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CHRONOTHERAPEUTIC EFFECTS OF GALLIC ACID IN THE RENAL MARKERS, LIPID PEROXIDATION, ENZYMATIC ANTIOXIDANTS AND NON ENZYMATIC ANTIOXIDANT ON VANCOMYCIN INDUCED NEPHROTOXIC RATS

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ABSTRACT

The present study investigates chronotherapeutic effects of renal markers and lipid peroxidation and antioxidant properties in nephrotoxic rats, adult male albino Wistar rats, weighing 180-200 g with a nephrotoxic dose of vancomycin (200 mg/kg/body weight, twice a day(12 hour interval), i.p injection). This study used rats that were treated with 7 groups especially treatment group (GA + VAN) divided in to 4 groups (i.e 06:00hrs, 12:00hrs, 18:00hrs, 24:00hrs). Results showed that 24:00h time point level is peak when compared with other time points. The present study demonstrates that gallic acid can be protective against vancomycin -induced nephrotoxicity.

KEYWORDS: Chronotherapy, Gallic acid, Vancomycin, Nephrotoxicity, Wistar rats.

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INTRODUCTION

Long time administration of drugs or antibiotics is involved in causing oxidative stress and high intracellular levels of reactive oxygen species (ROS) that can lead to damaged the cells at different peripheral organelles resulting disruption of circadian clock coordination that contribute to maximize the damage and significantly enhanced the severity of renal toxicity. A number of recent publications concerning experimental chronotoxicology have reported that highly nephrotoxic substances such as heavy metals or aminoglycosides [1] exhibit substantial circadian variations in their acute toxicity, as determined by mortality rates at different times during at the 24 h cycle. These responses do not apply only to the human body. The responses of antibiotics to bacteria and of similar disease to chemotherapeutic agents or radiotherapy are two examples of the way that circadian changes alter therapeutic response. This type of therapy uses to arrange the timing of drug administration according to the circadian rhythms of cell susceptibility and those of adverse effects of chemotherapy.

Chronotherapy consists of chemotherapy delivery according to biologic rhythms along the 24-hour scale [2]. These genetically based rhythms modulate cellular metabolism and proliferation in normal tissues [3,4]. As a result of chemotherapy, in laboratory rodents, the tolerability and therapeutic efficacy of various drugs, varied largely according to dosing time [2,5]. The aim of transferring this concept to the clinic was primarily to increase dose-intensity through an adjustment of drug delivery to 24-hour rhythms in tolerability. A specific technology (programmable-in-time injectors) allowed the administration of chronotherapy to patients who were fully ambulatory [6].Gallic acid (3,4,5-trihydroxybenzoic acid) (Figure 1) is a colorless crystalline organic acid found in gallnuts, sumach, tea leaves, oak bark, and many other plants. Since gallic acid has hydroxyl groups and a carboxylic acid group in the same molecule. Gallic acid is known to potentiate several pharmacological and biochemical pathways, having anti-inflammatory effects [7]. It possesses hepatoprotective [8], anticancer [9] activities.

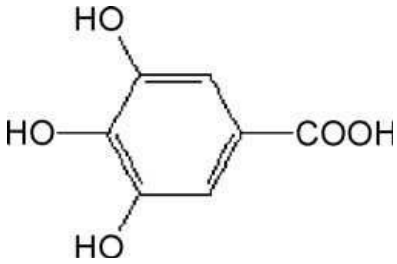


Figure 1
Structure of gallic acid (3, 4, 5-trihydroxybenzoic acid)

Drug-induced nephrotoxicity accounts for up to one-third of in-hospital AKI cases [10]. Traditionally, drug-induced nephrotoxicity (NT) has been associated with vancomycin since its introduction in the early 1950s. The first reports of vancomycin-associated nephrotoxicity were attributed to poor manufacturing processes [11]. Early lots of the compound were called “Mississippi mud” because impurities produced a muddy, brown appearance. After purification methods were implemented, vancomycin was approved for clinical use by the US Food and Drug Administration in 1958. Vancomycin’s approval by the Food and Drug Administration was based on 13 of 15 patients being treated successfully with vancomycin. Lingering safety concerns, as well as the availability of methicillin and cephalothin, limited vancomycin were used in early years. Vancomycin use began to increase after methicillin-resistant

Staphylococcus aureus was first described in 1961 [12]. Vancomycin-associated nephrotoxicity was reported in 0% to 5% of patients in the 1980s. Concomitant nephrotoxic agents increase rates of vancomycin- associated toxicity to as high as 35% [13].

MATERIALS AND METHODS

(i) Drug and Chemicals

Vancomycin was purchased from Ranbaxy laboratories limited, New Delhi, India. Gallic acid, thiobarbituric acid (TBA), phenazine methosulphate (PMS), nitroblue tetrazolium (NBT), adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD) were purchased from Sigma Chemical Company, St. Louis, USA. Butylated hydroxy toluene (BHT), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), phosphate buffered saline and ethylene diamine tetra acetic acid (EDTA) were purchased from S.D. Fine Chemicals Ltd., Mumbai, India. The rest of the chemicals and biochemicals utilized were obtained from local firms (India) and were of analytical grade. All other chemicals used in the study were of analytical grade.

(ii)Experimental Induction of Nephrotoxicity

Nephrotoxicity was induced in male Wistar rats (180-200g) by intraperitoneal injections of vancomycin (VAN) at a dose of 200mg/kg b.wt for 7 days [14].

(iii) Experimental Animals

Adult male albino Wistar rats, weighing 180-200 g bred in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, were used. The animals were housed in polycarbonate cages in a room with a 12 h day-night cycle, temperature of 22 ± 2°C and humidity of 45-64%. Animals were fed with a standard pellet diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. All animal experiments were approved by the ethical committee (Rajah Muthiah Medical college, Annamalai University, Institutional Animal Ethics Committee Central Animal House Registration Number 160/1999/CPCSEA, Vide. No. 771/2010),

(iv) Experimental Design

The animals were randomized and divided into seven groups of six rats each as follows

Group I	Control
Group II	Rats orally administered with gallic acid (200 mg/kg b.wt)
Group III	Rats treated with vancomycin (200 mg/kg b.wt, i.p injections)
Group IV	Rats treated with VAN +GA to be administered at 06:00 h
Group V	Rats treated with VAN +GA to be administered at 12:00 h
Group VI	Rats treated with VAN +GA to be administered at 18:00 h
Group VII	Rats treated with VAN +GA to be administered at 24:00 h

At the end of the experimental period, all animals were fasted overnight and sacrificed by cervical dislocation. Blood samples were collected for biochemical estimations (lipid peroxidation products, antioxidants). Tissues (kidney) were dissected out and washed in ice-cold saline, patted dry and weighed for various biochemical estimations. Erythrocytes were also prepared for the estimation of various biochemical preparations.

(v) Biochemical Measurements

In plasma, urea, uric acid and creatinine were measured using commercial kits (Sigma, St. Louis, MO, USA). Antioxidant properties of GPX and GSH level, SOD and CAT activity and lipid peroxidation as thiobarbituric acid reactive substances (TBARS) and Hydroperoxides (HP) in the renal cortex were measured spectrophotometrically as described before [15,16].

(vi) Statistical Analysis

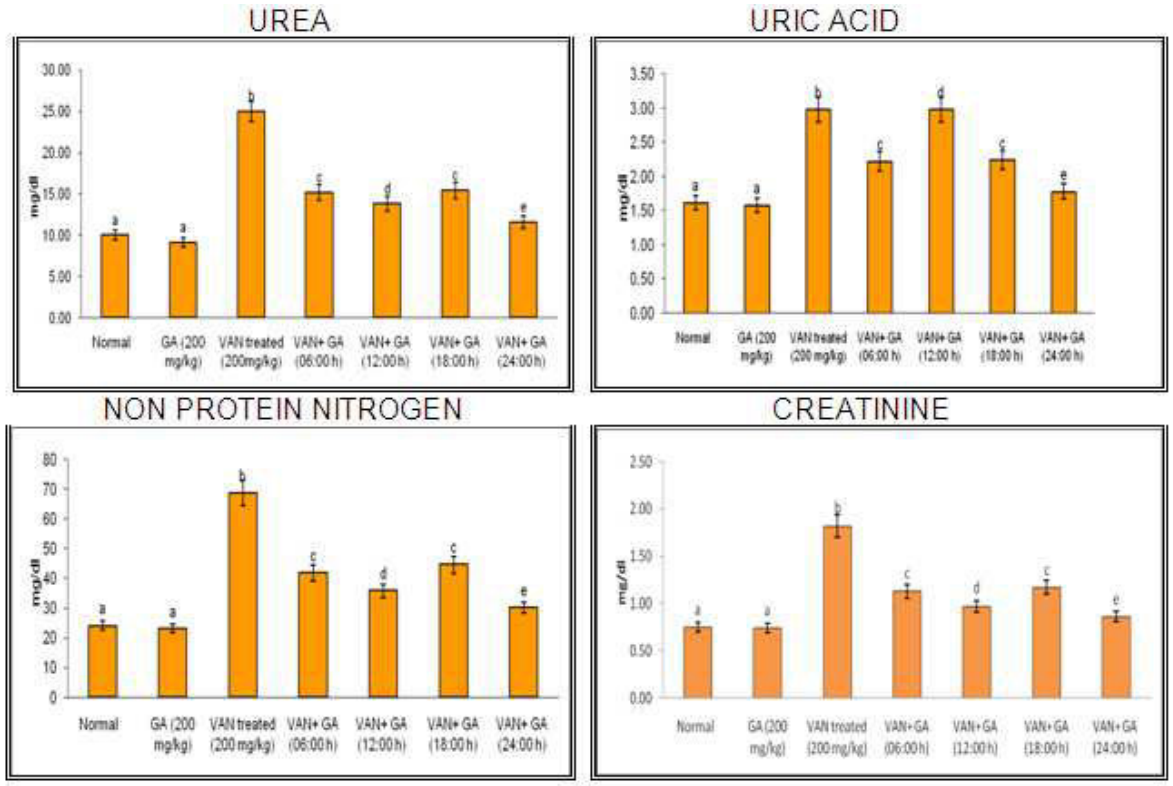
The data for various biochemical parameters were analyzed using analysis of variance (ANOVA) and the group means were compared with Duncans Multiple Range Test (DMRT) [17]. Values were considered statistically significant when $p < 0.05$.

RESULTS

The changes in the levels of urea, uric acid, non-protein nitrogen and creatinine in all groups and the levels were markedly elevated in VAN treated rats and no significant difference was observed in gallic acid administered rats when compared with control rats. The levels were significantly lower in vancomycin and gallic acid treated rats at different time intervals (06:00, 12:00, 18:00 and 24:00hrs) as compared to VAN treated group; but treatment with gallic acid at 24:00 h was found to be more effective than other time points. The levels of kidney markers such as urea, uric acid, non protein nitrogen and creatinine in plasma of normal and experimental groups were shown in Graph 1. Administration of gallic acid to VAN induced nephrotoxic rats restored the levels of kidney markers; however treatment with gallic acid at 24:00h was found to be more effective than other time points.

Graph 1

Chronotherapeutic effect of Gallic acid on changes in the plasma urea, uric acid, non protein nitrogen and creatinine of normal and experimental rats



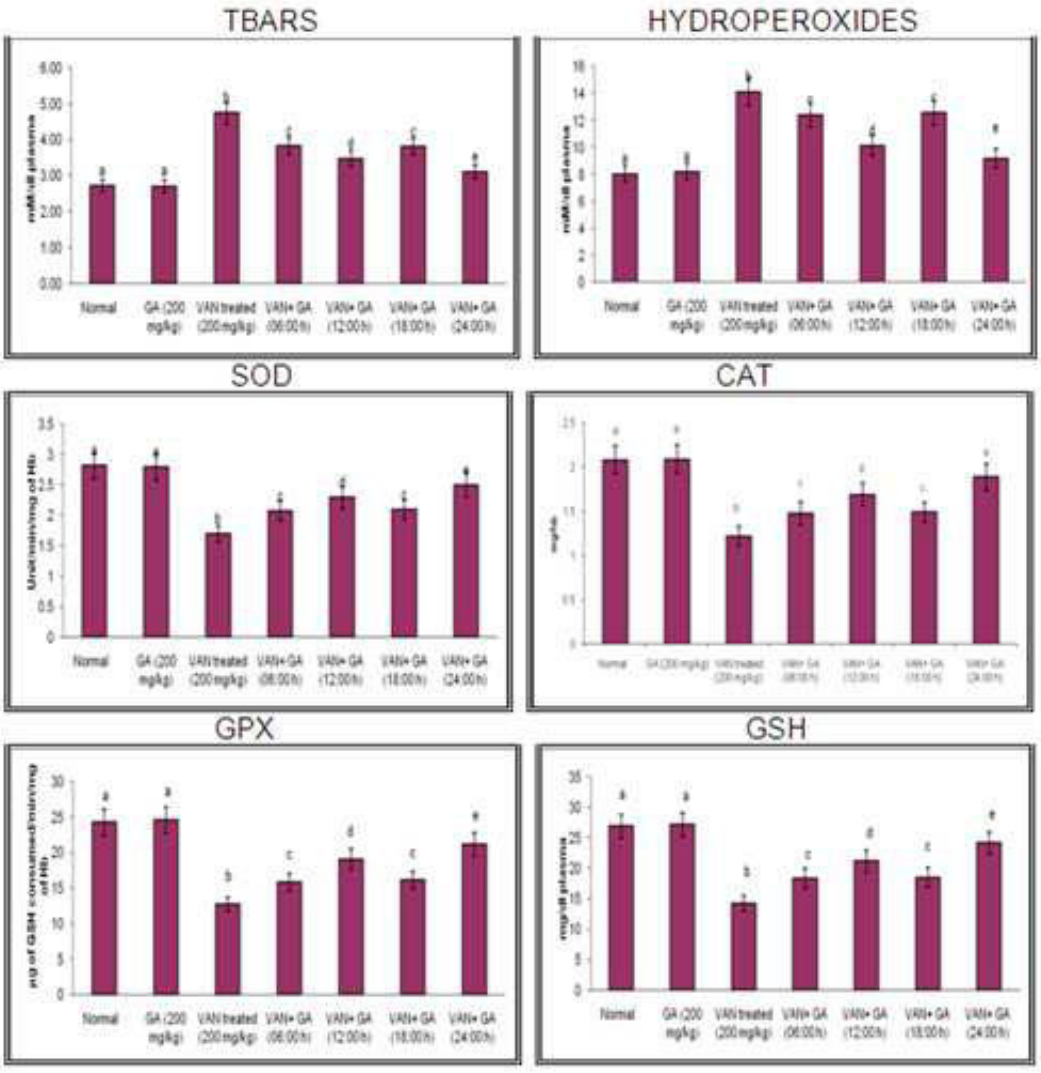
Values are given as mean ± S.D from six rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Graph 2 and 3 showing the concentration of TBARS and hydroperoxides in the plasma and tissues (kidney) of control and experimental animals. The levels of TBARS and hydroperoxides were significantly increased in VAN treated rats as compared to control rats. Administration of gallic acid significantly decreased the level of lipid peroxides when compared to VAN treated rats. The levels were significantly lower in gallic acid treated vancomycin rats at different time intervals groups (06:00, 12:00, 18:00 and 24:00 hrs) as compared to VAN treated rats; however treatment with gallic acid at 24:00 hrs was found to be more effective than at other time points.The chrononotherapeutic effects of Gallic acid at the levels of SOD, CAT, GPx and GSH in hemolysate and tissues (kidney) was shown

in Graph 2 and 3 Levels of these antioxidants were significantly increased in gallic acid treated vancomycin rats at different time intervals (06:00, 12:00, 18:00 and 24:00 hrs) as compared to VAN treated rats; however treatment with gallic acid at 24:00 h was found to be more effective than other time points.

Graph 2

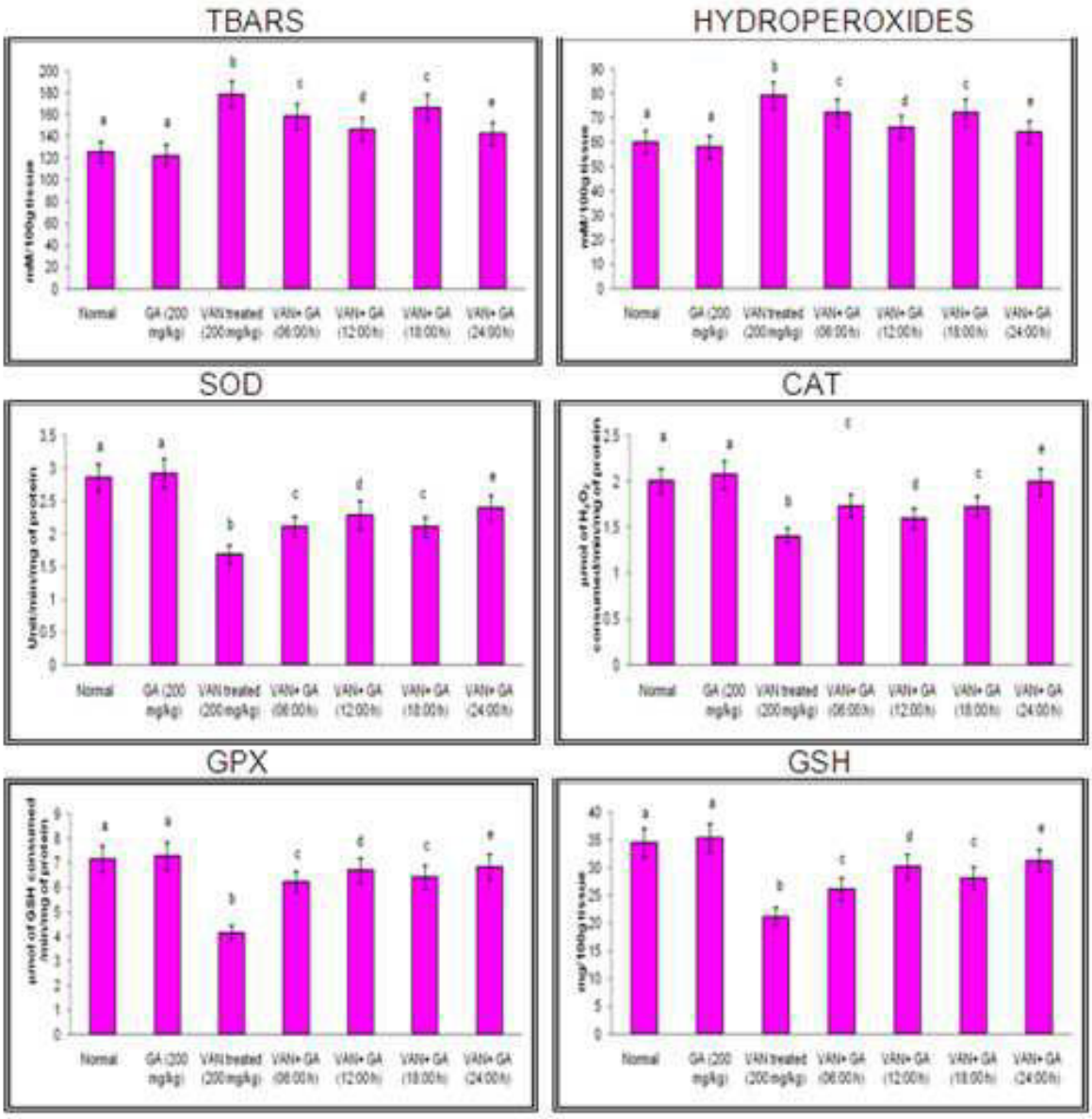
Chronotherapeutic effect of Gallic acid on changes in the TBARS,Hydroperoxides, SOD,CAT,GPX and GSH of normal and experimental rats in circulation



Values are given as mean ± S.D from six rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Graph 3

Chronotherapeutic effect of Gallic acid on changes in the TBARS, Hydro peroxides, SOD, CAT, GPX and GSH of normal and experimental rats in tissue



Values are given as mean ± S.D from six rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

DISCUSSION

Circadian rhythms are approximately 24hrs oscillations, which govern a wide variety of biological functions such as endocrine secretions, metabolism, cell division, renal activity, blood pressure, heart beat, visual activity, enzyme levels, body temperature *etc* [18,19]. A number of biochemical variables are found to be circadian in nature [20]. The biochemical variables chosen for this study exhibit marked fluctuations over the 24 hrs period and the results of the present study indicated that control and VAN treated group rats differ in the temporal characteristics. Alterations in period, amplitude, measure and acrophase were detected in DNA synthesis of spleen, liver and bone marrow of diseased mice [21]. Rhythmic alterations include diminished amplitude, phase shifts, period changes and erratic peak and troughs in endocrine, metabolic, immunological and rest – activity cycles [22]. Our results revealed that the rhythms in animals are not synchronized/exhibited at phasing with that of normal rats. This lack of synchronization reflected as an alteration of circadian clock function in nephrotoxic rats and may require specific measures for

chemotherapy to improve the therapeutic index of drugs. The diminished GSH levels and decreased activities of SOD, catalase and GPx in nephrotoxic rats reflected that decreased values could be due to over utilization of these antioxidants to scavenge the products of lipid peroxidation. GSH and catalase exhibit circadian rhythms and showed peaks at 08:00 in experimental animals [20] and the circadian rhythms of SOD and catalase were also previously reported in liver and blood [23]. Circadian fluctuations in plasma [24] and tissue GSH concentrations including the liver, brain [25], heart, stomach [20], kidney [26], gut [27] etc., were reported. However, lipid peroxidation in cell membranes and subcellular organelles has been proposed as a primary mechanism for cellular membrane dysfunction and tissue injury associated with free-radical initiated processes. Elevated concentrations of lipid peroxides may disturb relations between protective and aggressive factors at the tissue and molecular level leading to tissue damage [28]. Although much is known about the chemistry of lipid peroxidation and cellular defense mechanisms, chronobiological studies are needed to quantify the various cellular components involved in these processes to achieve better management, prognosis and treatment. Chronomes of putative anti and pro-oxidants have recently been mapped to explore their putative chronotherapeutic role as markers in cancer chemoprevention and the management of other diseases [29]. The circadian patterns of the TBARS peak time were found to be controlled group in Wistar rats at 19:16 h [20]. Prominent circadian variations of antioxidants in nephrotoxic rats were observed, pointing to an overall decrease in antioxidant defense mechanisms during nephrotoxic. A decrease in enzymic and non-enzymic antioxidants in various diseases including nephrotoxicity has been reported [30]. SOD is the first among the scavenger enzyme series to ameliorate the damage caused in cells by free radicals [31]. Catalase and GPx are involved in the removal of hydrogen peroxides and several other toxic peroxides. These antioxidant enzymes form the primary enzymatic defense against toxic oxygen reductive metabolites. Such metabolites have been implicated in the damage brought about by ionizing radiations, as well as in the effects of several cytostatic compounds [32]. The reduced activity of these primary defensive enzymes could be due to a direct and greater involvement of ROS in the pathogenesis of nephrotoxicity disturbing the prooxidant vs antioxidant ratio, thereby participating in the tissue damage. Palozza *et al.,* [33] reported that the temporal variations in the hepatic concentration of GSH could be responsible for time dependent variations in GPx and other GSH dependent enzyme activities. The demonstration of a circadian rhythm in all variables investigated herein suggests that these variables could also serve as putative markers (i) to optimize the timing of treatment administration and (ii) to assess responses to treatment. The increased lipid peroxides and decreased antioxidant enzyme activities clearly indicate the involvement of free radicals in the etiopathogenesis of the disease. Chronobiological studies provide the capability of therapeutic intervention at a time when this intervention is useful and best tolerated and avoidance when it is not [34]. The chronobiologic approach to treatment, by exploring the rhythmic nature of oxidants and antioxidants, is especially critical and meaningful when potentially damaging or toxic agents have to be used. But far beyond this application, the time factor has to be introduced in just about all aspects of clinical pharmacology and many "time honored" customs like "three times a day" medications will have to be replaced by more meaningful, and often more effective and less toxic chronobiologic treatment schedules [35]. The choice of the "right time" will require chronobiologic knowledge, interpretation and experience since treatment at the "wrong time" can be potentially harmful [36,34].

CONCLUSION

In conclusion, chronotherapeutic effect of gallic acid (24:00 h) in nephrotoxic rats may be due to various influencing factors like (i) the chronopharmacokinetics of Gallic acid showing

significant variation in absorption, distribution, metabolism and renal elimination due to variations in glomular filtration rate (GFR) over 24h period, (ii) temporal variations of metabolic enzymes involved in the degradation of gallic acid, (iii) temporal variations of kidney marker enzymes, lipid peroxidation products and antioxidants, and (iv) 24h variation in bioavailability of Gallic acid. However elucidating the underlying mechanism(s) and further investigations are desirable.

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