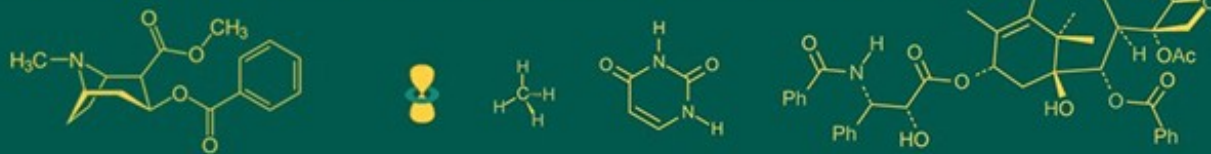


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Modulatory effects of *Psidium guajava* leaf extract on haematobiochemical alterations in adenine-induced chronic kidney disease in rats

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ABSTRACT

The present study was conducted to evaluate the haematobiochemical alterations associated with adenine-induced chronic kidney disease (CKD) and to assess the nephroprotective potential of aqueous *Psidium guajava* leaf extract in Wistar rats. Forty male Wistar rats were randomly divided into five groups, comprising eight animals each ($n = 8$). Group I served as the normal control and received 0.5% carboxymethyl cellulose (CMC) by oral gavage for 28 days. Group II served as the CKD control and was administered adenine at 100 mg/kg body weight (b.wt.) by oral gavage for 28 consecutive days. Group III served as the extract control and received aqueous *Psidium guajava* leaf extract at 300 mg/kg b.wt. for 28 days. Group IV, the prophylactic group, received adenine (100 mg/kg b.wt.) concomitantly with aqueous *P. guajava* leaf extract (300 mg/kg b.wt.) for 28 days, whereas Group V, the therapeutic group, received adenine (100 mg/kg b.wt.) for 28 days and aqueous *P. guajava* leaf extract (300 mg/kg b.wt.) from day 15 to day 28. Adenine administration resulted in a significant decrease in haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), lymphocyte count, serum total protein, albumin, globulin and calcium levels, along with a significant increase in total leukocyte count (TLC), neutrophil count, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and phosphorus levels in the CKD control group. Treatment with aqueous *P. guajava* leaf extract significantly ameliorated these adenine-induced haematobiochemical alterations, with more pronounced improvement observed in the prophylactic group than in the therapeutic group. The findings suggest that aqueous *P. guajava* leaf extract possesses nephroprotective potential and may help attenuate haematological and biochemical alterations associated with adenine-induced CKD in rats.

Keywords: Adenine, Chronic Kidney disease, *Psidium guajava*, Haemato-Biochemical

Introduction

Chronic kidney disease (CKD) is a major non-communicable disorder characterized by persistent, progressive, and irreversible impairment of renal structure and function, resulting in a gradual decline in renal excretory capacity. CKD represents a significant clinical concern in both human and small-animal medicine and is associated with substantial morbidity, reduced life expectancy, and impaired quality of life (Levey *et al.*, 2007) [1] (Levey *et al.*, 2007) [1]. Although CKD can occur at any age, it is predominantly observed in geriatric dogs and cats, in which advanced disease is frequently associated with a poor to guarded prognosis. (Bronson, 1982; Lund *et al.*, 1999) [2, 3] (Bronson, 1982; Lund *et al.*, 1999) [2, 3].

The pathogenesis and progression of CKD involve complex and interrelated mechanisms, with inflammation and oxidative stress playing important roles in the development of renal injury and disease progression [4] (Ali *et al.*, 2015) [4]. Progressive loss of functional nephrons may result from pathological alterations involving the glomeruli, renal tubules, vasculature, and interstitial compartment. The consequent reduction in functional nephron mass compromises renal filtration and excretory capacity, leading to the systemic accumulation of uremic toxins and metabolic waste products. This uremic milieu contributes to persistent systemic inflammation and enhanced generation of reactive oxygen species (ROS), thereby promoting oxidative stress and further structural and functional renal damage (Melekoglu *et al.*, 2021) [5] (Melekoglu *et al.*, 2021) [5].

Several experimental models have been developed to investigate the pathogenesis and progression of renal fibrosis and chronic kidney disease (CKD). Among these, adenine-induced nephropathy is a well-established, non-surgical experimental model of CKD in rodents (Yokozawa *et al.*, 1986) [6] Yokozawa *et al.* (1986) [6]. Prolonged oral administration of adenine results in its metabolism to 2,8-dihydroxyadenine (2,8-DHA), a poorly soluble metabolite that precipitates within the renal tubules, particularly in the proximal tubular compartment. The subsequent formation and accumulation of 2,8-DHA crystals within the tubular lumen and renal parenchyma contribute to tubular obstruction and epithelial injury, followed by inflammatory

responses, ischemic changes, and progressive tubulointerstitial fibrosis which leads to progressive course of disease (Ali *et al.*, 2018) [7] (Ali *et al.*, 2018) [7].

India is widely recognized for its rich diversity of medicinal plants and has historically been referred to as an “emporium of medicinal plants.” *Psidium guajava* L., a member of the family Myrtaceae, is a perennial evergreen shrub commonly known as guava and is widely utilized for its nutritional and medicinal properties. Various parts of the plant, particularly the leaves, have been reported to possess a broad spectrum of pharmacological activities, including antioxidant, free-radical-scavenging, anti-inflammatory, antiproliferative, antidiarrhoeal, antihypertensive, hepatoprotective, antimicrobial, hypoglycaemic, and antimutagenic effects. The therapeutic potential of *P. guajava* has been attributed, at least in part, to its diverse phytochemical constituents, particularly flavonoids and polyphenolic compounds such as quercetin, avicularin, guajaverin, rutin, catechin, gallic acid, kaempferol and ellagic acid (Chang *et al.*, 2017; De Araujo *et al.*, 2014) [8, 9] (Chang *et al.*, 2017) [8] (De Araujo *et al.*, 2014) [9]. These bioactive compounds are considered important contributors to the antioxidant and anti-inflammatory properties of the plant and may underlie its potential protective effects against various pathological conditions. Therefore, the present study was undertaken to evaluate the haematobiochemical alterations associated with adenine-induced chronic kidney disease and to investigate the nephroprotective potential of aqueous *Psidium guajava* leaf extract in rats.

2. Materials and Methods

2.1 Collection of plant material

Fresh leaves of *Psidium guajava* were collected from the campus of the Post Graduate Institute of Veterinary and Animal Sciences, Akola. The plant material was identified and authenticated by an expert taxonomist.

2. Preparation of aqueous extract *P. guajava* leaves

The collected leaves were thoroughly washed under running tap water to remove adhering dirt and extraneous matter, followed by shade-drying under ambient conditions. The dried leaves were subsequently pulverized into a coarse powder. For the preparation of the aqueous leaf extract, 200 g of the coarse powder was suspended in 2000 mL of distilled water and subjected to extraction in a boiling water bath at 80 °C for 60 min. The extract was then allowed to cool to room temperature and filtered sequentially through muslin cloth and Whatman No. 1 filter paper. The resulting filtrate was concentrated to dryness by evaporation at room temperature under a fan. The dried crude extract was collected and stored in sterile conditions at 4 °C until further use (Babatola *et al.*, 2019) [10] (Babatola *et al.*, 2019) [10].

3. Animals

The present experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the Post Graduate Institute of Veterinary and Animal Sciences, Akola, prior to commencement of the study (IAEC Approval No. 312/11/03/2000/2023; dated 20 October 2023). A total of 40 healthy male Wistar rats, weighing approximately 150–200 g, were procured from LACSMI Biofarms Pvt. Ltd., Pune, Maharashtra, India (CCSEA Registration No. 1277/PO/ReBiBt/S/20/CCSEA).

Prior to the initiation of the experiment, all animals were acclimatized to the laboratory environment for seven days. Throughout the experimental period, the animals were provided with standard commercial pelleted feed and clean drinking water *ad libitum*.

4. Experimental procedure

Following a one-week acclimatization period, the 40 male Wistar rats were randomly allocated into five groups, comprising eight animals per group. Group I (normal control) received 0.5% carboxymethyl cellulose (CMC) solution throughout the experimental period. Group II (CKD control) was administered adenine at a dose of 100 mg/kg

body weight (b.wt.) for 28 consecutive days to induce chronic kidney disease (CKD). Group III (extract control) received an aqueous extract of *Psidium guajava* leaves at a dose of 300 mg/kg b.wt. for 28 consecutive days. Group IV (prophylactic group) received adenine (100 mg/kg b.wt.) concomitantly with the aqueous *P. guajava* leaf extract (300 mg/kg b.wt.) from day 1 to day 28. Group V (therapeutic group) was administered adenine at 100 mg/kg b.wt. for 28 consecutive days and subsequently treated with the aqueous *P. guajava* leaf extract at 300 mg/kg b.wt. from day 15 to day 28 (Table 1).

Blood samples were collected from animals in all experimental groups on day 14 and at the end of the experimental period (day 28) from the retro-orbital plexus using a sterile capillary tube under anaesthesia induced with thiopentone sodium (40 mg/kg b.wt., intraperitoneally) (Sorg *et al.*, 1964) [11] (Sorg *et al.*, 1964) [11]. The collected blood samples were divided into two portions and transferred into appropriate collection tubes. Blood intended for haematological analysis was collected in K3-EDTA-containing tubes, whereas blood intended for biochemical analysis was collected in clot-activator tubes. Blood smears were prepared immediately from a fresh drop of blood at the time of collection and subsequently used for differential leukocyte counting.

For biochemical analysis, blood samples collected in clot-activator tubes were allowed to clot and then centrifuged at 3000 rpm for 15 min. The separated serum was transferred into sterile Eppendorf tubes and stored at –20 °C until further analysis.

5. Haematological parameters

The haematological parameters viz. Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Total Leukocyte Count (TLC) and Differential Leukocyte Count (DLC) were estimated as per the standard method (Medway *et al.*, 1969) [12] Medway *et al.* (1969) [12].

2.6 Biochemical markers

The biochemical parameters viz. BUN, creatinine, AST, ALT, total protein, albumin, calcium and phosphorous were estimated from serum samples using AGD diagnostic kits supplied by AGD Biomedicals (P) Ltd. Andheri (E), Mumbai, India on Autoanalyzer (Make- AGD Biomedical, Model No. AGD 2020) as per standard protocol/method described in kit manual. The kidney and liver function markers were evaluated on 14th day of experiment to determine the induction of chronic kidney disease by adenine. Following the conclusion of the experiment, all the biochemical parameters were assessed using serum samples obtained from rats of various treatment groups.

7. Statistical analysis

The data obtained for haematological and biochemical parameters was analyzed by applying complete randomized design (Snedecor *et al.*, 1989) [13] Snedecor *et al.* (1989) [13].

2. Results

3.1 Haematological parameters

The mean Hb, PCV and TEC values in CKD positive control group revealed a notable decrease in comparison to normal healthy and extract control groups followed by prophylactic and therapeutic groups. On the contrary, substantial increase in Hb, PCV and TEC was observed in prophylactic group, followed by therapeutic group when compared with CKD control group (Table 2). The mean TLC in CKD positive control group was significantly higher, followed by therapeutic and prophylactic groups. A significant decline in TLC was observed in prophylactic group followed by therapeutic group when compared with CKD control group. There was a significant increase in neutrophil count in CKD positive control group, followed by therapeutic group. The mean percentage of neutrophils was significantly

reduced in prophylactic group when compared to CKD control and therapeutic groups. There was a significant decrease in lymphocyte percentage in CKD control followed by therapeutic and prophylactic

group. Moreover, the lymphocyte count in therapeutic group was analogous to those of CKD control and prophylactic groups (Table 3).

Table 1. The details of experimental design

Groups	Treatment	No. of Animals	Experimental Period
	Adenine@ mg/kg b.wt. PO in 0.5% CMC solution once a day		
I	0.5% CMC solution	08	28 days
II	@ 100 mg/kg b.wt	08	28 days
III	-	08	28 days
IV	@100 mg/kg b.wt (1-28 days)	08	28 days
V	@ 100 mg/kg b.wt (1-28 days)	08	28 days
	Aqueous extract of guava leaves @mg/kg b.wt. once a day		
	-	08	28 days
	@300 mg/kg b.wt	08	28 days
	@300 mg/kg b.wt (1-28 days)	08	28 days
	@300 mg/kg b.wt (14-28 days)	08	28 days

Table 2. Mean ($\pm$ SE) values of various haematological parameters related to erythrocytes of rats in different treatment groups

Groups	Hb (g/dL)	PCV (%)	TEC (10^6 /cumm)	MCV (fL)	MCH (pg)	MCHC (%)
I	14.51 $\pm 0.17^a$	48.00 $\pm 1.07^a$	8.92 $\pm 0.13^a$	53.88 ± 1.21	16.30 ± 0.25	30.32 ± 0.63
II	11.79 $\pm 0.29^d$	39.33 $\pm 0.96^d$	7.09 $\pm 0.11^d$	55.48 ± 0.93	16.65 ± 0.44	30.09 ± 1.02
III	14.83 $\pm 0.13^a$	48.88 $\pm 0.83^a$	8.91 $\pm 0.17^a$	55.01 ± 1.64	16.69 ± 0.46	30.38 ± 0.50
IV	13.81 $\pm 0.32^b$	45.25 $\pm 1.06^b$	8.28 $\pm 0.19^b$	54.90 ± 1.94	16.78 ± 0.69	30.57 ± 0.62
V	12.68 $\pm 0.23^c$	42.38 $\pm 0.71^c$	7.54 $\pm 0.09^c$	56.20 ± 0.67	16.82 ± 0.33	29.94 ± 0.58
Level of significance	**	**	**	-	-	-
CD (0.05)	0.687	2.687	0.406	-	-	-

Table 3. Haematological values related to leukocytes in different treatment groups

Groups	TLC	Neutrophil	Lymphocyte	Eosinophil	Monocyte
I	11.05 $\pm 0.72^d$	11.88 $\pm 0.85^c$	85.75 $\pm 1.49^a$	0.50 ± 0.27	1.88 ± 0.30
II	21.48 $\pm 0.69^a$	20.00 $\pm 1.00^a$	78.38 $\pm 0.71^c$	0.25 ± 0.16	1.38 ± 0.26
III	10.93 $\pm 0.64^d$	10.25 $\pm 0.80^c$	87.63 $\pm 0.46^a$	0.38 ± 0.18	1.75 ± 0.37
IV	14.13 $\pm 0.66^c$	14.88 $\pm 1.06^b$	82.75 $\pm 0.98^b$	0.50 ± 0.27	1.88 ± 0.30
V	19.10 $\pm 0.70^b$	17.63 $\pm 0.75^a$	80.63 $\pm 0.71^{bc}$	0.38 ± 0.18	1.25 ± 0.37
Level of significance	**	**	**	-	-
CD (0.05)	1.957	2.586	2.683	-	-

3.2 Biochemical parameters

On the 14th day, serum BUN, creatinine, AST and ALT revealed significant increase in CKD positive control group followed by therapeutic group. Furthermore, a significant decline in BUN, creatinine, AST and ALT levels was observed in prophylactic group when compared to CKD control and therapeutic groups. At the end of the experiment significantly escalated levels of BUN, creatinine, AST and ALT were

observed in CKD control group as compared to all other treatment groups. Prophylactic and therapeutic groups exhibited a significant decrease in BUN, creatinine, AST and ALT levels compared to CKD control group but displayed significantly augmented levels of BUN, creatinine, AST and ALT compared to normal and extract control groups. However, the decrease was more marked in prophylactic group than therapeutic group when compared with CKD positive control group (Table 4).

Table 4. BUN, Creatinine, AST and ALT at 14th day and at the end of experiment

Groups	BUN (mg/dL)		Creatinine (mg/dL)		AST (IU/L)		ALT (IU/L)	
	Day 14	At the end of study	Day 14	At the end of study	Day 14	At the end of study	Day 14	At the end of study
I	12.26 ±0.11 ^c	14.97 ±0.67 ^d	0.56 ±0.02 ^c	0.60 ±0.02 ^d	140.70 ±1.93 ^c	148.59 ±3.21 ^d	33.97 ±2.00 ^c	35.86 ±2.14 ^d
II	25.86 ±0.11 ^a	68.26 ±2.28 ^a	1.17 ±0.03 ^a	2.09 ±0.05 ^a	154.45 ±2.06 ^a	188.25 ±2.77 ^a	50.93 ±2.20 ^a	77.54 ±2.30 ^a
III	12.32 ±0.08 ^c	14.36 ±0.52 ^d	0.53 ±0.02 ^c	0.63 ±0.05 ^d	140.15 ±2.18 ^c	147.79 ±2.87 ^d	33.85 ±2.01 ^c	35.79 ±2.13 ^d
IV	20.08 ±0.06 ^b	38.33 ±0.96 ^c	0.98 ±0.03 ^b	1.17 ±0.04 ^c	146.52 ±2.27 ^b	163.53 ±2.36 ^c	41.80 ±2.27 ^b	51.61 ±2.24 ^c
V	25.77 ±0.10 ^a	54.72 ±0.78 ^b	1.18 ±0.03 ^a	1.62 ±0.03 ^b	154.57 ±2.06 ^a	177.48 ±2.80 ^b	51.32 ±2.63 ^a	64.83 ±2.44 ^b
Level of significance	**	**	**	**	**	**	**	**
CD (0.05)	0.275	3.503	0.055	0.118	6.197	8.086	6.511	6.466

The serum total protein, albumin, globulin and calcium in CKD positive control group showed significant decrease when compared with other treatment groups. Contrarily, a substantial increase in total protein, albumin, globulin and calcium was apparent in prophylactic and

therapeutic groups in comparison to CKD control group. A notable reduction in serum phosphorus levels was observed in the prophylactic group, followed by the therapeutic group, compared with the elevated levels observed in the CKD control group (Table 5).

Table 5. Serum biochemical parameters in different groups at the end of the experiment

Groups	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Calcium (mg/dL)	Phosphorous (mg/dL)
I	7.50 ±0.09 ^a	4.13 ±0.12 ^{ab}	3.37 ±0.14 ^a	10.33 ±0.42 ^a	8.61 ±0.17 ^d
II	5.47 ± 0.10 ^d	2.81 ±0.09 ^d	2.66 ±0.13 ^b	8.72 ±0.45 ^b	18.00 ±0.41 ^a
III	7.52 ± 0.12 ^a	4.15 ±0.04 ^a	3.37 ±0.14 ^a	10.64 ±0.43 ^a	8.43 ±0.24 ^d
IV	6.84 ± 0.08 ^b	3.87 ±0.06 ^b	2.97 ±0.09 ^{ab}	10.28 ±0.27 ^a	11.51 ±0.33 ^c
V	6.10 ± 0.11 ^c	3.31 ±0.11 ^c	2.79 ±0.19 ^b	9.67 ±0.30 ^{ab}	15.11 ±0.17 ^b
Level of significance	**	**	**	**	**
CD (0.05)	0.291	0.258	0.410	1.094	0.800

Discussion

The current investigation evaluated the impact of the aqueous extract derived from *P. guajava* leaves on adenine-induced chronic kidney disease through the assessment of haematobiochemical parameters. The present findings of significant decrease in Hb, PCV and TEC in CKD positive control group are in accordance with previously reported studies (Gori *et al.*, 2021; Vázquez-Méndez *et al.*, 2020) [14, 15] Gori *et al.* (2021) [14], Vázquez-Méndez *et al.* (2020) [15]. Adenine intoxication induces chronic kidney damage and diminishes the population of functional fibroblasts that produce erythropoietin consequently leading to decrease in haemoglobin, haematocrit and erythrocyte indices (Vázquez-Méndez *et al.*, 2020) [15] Vázquez-Méndez *et al.* (2019). Studies have demonstrated that uremic anaemia is primarily caused by relative erythropoietin deficiency resulting from injury and fibrosis, as well as the accumulation of myofibroblasts in the kidney (Ali *et al.*, 2014) [16] (Ali *et al.*, 2014) [16]. Administration of aqueous extracts of *Psidium guajava* leaves @250 mg and @500 mg/kg to albino rats resulted in substantial rise in the Hb, PCV and TEC values (Kullu *et al.*, 2013; Uboh *et al.*, 2010) [17, 18] Kullu *et al.* (2013) [17], Uboh *et al.* (2010) [18]. The increase in total leukocyte count might be attributed to ongoing inflam-

matory activity in CKD rats. Moreover, previous study also reported similar non significant changes in the mean values of monocytes in adenine induced CKD rats and different treatment groups (Patel *et al.*, 2020) [19] Patel *et al.* (2020) [19].

The findings of BUN and creatinine level were similar to the previous study which documented significant rise ($p < 0.0001$) in the adenine intoxicated rats after administration of adenine compared to the control group (Melekoglu *et al.*, 2021; Gori *et al.*, 2021; Vázquez-Méndez *et al.*, 2020) [5, 14, 15] Melekoglu *et al.* (2021) [5], Gori *et al.* (2021) [14], Vázquez-Méndez *et al.* (2020) [15]. Adenine intoxication results in renal impairment that leads to a reduction in the elimination of BUN and creatinine, as evidenced by an elevation in serum BUN and creatinine levels (Winearls, 2009; Shuvy *et al.*, 2011) [20, 21] (Winearls, 2009; Shuvy *et al.*, 2011) [20, 21]. It is reported that administration of aqueous extract of *Psidium guajava* leaves resulted in significant reduction ($p < 0.05$) in the levels of serum urea and creatinine in cyclosporine induced hypertensive rats which are in close association with current findings from prophylactic and therapeutic groups (Babatola *et al.*, 2021) [22] Babatola *et al.* (2021) [22]. Earlier studies also reported significant increase in the serum AST and ALT levels in adenine treated rats which closely resemble to our current findings (Ali *et al.*, 2015; Ali *et al.*, 2016) [4, 23] Ali *et al.* (2015) [4] and Ali *et al.* (2016) [23]. Congruence with the present find

ings of prophylactic and therapeutic groups earlier researchers found that supplementation of aqueous extract of *Psidium guajava* leaves restored elevated level of AST and ALT in rats treated with CCl₄ (Debnath *et al.*, 2021) [24] (Debnath *et al.*, 2021) [24].

The results of significant decrease in serum total protein and albumin levels of adenine received rats were in agreement with previous studies (Li *et al.*, 2018; Sueyoshi *et al.*, 2019) [25, 26] Li *et al.* (2018) [25] and Sueyoshi *et al.* (2019) [26]. The decline in serum total protein and albumin levels could potentially be linked to the disruption of renal histoarchitecture induced by adenine, resulting in the excretion of protein through urine and the development of proteinuria. Contrary to the findings observed in CKD control rats significantly elevated globulin levels was observed in adenine intoxicated rats compared to control animals (Sueyoshi *et al.*, 2019) [26] Sueyoshi *et al.* (2019) [26].

The present findings of significantly decreased serum calcium levels and significantly increased phosphorous levels in adenine treated rats are in harmony with previous studies (Gori *et al.*, 2021; Sueyoshi *et al.*, 2019) [14, 26] Gori *et al.* (2021) [14] and Sueyoshi *et al.* (2019) [26]. However, in contrast, earlier studies reported that serum calcium levels were not altered in the adenine treated rats (Chang *et al.*, 2017; Sun *et al.*, 2013) [8, 27] Chang *et al.* (2017) [8] and Sun *et al.* (2013) [27]. Furthermore, another researcher also did not find any significant change in serum calcium levels in CKD induced group mice as compared to control group (Tani *et al.*, 2017) [28] Tani *et al.* (2017) [28]. Moreover, contrarily a study did not show any significant variation in the serum phosphorous in adenine treated mice (Sun *et al.*, 2013) [27] Sun *et al.* (2013) [27].

5. Conclusion

In conclusion to the above findings of the study, Adenine @ 100 mg/kg b.wt. orally for 28 days induced chronic kidney disease with adverse effects on haematobiochemical parameters in rats. Both prophylactic as well as therapeutic administration of aqueous extract of *Psidium guajava* leaves @ 300 mg/kg b.wt. showed beneficial protective and therapeutic effect on haematobiochemical parameters. Significant improvement in haematobiochemical parameters of the prophylactic and therapeutic groups might be attributed to antioxidant, anti-inflammatory and nephroprotective effect of bioactive compounds present in the *Psidium guajava* leaves.

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