needed to elucidate the actual compounds eliciting these properties.

KEYWORDS: Piroxicam, Kidney, electrolyte, Chasmanthera dependens.

INTRODUCTION
Considering that the kidney plays vital roles in maintaining homeostasis, its optimal functioning is highly essential for human survival. Important metabolic activities such as acid - base regulation, hormone synthesis, and toxic waste excretion usually take place within the renal compartments. Consequently, an alteration in any of these physiological activities may signify a compromised renal integrity while also predisposing to ill health. It is estimated that the plague of chronic kidney diseases (CKD) is affecting about 13.4% of the world population. As a significant part of this heart-rending prevalence, over 4 million patients are requiring renal replacement therapy due to end stage kidney disease (ESKD).[1] Among the non-communicable diseases, chronic kidney disease is one of the leading causes of morbidity and mortality worldwide. While chronic diseases like diabetes mellitus and hypertension can to potentially degenerate to CKD, the nephrotoxicity induced by certain orthodox medication can also be a contributing factor. Piroxicam is a non - steroidal anti-inflammatory drug (NSAID) which is commonly prescribed for relieving pathological symptoms of inflammation and nociception. Regrettably, continuous use of piroxicam has been widely reported to elicit life threatening toxicity response to vital organs such as the brain,[2] liver[3] gastrointestinal tract and the kidney.[3] The adverse drug reactions associated with piroxicam is usually mediated through the generation of highly reactive oxygen radical and corresponding upregulation of apoptosis signalling.[5,6] Besides, significant alteration in electrolyte concentration during piroxicam regimen is a classic symptom of its renotoxic adverse effects.[7] These vacuum in contemporary medical practice makes the search for pharmacological agents with renoprotective potency inevitable. Unfortunately, there is no available conventional medication that is effective enough to stem the tide of

piroxicam induced nephrotoxicity. Over the years, there has been continuous exploration of plant bioactive mechanisms which has the potency to modulate biochemical pathways of drug induced toxicity. In attempt to discover safer drug candidates, recent studies have demonstrated the efficacy of various plant bioactive agents in curtailing the debilitating side effects of synthetic medications.[8]

Chasmanthera dependens is a tropical plant belonging to Menispermaceae plant family. It is a climbing shrub producing stems of at least 5 metres long. Commonly referred to as ato by the Yoruba ethnic group of Nigeria, *C. dependens* is well reputed in folklore medicine for its efficacy in relieving pathological symptoms. Notably, its antiulcerogenic,[9] hepatoprotective,[10] anti-inflammatory, analgesic,[11] renoprotective,[12] antioxidative and antimalarial,[13] activities have been demonstrated. Its remarkable pharmacological properties have been attributed to its constituent phytochemicals.[14] Although previous investigations have demonstrated the potential pharmacological properties of *C. dependens* in various experimental models, its roles in attenuating drug induced electrolyte imbalance are scarcely explored. This study was therefore aimed at elucidating the effects of tannin-rich extract of *Chasmanthera dependens* on biochemical indices associated with Piroxicam-induced nephrotoxicity.

MATERIALS AND METHOD

Reagents

Acetone, methanol, diethyl ether, trichloroacetic acid (TCA), hydrochloric acid (HCl), Tris-base, Dipotassium hydrogen phosphate (K_2HPO_4), Potassium diphosphate (KH_2PO_4), potassium chloride (KCl), , disodium hydrogen phosphate (Na_2HPO_4), sodium diphosphate (NaH_2PO_4), reduced glutathione (GSH), Ellman's reagent , thiobarbituric acid (TBA) were procured from Sigma Chemical Co, (St Louis, MO, USA).

Drug

Piroxicam was procured from HINGBO Dahongying pharmaceuticals Co Ltd, China. The piroxicam capsules were dissolved in aqueous proportion of deionized water to achieve the requisite concentrations for dosing to the rats based on their body weight.

Plant Collection and Authentication

Fresh stems of *Chasmanthera dependens* were harvested from a local garden at Oke Oba area, Iwo, Osun state Nigeria. The plant was authenticated by Mr. Esimekhuai, D.P.O, a plant taxonomist at the department of botany, University of Ibadan, Ibadan Oyo state, Nigeria. A voucher was thereafter issued with the number UIH-22478.

Preparation of tannin extract of *chasmanthera dependens*

The stems of *Chasmanthera dependens* were macerated into smaller pieces and air-dried at room temperature. The dried samples were pulverized with an electric blender (Bajaj bravo 3 jars mixer grinder/blender, India), weighed and kept in air –tight container prior to extraction. The methanol extract was prepared by immersing the coarse powdered stem barks (100 gm) in 1 L methanol for 48 hours using soxhlet apparatus (Quicket UK). The mixture was filtered using Whatman No. 1 (Whatman Ltd, Germany) paper and evaporated under reduced pressure using a rotary evaporator. The filtrates were freeze dried using lyophilizer to yield a dark brown extract. The dried extract was stored in an air tight container until further needed. About 2 litres of 70% acetone was then used to wash off the residue. The washed solution was combined to the filtrate. The filtrate was subjected to extraction using diethyl ether, and this was repeated five times, until a complete separation of diethyl ether and tannin, which is the upper layer and lower layer respectively were obtained. The tannin - rich extract of *Chasmanthera dependens* (TRECDS) was then collected using a separating funnel. The collected tannin was evaporated using a rotary evaporator (Yamato RE801C-W, America).

Animal Procurement and Care

Thirty two (32) adult male Wistar rats (weighing 110-150 g) were procured from the animal house facility at Bowen University in Iwo, Osun state. Prior to the commencement of the experiment, the rats were housed in properly ventilated plastic (medwise) cages for two weeks for acclimatization. The rats were kept under standard laboratory condition of 12 hour day/night cycle, while standard room temperature was also maintained. The rats were given unrestricted access to standard rat pellet (growers mash) and drinking water. Also, rats were treated humanely, as specified in the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, 1985).

Animal Grouping and Route of administration

The rats were randomly divided into four groups (n=8). Rats in group one received 0.5 ml (0.9 % v/v) of normal saline and served as normal control group. Rats in group two received 20 mg/kg body weight of piroxicam only once daily. Rats in groups three and four received 20 mg/kg body weight of piroxicam along with concomitant administration of 200 mg/kg and 400 mg/kg body weight TRECDS. Piroxicam and TRECDS were administered orally for ten days using gastric gavage.

Animal sacrifice

A day following the last administration (day 11), rat in all the experimental groups was sacrificed by cervical dislocation. A ventral incision was made, and the kidney of each rat was excised. The kidney was then rinsed with ice cold 0.01 M phosphate buffer (pH 7.4) and weighed. Using four times the volume of the phosphate buffer, the kidney was homogenized and then centrifuged at 3000 rpm for 30 minutes. The supernatant was collected, stored at -20^0 C and was later used for all the biochemical analyses.

Biochemical analyses

Oxidant/Antioxidant assay

The extent of lipid peroxidation in the kidney tissue was determined in the form of malondialdehyde (MDA), a product of thiobarbituric acid reactive substance (TBARS) while reduced glutathione (GSH) was analysed based on previously described assay method.[15]

Estimation of total Protein and Albumin

The total protein and Albumin concentrations were determined using Landwind LW C100 plus Auto Chemistry Analyser based on biuret and bromocresol green principles respectively.

Calcium estimation

The calcium concentration was determined using Landwind LW C100 plus Auto Chemistry Analyser based on cresolphthalein principle.

Electrolytes

The concentrations of sodium, potassium and bicarbonate in the kidney homogenate were estimated using SFRI ISE 4500 automatic analyser.

Statistical analysis

Data were analysed using statistical package for social science (SPSS) (version 20.0 for Microsoft windows 10). All data were presented as mean ± SEM, and analysed using one way analysis of variance (ANOVA). This was followed by post hoc analyses using Duncan's multiple comparison test. Significance was set at $p < 0.05$.

RESULTS

Figures 1-9 depicts the mean concentration of biochemical parameters investigated across the treatment groups as compared to controls. According to figure 1, the mean concentration of sodium was statistically significant across the treatment groups when compared to control ($p<0.05$). PIRO + T1 and PIRO + T2 groups had a significant reduction in sodium concentration similar to PIRO group. Potassium concentration was significantly decreased in the PIROX group while there was a significantly higher potassium concentration among the PIRO + T2 groups (figure 2). The effects of piroxicam and TRECDS on bicarbonate concentration are depicted in figure 3. PIROX, PIRO + T1 as well as PIRO + T2 groups had a significantly ($p <0.05$) higher level of bicarbonate relative to the control group. Figure 4 shows the mean concentration of total protein across the treatment groups as compared to control. PIROX alone treated group and PIRO T1 group showed significant increase in concentrations of total protein while there was a decrease in the concentration of total protein in group treated with TRECDS (PIRO + T2). The mean concentration of albumin across the treatment groups relative to control is presented in figure 5. The mean concentrations of Albumin in PIROX alone treated group and PIRO+T1 showed significant increase in concentrations of Albumin which was reduced in groups treated with TRECDS (PIRO + T2). Meanwhile, figure 6 depicts the mean concentration of calcium across the treatment groups as compared to control. PIROX alone treated group and PIRO + T2 showed a significant increase in concentrations of calcium. PIROX group had significant increase in the concentration of phosphate with a reduction when PIRO + T1 was administered and a further increase with PIRO + T2 ($p<0.05$) (figure 7). According to figure 8, PIROX alone treated group showed a significant decrease in concentrations of GSH while there was a reversal to normal concentration among the groups treated with TRECDS (PIRO+T1 and PIRO + T2). The effect of *C. dependens* on renal malondialdehyde level is as shown in figure 9. PIROX alone treated group showed a significant increase in MDA level while it was significantly reduced in groups treated with TRECDS (PIRO+T1 and PIRO + T2) (figure 9).

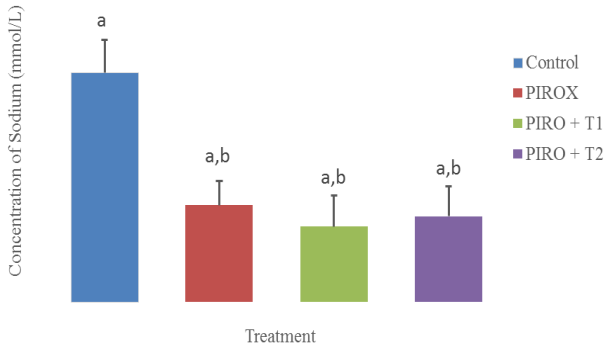

Figure 1: Effect of tannin-rich extract of *Chasmanthera dependens* stem on Sodium level in piroxicam-induced nephrotoxicity. Error bar indicates standard error of mean. Means with letters (a, b) are significant from one another ($p<0.05$).

PIRO = Piroxicam 20mg/kg

TAN1=200mg/kg of Tannin-rich extract of *Chasmanthera dependens* stems

TAN 2 = 400mg/kg tannin-rich extract of *Chasmanthera dependens* stem

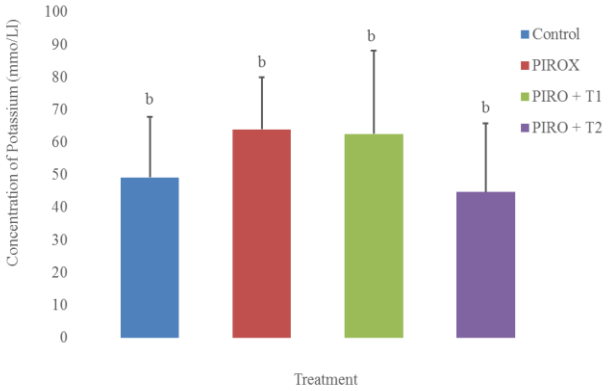

Figure 2: Effect of tannin-rich extract of *Chasmanthera dependens* stem on potassium level of kidney homogenate in piroxicam-induced nephrotoxicity in adult male Wistar rats. Values are significant when compared with control p< 0.05.
PIRO = Piroxicam 20mg/kg
TAN1=200mg/kg of Tannin-rich extract of *Chasmanthera dependens*
TAN 2 = 400mg/kg tannin-rich extract of *Chasmanthera dependens* stem

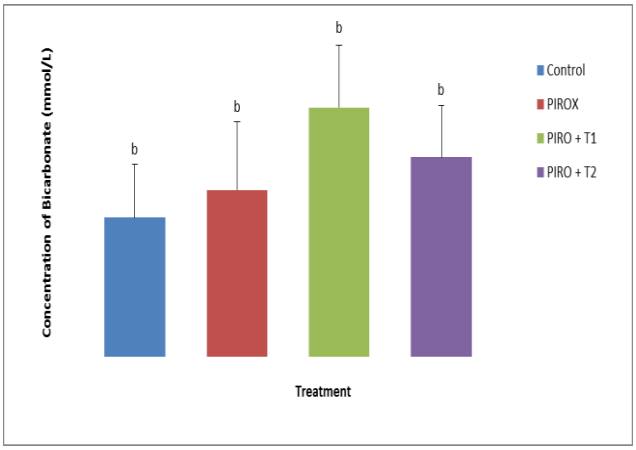

Figure 3: Mean concentration of Bicarbonate across the treatment groups as compared to control. Values are significant (^{b} p < 0.5).
PIROX = Piroxicam 20mg/kg
TAN1=200mg/kg of Tannin-rich extract of *Chasmanthera dependens* stem
TAN 2 = 400mg/kg tannin-rich extract of *Chasmanthera dependens* stem

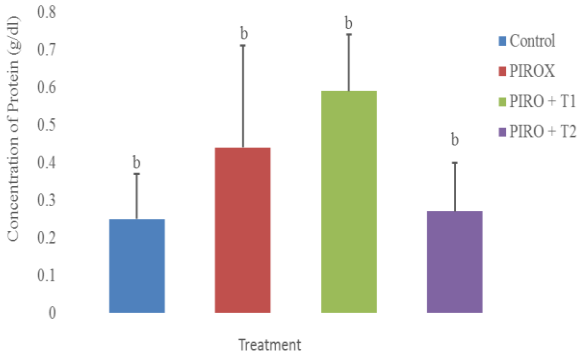

Figure 4: Effect of tannin-rich extract of *Chasmanthera dependens* stem on total protein level in piroxicam-induced nephrotoxicity. Values are significant when compared with control p< 0.05.
PIRO = Piroxicam 20mg/kg,
TAN1=200mg/kg of Tannin-rich extract of *Chasmanthera dependens* stem
TAN 2 = 400mg/kg tannin-rich extract of *Chasmanthera dependens* stem

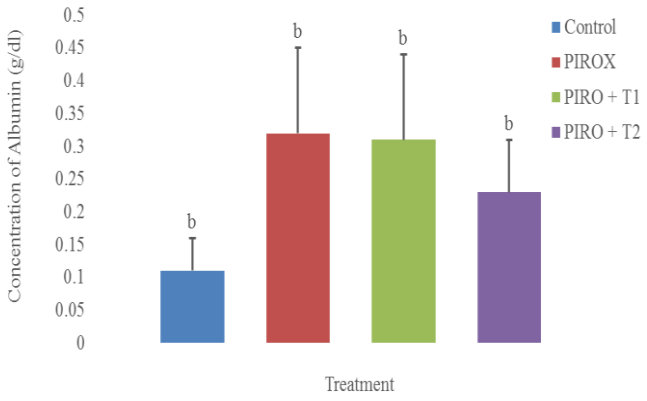

Figure 5: Effect of tannin-rich extract of *Chasmanthera dependens* stem on Albumin level of kidney homogenate in piroxicam-induced nephrotoxicity in adult male Wistar rats. Values are significant when compared with control p< 0.05.
PIRO = Piroxicam 20mg/kg,
TAN1=200mg/kg of Tannin-rich extract of *Chasmanthera dependens* stem
TAN 2 = 400mg/kg tannin-rich extract of *Chasmanthera dependens* stem

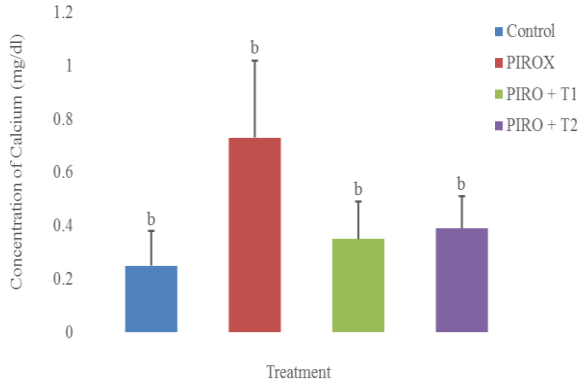

Figure 6: Effect of tannin-rich extract of *Chasmanthera dependens* stem on Calcium level of kidney homogenate in piroxicam-induced nephrotoxicity. Values are significant when compared with control (^{b}p< 0.05).
PIRO = Piroxicam 20mg/kg
TAN1=200mg/kg of Tannin-rich extract of *Chasmanthera dependens*
TAN 2 = 400mg/kg tannin-rich extract of *Chasmanthera dependens* stem

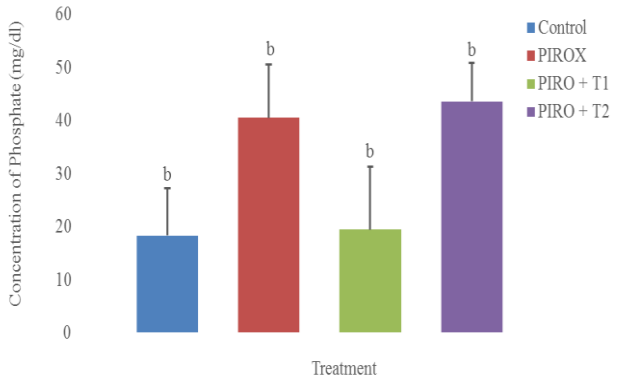

Figure 7: Concentration of Phosphate across the treatment groups as compared to control. Values are significant when compared with control (^{b}p< 0.05).
PIRO = Piroxicam 20mg/kg
TAN1=200mg/kg of Tannin-rich extract of *Chasmanthera dependens* stem
TAN 2 = 400mg/kg tannin-rich extract of *Chasmanthera dependens* stem

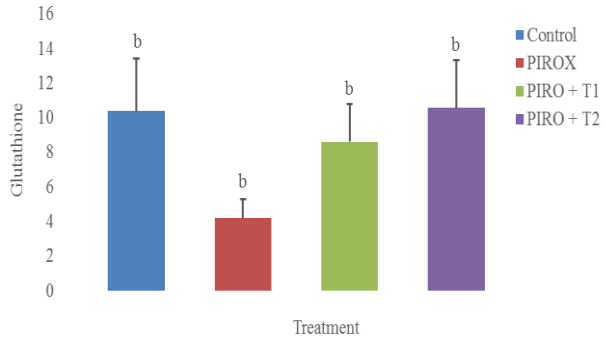

Figure 8: Effect of tannin-rich extract of *Chasmanthera dependens* stem on GSH level of kidney homogenate in piroxicam-induced nephrootoxicity in adult male Wistar rats. Values are significant when compared with control ($^{b}p< 0.05$).
PIRO = Piroxicam 20mg/kg,
TAN1=200mg/kg of Tannin-rich extract of *Chasmanthera dependens*
TAN 2 = 400mg/kg tannin-rich extract of *Chasmanthera dependens* stem

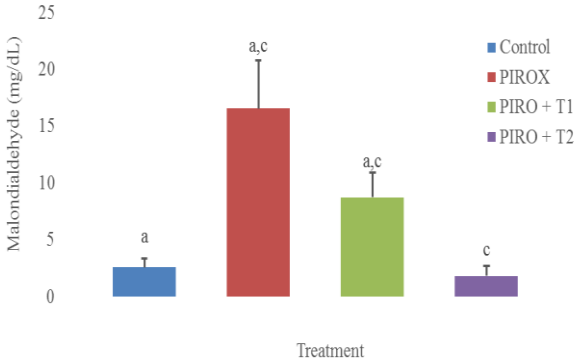

Figure 9: Effect of tannin-rich extract of *Chasmanthera dependens* stem on LPO level of kidney homogenate in piroxicam-induced nephrootoxicity in adult male Wistar rats. Values are significant when compared with control ($^{b}p< 0.05$).
PIRO = Piroxicam 20mg/kg,
TAN1=200mg/kg of Tannin-rich extract of *Chasmanthera dependens*
TAN 2 = 400mg/kg tannin-rich extract of *Chasmanthera dependens* stem

DISCUSSION

The renal compartments play remarkable roles in the effective elimination of drug metabolic by-products from the blood. However, empirical data have shown that these toxic wastes can take a deleterious toll on several important organelles in the kidney cells, thereby distorting homeostasis. As a result, the clinical application of many conventional NSAIDS often presents a drawback, owing to life threatening contraindications.[16] A matter of clinical concern is the fact that, therapeutic regimen consisting of NSAIDS has been linked with significant alterations in various biochemical indices, which are hallmarks of specific organ failure.[17] This may potentially predispose to life threatening, irreversible damage to vital organs such as the kidney.

Under normal physiological conditions, electrolytes usually play fundamental roles in maintenance of homeostasis. These include neurotransmitter signalling, acid-base balance, regulation of blood pressure, maintenance of healthy bones and teeth.[18,19] Apart from being a biochemical marker of impaired renal function, any significant alteration to the electrolyte concentration can modulate biochemical cascades of disease initiation and progression. Data obtained from the present study agrees absolutely with those of foremost investigators,[20,21] in the sense that piroxicam administration generated significant imbalance to electrolyte concentration among the untreated group. This implies a possible mechanism for piroxicam induced nephrotoxicity.

Sodium is one of the most important electrolytes in the body and a major extracellular cation. It is of important in maintenance of blood pressure via the angiotensin-renin system.[22] The present study showed that piroxicam significantly reduced sodium concentration relative to the control group. This result is consistent with that of a previous investigator.[23] In consistence with these findings, clinical application of NSAIDs such as piroxicam are known to commonly predispose to a

pathological condition known as hyponatremia.[24] This is particularly possible through the modulation of vasopressin V2 receptors, and consequent development of nephrogenic syndrome of inappropriate antidiuresis (NSIAD).[25] Unfortunately, concomitant supplementation with tannin rich extract of *C. dependens* did not restore the sodium concentration to normal level. This may likely suggest that the plant extract is lacking in essential bioactive compounds which can modulate upstream molecular pathways associated with piroxicam induced hyponatremia.

Potassium is a major intracellular cation which is vital to the healthy functioning of all body's cells, tissues and organs.[26] The present study showed that the piroxicam untreated group of animals had a significantly higher potassium levels relative to the control group. This observation is consistent with a previous investigation.[27] Interestingly, the coadministration of tannin rich extract of *C. dependens* with piroxicam significantly reduced the potassium level in a dose dependent manner. This showed that *C. dependens* may likely be a natural repository of essential compounds which can potentially mitigate drug induced hyperkalemia.

Bicarbonate is very vital for the maintenance of acid-base equilibrium in the body. Disturbance of acid-base homeostasis is one of the biochemical features of kidney damage.[28] Administration of piroxicam alone resulted in a spike in bicarbonate level. However, this adverse effect was not significantly attenuated by tannin rich extract of *C. dependens*. It is well-known that as kidney function declines, there is a progressive deterioration in mineral homeostasis, with a disruption of normal serum and tissue concentrations of phosphorus, calcium, and changes in circulating levels of hormones.[29] Phosphorus is an essential mineral required for cell structure and signaling, energy transfer, acid-base equilibrium. Phosphorus can also function as essential component of important biomolecules such as phospholipids, nucleotides, and nucleic acid.[30] Drug induced derangement in the concentration of phosphorus can impose adverse consequences on the skeletal, renal, and cardiovascular systems. These disturbances of bone mineral metabolism have been linked with elevated risk of impaired kidney function, end-stage renal disease (ESRD), cardiovascular disease (CVD)[31] and also a high mortality rate. In the present study, there was a significant increase in the level of phosphate in group of rats treated with piroxicam alone, relative to the control group. Notwithstanding, concomitant administration of 200 mg/kg TRECDS was able to curtail the elevated phosphate level significantly, but not the 400mg/kg dose. Moreover, the piroxicam administration also caused an increase in the calcium concentration in comparison with the control. This metabolic derangement was significantly remediated by TRECDS. This suggests that TRECDS may be a promising novel natural supplement which can be explored for the management of disorders associated with calcium deficiency.

Albumin is an important plasma protein that plays vital metabolic roles including maintenance of oncotic pressure, as well as selective binding and transport of lipid and steroid hormones.[32] It is known that a treatment regimen consisting of prolonged NSAIDS usually predispose at least 60% of the patients to protein losing enteropathy. Data obtained from the present study showed that piroxicam administration significantly increased the albumin concentration while also decreasing the total protein concentration. Reduced renal protein concentration in piroxicam induced nephrotoxicity has been reported in a previous study.[33] However, TRECDS treatment resulted in significant reduction of albumin and increase in total renal protein concentration in a dose dependent manner.

One of the proposed mechanisms for drug induced organ damage is the free radical hypothesis.[34] While the biotransformation of certain drugs by hepatic metabolizing enzymes may generate intermediates which possess ionizing properties, the production of oxidative radical specie as a by-product may also be inevitable.[35] The resultant free radical can compromise the structure and function of important biomolecule, including lipid bilayer of cellular membrane. Consequently, the continuous generation of lipid peroxidation products can upset homeostasis in the cell and tissues within the affected organ. In the present study, piroxicam administration significantly reduced the renal GSH concentration while also increasing malondialdhyde. This showed a compromised antioxidant defense mechanism and a corresponding upregulation of oxidative attack to the renal compartments. Similar observation has been documented by previous investigators.[36] GSH is an antioxidant enzyme responsible for the maintenance of redox homeostasis and prevention of tissue and organ damage by ROS.[37] Data obtained from this study showed that the GSH level was significantly restored following concomitant administration with TRECDS, while also curtailing the renal level of lipid peroxidation. By implication, the tannin rich extract of *C. dependens* may have elicited these pharmacological activities owing to its constituent bioactive mechanisms. According to a previous report, *C. dependens* possess a remarkable antioxidant capacity,[38] which may possibly justify the antiperoxidative property that was demonstrated in the present study. Although the actual antioxidant value of *C. dependens* are scarcely explored, its remarkable pharmacological potentials suggested that it is a promising source of essential compounds which are modulate multiple disease processes.

CONCLUSION

The present study demonstrated that the tannin rich extract of *C. dependens* was remarkably nephroprotective by curtailing electrolyte imbalance and reversing oxidative stress. Future studies are needed to isolate and characterise the specific bioactive agents eliciting these pharmacological properties.

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