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EVALUATION OF COMBINATIONAL TREATMENT WITH QUERCETIN AND VITAMIN E AGAINST AMPHOTERICIN B INDUCED NEPHROTOXICITY

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ABSTRACT Free radical induced oxidative injury can play a key role in amphotericin B induced nephrotoxicity. Quercetin is strong free radicals trapper found in many plants and foods, for example apples, green tea, red wine, and onions. Vitamin E is a fat-soluble vitamin that exhibits significant role as an antioxidant. The present study was aimed to investigate the effect of quercetin and vitamin E against amphotericin B induced nephrotoxicity in rats. Nephrotoxicity was assessed by measuring various marker enzymes including Lactate dehydrogenase (LDH) Cathepsin-D, N-acetyl-β-D glucosaminidase, Alkaline phosphatase, Acid phosphatase, β-D-glucuronidase, Blood urea nitrogen, uric acid, creatinine and protein. The amphotericin B induced nephrotoxic animals exhibited elevation of γ-glutamyltranspeptidase Lactate dehydrogenase, Cathepsin-D, N-acetyl-β-D glucosaminidase, Alkaline phosphatase Acid phosphatase β-D-glucuronidase and lymphocytes levels whereas these enzyme levels were reverted back to near normal upon the administration of quercetin and vitamin E. the levels of urea, uric acid, creatinine and protein levels were increased in nephrotoxic condition and these levels were bring back to near normal upon treatment with quercetin and vitamin E alone or in combination through its antioxidant properties. In addition to that inflammatory markers such as TNF α, Ifi44 and IL-1 α were significantly decreased in quercetin and vitamin E alone or in combination groups.

KEYWORDS : Quercetin, vitamin E, nephrotoxicity, amphotericin B, antioxidant.

INTRODUCTION:

The kidney participates in whole-body homeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and regulation of blood pressure. The kidney accomplishes these homeostatic functions both independently and in concert with other organs, particularly those of the endocrine system. Various endocrine hormones coordinate these endocrine functions; these include renin, angiotensin II, aldosterone, antidiuretic hormone and atrial natriuretic peptide, among others (Ariel Gomez *et al*,1995; John Atherton, 2012).Most of the drugs that can cause acute tubular necrosis are excreted by the kidney; these include aminoglycoside antibiotics (Simmons et al.,1981),amphotericin B, cisplatin (causing renal failure in up to 25% of patients after a single dose) (Madias and Harrington,1978) Aminoglycoside antibiotics, used in severe gram-negative bacteria, cause nephrotoxicity in 10% to 20% of therapeutic courses (Swan, 1997).The mechanism is proximal tubular injury leading to cell necrosis. Binding of these drugs to the proximal tubule depends on amino groups in each aminoglycoside agent. Risk factors include a long duration of treatment, high trough concentrations (>2 mg/L), repeated courses of aminoglycoside therapy a few months apart, advanced age, malnutrition, volume depletion, liver disease, pre-existing renal disease, potassium and magnesium depletion, and concomitant exposure to other nephrotoxic drugs such as amphotericin B, cyclosporine, or diuretics. Gentamicin is the most nephrotoxic of the aminoglycosides, followed in descending order by tobramycin, amikacin, netilmicin, and streptomycin. Amphotericin B is still the gold standard therapy for life-threatening systemic fungal sepsis, but many patients develop acute renal failure associated with urinary magnesium and potassium wasting, hypokalemia, renal tubular acidosis and polyuria due to nephrogenic diabetes insipidus (Wingard *et al*, 1999).The nephrotoxicity is related to direct tubular damage by deoxycholate used as a solubilizing agent for amphotericin B as well as renal vasoconstriction (Hamer and Nahas, 2006).

Epidemiological data advocate that an elevated intake of quercetin containing foods may decrease the risk of developing several types of cancers (e.g. lung, gastric, pancreatic and ovarian cancer) and cardiovascular diseases. But the comparatively low number of studies and the possible presence of other bioactive constituents in quercetin containing foods make these data insufficient to draw any conclusion regarding the possible protective effect of quercetin in these diseases. Several experimental data have shown, however, that quercetin possesses a wide range of biological activities involved in the avoidance and treatment of these and other diseases. The ability of quercetin to decline superoxide anion, hydroxyl radical and peroxinitrite levels at sub micro molar concentrations may play a key role in its antioxidant activity. Quercetin has also been found to wield antioxidant effects through other mechanisms of action, although most of these effects have been observed at higher concentrations (Abdulrahman *et al*., 2018). Quercetin can inhibit the activity of enzymes that generate ROS, such as the enzyme xanthine oxidase (Ozyurek et al, 2009).Like other flavonoids, quercetin can also decrease the formation of hydroxyl radical through the Fenton's reaction by chelating ferrous or cuprous ions (Mira et al,2002; Ren et al,2008).

Interferons (IFNs) are significant cytokines complicated in inflammatory process. Ifi44 was refereed as microtubule-associated protein 44 and it has been revealed to have antiproliferative activities. Ifi44, also termed p44 or interferon-inducible protein 44 as it collections to make microtubular forms, which is portion of the type I IFN-inducible gene group. Consequently, Ifi44 could be connected with the inflammatory response complicated in gentamycin associated nephrotoxicity. Therefore the present study was aimed to investigate the role of quercetin and vitamin E alone or in combination against amphotericin B induced nephrotoxicity in experimental rats.

MATERIALS AND METHODS:

Chemicals and reagents:

Amphotericin B, quercetin, and vitamin E were obtained from Sigma, USA. All the chemicals and reagents used in the experiments were of analytical grade.

2.1. Experimental animals

Experimental Wistar albino rats with body weight of 180±20 g, were used for tis investigation. Animals were familiarized and accommodated under organized conditions (12 h light/12 h darkness) and temperature of 20–25°C. Normal animal pellet diet and water *ad libitum* were given to the animals throughout the experimental duration. The present investigation was agreed by the Institutional Animal Ethics Committee (IAEC: VMKVMC/02/2017).

2.3. Experimental groups

The experimental animals were divided in to five groups of 6 rats each. All the rats will be housed in polypropylene cages and will be maintain 12 hours light and dark cycle with constant temperature and humidity. The animals were fed with commercial rat diet with water *ad libidum*. Group 1 was administered with vehicle and group 2 with amphotericin B; Group 3 amphotericin-B + Quercetin; Group 4 amphotericin-B + Vitamin E, Group5 amphotericin-B + quercetin+ Vitamin E for 45

days. After that 24 hrs urine sample will be collected for biochemical analysis. Then animals will be anaesthetized with anesthetic ether and blood will be collected through retro orbital puncture/tail vein and the serum will be separated and subjected to biochemical analysis.

Biochemical assays

Lactate dehydrogenase (LDH) activity was estimated by the methodology of King (1965a). Cathepsin-D activity was estimated by the methodology of Sapolsky et al., (1973). N-acetyl-β-D glucosaminidase (NAG) activity was determined by the methodology of Maruhn (1976). Alkaline phosphatase was estimated by the methodology of Bergmeyer (1963) as described by Balasubramanian *et al*., (1983). Acid phosphatase was estimated by the methodology of Bergmeyer (1963) as described by Balasubramanian *et al*., (1983), β-D-glucuronidase activity was estimated by the method of Delvin and Gianetoo (1970) Blood urea nitrogen was assessed by the methodoogy of Natelson et al. (1951), uric acid was quantified by the methodology of Caraway (1963), creatinine was estimated by the method of Slot (1965), protein was assessed by the methodology of Lowry *et al*., (1951).

RNA isolation and Gene expression investigations:

The total RNAs were isolated from kidneys tissue by using trizol reagent and the amount was quantified by using a UV spectrophotometer. The extracted RNA has the purity was found between 1.9-2.1 and the cDNA was synthesized by real time PCR methodologies. β actin was used as an internal control and the primers were synthesized by Sigma and were tabulated.

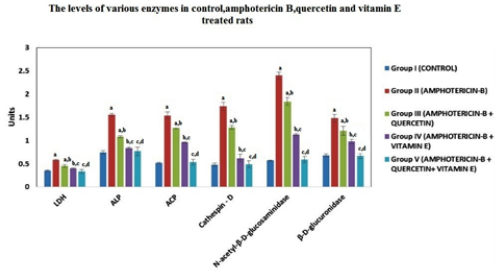
S No	Gene	Forward Primer (5'-3')	Reverse Primer (5′-3′)
1	Ifi44	AGC CGT ATG GAG ACC TGG	TGA GTG ATG CTG CCC TTG
2	TNF-α	CGGGCTCAGAATTTC CAACA	CGCAATCCAGGCCACTAC TT
3	IL-1-α	CATCCGTGGAGCTCT CTTTACA	TTAAATGAACGAAGTGA ACAGTACAGATT
4	β actin	TGAGAGGGAAATCG TGCGTG	TGCTTGCTGATCCACATC TGC

RESULTS:

The actions of several enzymes in the kidney tissues of control, amphotericin B, quercetin and vitamin E treated rats were plotted on the graph. Substantial elevation in the actions of marker enzymes for example γ-GT, LDH, Cathepsin-D, N-acetyl-β-D-glucosaminidase, Alkaline phosphatase, Acid phosphatase, β-D-glucuronidase were observed (P<0.05), which may be due to tubular destruction of the kidney when compared with control animals.

Moreover actions of these marker enzymes were deteriorated back to near normalcy (P<0.05) upon treatment in group III and IV animals when compared amphotericin B treated rats. Moreover actions of these marker enzymes were deteriorated back to near normalcy condition (P<0.05) upon with quercetin and vitamin E treatment in group III and IV animals when compared to amphotericin B treated group of animals. Combinational treatment of quercetin and vitamin E animals exhibited analogous values compared to group I control animals (P<0.05).

Figure 1

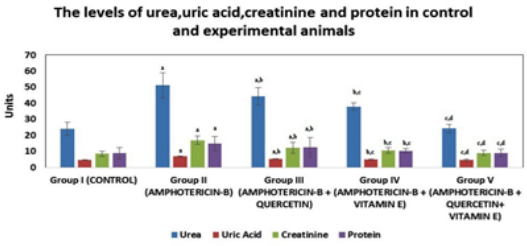


Each bar expressed as mean + SD for six animals in each group

a - Group I Vs Group II, III and IV;
b - Group II Vs Group III and IV;
c - Group III Vs Group IV; d- Group IV Vs Group V

The significance at the level of p<0.05

Figure 2



Each bar expressed as mean + SD for six animals in each group
a - Group I Vs Group II, III and IV;
b - Group II Vs Group III and IV;
c - Group III Vs Group IV; d- Group IV Vs Group V

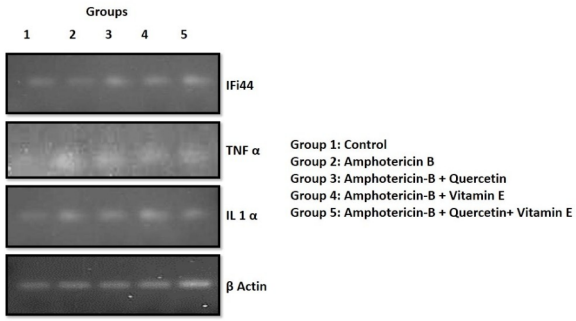
The significance at the level of p<0.05

Figure 2 represent the urinary components levels in urine of control and amphotericin B, quercetin and vitamin E treated rats. Because of renal destruction caused by amphotericin B in group II animals exhibited increased levels of urea, uric acid, creatinine and protein (P<0.05), when matched with group I control animals. Administration of quercetin and vitamin E significantly deteriorated back of the levels of the urinary constituents (P<0.05) in group III and IV animals compared with group II animals. No such notable variations were observed in quercetin and vitamin E alone treated groups while matched with group I control animals.

The blood urea nitrogen, uric acid and serum creatinine in serum of control and amphotericin B, quercetin and vitamin E treated rats were assessed. It was perceived that augmented levels of blood urea nitrogen, uric acid and serum creatinine were detected in the amphotericin B intoxicated group animals compared with group I control animals (P<0.05). Administration of quercetin considerably decreased the levels of blood urea nitrogen (BUN), uric acid and serum creatinine (P<0.05) matched to that of amphotericin B treated group of animals. On the conflicting, the protein levels in serum were significantly decreased (P<0.05) in amphotericin B treated group of animals. Treatment alone and combination of quercetin and vitamin E the protein level was brought back to near normal level when compared to control animals (P<0.05).

The effect of amphotericin B, quercetin and vitamin E on the gene expression levels:

Amphotericin B group showed considerably increased the IL- 1α gene expression in kidney tissues (P < 0.001) compared to control groups. Remarkably, administration of quercetin and vitamin E to amphotericin B treated animals reveals the down regulation of IL-1α expression. The expression of TNF-α in kidney tissues were analyzed. Amphotericin B alone was resulted in substantial decresed in TNF α, Ifi44 and IL-1 α expression level (P < 0.001) compared to control group. The quercetin and vitamin E administered group showed down regulation of TNF α, Ifi44 and IL-1 α gene levels to its normal levels compared to amphotericin B treated animals.



DISCUSSION:

Experimental investigations in canine models and rodents models revealed that amphotericin B deoxycholate infusion initiated renal arterioles vasoconstriction beside with a serious renal blood flow drop

as well as GFR (Sawaya *et al.* 1995). The renin–angiotensin is significant in intervening amphotericin B mediated renal vasoconstriction. It has been showed that the production nitric oxide (NO) may deliberate safeguard alongside amphotericin B mediated renal vasoconstriction (Sawaya *et al.,* 1995). Administration of N-nitro-L-arginine doesn't show any significant consequence on renal blood flow and the GFR reduction detected in an experimental rats of amphotericin B induced nephrotoxicity. A latest investigation revealed that administration of N-acetylcysteine intravenously may enhance the acute renal dysfunctions in rodents persuaded by a single administration high-dose amphotericin B. N-acetylcysteine defense in the investigation seemed to be connected to vasodilatation; there was no substantial defense from amphotericin B induced injury to distal tubule function as reported by Feldman *et al*., (2005). Sawaya *et al* (1995) reported that the arachidonic acid metabolites as well as prostaglandins also be complicated in the pathological consequences of amphotericin B induced nephrotoxicity. The intra-arterial infusion of a thromboxane opponent decreased an action in GFR and renal blood flow connected with amphotericin B infusion in rodents. Vasoconstriction of renal system may be also facilitated by administrative effect of amphotericin B on vascular smooth muscle cells. Amphotericin B exercised thru vasoconstrictor effects on the isolated blood vessels of rat kidneys and endothelium- liberated vasoconstriction established in the investigation was responded by atrial natriuretic peptide, aminophylline, and calcium channel blockers (Sawaya et al. 1991). Pretreatment with verapamil, the calcium channel blocker barred renal vasoconstriction related with amphotericin B infusion in rodents (Tolins and Raij 1988a). Sawaya *et al*. (1995) reported that calcium channel blockers could preclude vasoconstriction might be that amphotericin B induced destruction to cell membranes consequences in depolarization of cell, charted by calcium influx via voltage-gated calcium channels.

Gholampour and Saki (2019) revealed that quercetin prohibited ferrous sulfate- prompted nephrotoxicity and hepatotoxicity as showed by reduced actions of serum liver marker enzymes and reduced serum bilirubin levels, elevated levels of serum albumin, glucose, total protein, cholesterol, and triglyceride, in addition to elevated creatinine elimination and lesser fractional elimination of sodium contents. The defensive consequence of quercetin depend on, at least incompletely, on its anti-oxidative capabilities which results in reduced lipid peroxidation along with iron-chelating capabilities.

The protective effect of quercetin against doxorubicin (DXR) induced nephrotoxicity and cardiotoxicity was investigated in experimental animals. DXR created a substantial raise in the level of malondialdehyde (MDA) and considerably reticent the glutathione (GSH) activity in the heart and the kidney charted by the action of the enzymes catalase (CAT) in the heart tissue with a substantial elevation in the levels of aspartate transaminase (AST), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine and a reduction in serum GSH levels indicating acute cardiac toxicity. Pre-treatment with quercetin considerably reduced the concentration of MDA and amended the reticence of cardiac GSH and CAT activity. Quercetin treatment also considerably amended the AST, LDH, BUN, creatinine and GSH in serum levels of DXR-treated rats. Thus the study reveals that quercetin significantly defend against DXR-induced enzymatic changes in serum, cardiac and renal tissue damage (Abdul *et al*, 2016).
The defensive role of a natural quercetin on snake venom-induced hepatic and renal toxicities in rats was investigated. A single administration of snake venom at the concentration 4.76 mg/kg caused substantial rise in serum biomarkers of kidney and liver function. Quercetin pre-treatment of at the concentration of 10 mg/kg significantly reduced the toxic effects of snake venom on efficient damage in kidneys and liver of experimental animals. Management of quercetin also upturned snake venom -induced reduction in total thiols rise in lipid peroxidation. Therefore it could be concluded that quercetin considerably introverted the acute stage toxic properties of snake venom on kidneys and liver of experimental rats by inhibiting the reactive oxygen species (ROS) in the kidneys and liver. Quercetin is also recognized for its anti-hemorrhagic, anti-edema, anti-inflammatory, properties and consequently recognized against toxicity induced by snake venom (Abdulrahman *et al,* 2018).

Microtubule-associated protein 44 otherwise known as interferon-inducible protein 44 or p44 (Ifi44). Ifi44 is considered to be an inflammatory consensus gene. Therefore, Ifi44 may be connected with the inflammatory conditions involved associated with nephrotoxicity. The levels of TNF-α were elevated followed by cisplatin administration. The elevation of TNF-α in serum were described by Kelly et al. in ischemia-reperfusion injury model. As will be deliberated, increased renal TNF-α expression seems to play a significant role in cisplatin induced nephrotoxicity. Interleukin 1 cytokine family encoded the protein IL 1 alpha. It is a pleiotropic cytokine linked in numerous immune reactions, inflammatory progressions, as well as hematopoiesis. Monocytes and macrophages produced this cytokine as a pro-protein. This could be proteolytically processed and cleaved during cell injury, and consequently induces apoptosis. Tumor Necrosis Factor-α (TNF α) which induces programmed cell death though the TNF-α receptor 1 as well as an intracellular signaling cascade linking both expression of caspase-8 and caspase-3.The present investigations reveals the up regulation of NF-κB and TNF-α, may contribute to the initiation of inflammatory responses by reactive oxygen species. Additionally, IL-1 α with anti-inflammatory response could weaken the TNF-α activation induced through NF-κB pathway (Sato *et al*,1997; Peluso *et al*,2013). The present investigation reveals that the TNF α, IL-1 α and IFi44 expression was significantly decreased against the treatment with quercetin, vitamin E as well as in synergistic effect of quercetin and vitamin E.

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