Anti-inflamatory and anti-oxidant effect of vinpocetine and cilostazol on glycerol induce acute renal injury.

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Abstract

Acute renal failure, also identified now a days as acute kidney injury (AKI), is the unexpected and often abrupt loss of kidney function. Injection of glycerol, which causes myoglobinuria similar to clinical rabidomyolysis and is characterized by rapid increases in blood urea nitrogen and serum creatinine, glycerol-induced acute kidney injury is caused by renal ischemia and myoglobin nephrotoxicity. Vinpocetine is an herbal supplement, It is specific PDE type 1 inhibitor used to treat various neurological disorders, have anti-inflamatory and antioxidant properites. Cilostazol It inhibits platelet aggregation, it has specific PDE-3 inhibition, reduces vascular smooth muscle cell proliferation, and promotes vasodilation. In this study, we investigated effect and mechanism of vinpocetine and cilostazol drugs in animal model of glycerol induce AKI. Rats were divided in five groups, during 14-days trial, control group received 2ml/kg normal saline; induction group received 10ml/kg intramuscular injection of glycerol; vinpocetine group received 5mg/kg via gavage, cilostazol group received 50mg/kg, and combination group received half dose vinpocetine (2.5mg/kg) and cilostazol (25mg/kg). We observed that induction group have higher levels of urea and creatinine as well as increase in their inflammation and oxidative stress levels, also renal tissue show morphological changes typical of AKI, while pretreated with vinpocetine, cilostazol, combination groups reduce glycerol induce acute renal damage.From these trials we found that vinpocetine and cilostazol can reinforce protection of renal rat by inhibition of (Kim-1, NGAL, and NF-kB), and by reducing MDA levels and elevation of GSH levels.

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Introduction

Acute renal failure, also identified now a days as acute kidney injury (AKI), is the unexpected and often abrupt loss of kidney function[(1)], It is a condition that develops rapidly over the several hours or days and typically occurs in critically ill patients who are still in the hospital, Its affects approximately 1-5 percent of all hospitalized patients[(2)]. it considered major problem affect health of human[(3)]. Rabidomyolysis (RM) is a syndrome characterized by skeletal muscle degeneration and muscle enzyme leakage causing high mortality, The development of RM is associated with causes such as crush syndrom, exhaustive exercise, medications (glycerol, gentamycin, amphetamin), infections and toxins[(4)]. Glycerol induce AKI in rat is the most commonly used model for studying this

type of ARF[5]. Injection of glycerol, which causes myoglobinuria similar to clinical RM and is characterized by rapid increases in blood urea nitrogen and serum creatinine [6], which are associated with a significant decrease in GFR within 3 hours of glycerol administration[7].Ischemic injury, tubular nephrotoxicity caused by myoglobin, and the renal actions of cytokines released after rhabdomyolysis consider main cause among the pathogenicity of glycerolinduced myoglobinuric AKI caused by renal ischemia and myoglobin nephrotoxicity[8]. Vinpocetine is an herbal supplement used to treat various neurological disorders such as Alzheimer's and Parkinson's disease[9], that improved oxidative stress through reduction of MDA serum level and elevation of GSH significantly[10], and it reduced glomerular and renal tubular injury via reduction of proinflammatory biomarkers including Kim-1, NGAL, NF-Kb [11]. Also vinpocetine improves cerebral metabolism through increasing of cerebral blood flow and augmentation of oxygen consumption. It is specific PDE type 1 inhibitor which reduces blood viscosity and improves peripheral circulation[11]. Cilostazol inhibits platelet aggregation, reduces vascular smooth muscle cell proliferation, and promotes vasodilation[12]. It has also been shown to have anti-inflammatory, anti-atherosclerotic, and endothelial protective properties. It has specific PDE-3 inhibition decreases cAMP degradation and increases the availability of this signal molecule[13].

Materials and methodes

1- Animals

Thirty wistar albino male rats weight ranged from 120gm to 200gm. animals were placed in cages with free reach to food and water. The cages were located in a calm and temprature controlled environment in which a 12:12 hour light/dark cycle was sustained. The animals were allowed ten days. period to acclimate before being utilized in this experiment. They were handled according to the ethics committee in file NO: 21 approved in the Pharmacy College / Mustansiriyah University at date 29-11-2021.

2- Drugs and chemicals

Vinpocetine obtained from Sigma-Aldrich (USA)dissolved in dimethylsulfoxide DMSO(5mg/ml) because vinpocetine was water insoluble according to manufacturer instruction. Glycerine obtained from SDI (IRAQ), cilostazol obtained from Sigma-Aldrich (USA), it poorly water soluble so dissolved in DMSO and given orally (10 mg/ml) according to manufacturer instruction.

3- Experimental design

The animals were randomly allocated in five groups, each group have six rats, Group 1 (Control group) rats in this groups received distlled water (2ml/kg/day) orally for 14 days and on day 7 normal saline intramuscular injection (IM) in hind limp. Group 2 (induction group) rats received distlled water (2ml/kg/day) and on day 7 received glycerol 50% (10ml/kg) IM in hind limp to induce AKI. Group 3 (vinpocetine group) rats in this group received vinpocetine (5mg/kg) orally for 14 day and in day 7 received glycerol 50% (10ml/kg) IM in hind limp. Group 4 (cilostazol group) rats in this group received cilostazol(50mg/kg) orally for 14 day and in day 7 received glycerol 50% (10ml/kg) IM in hind limp. Group 5 (combination group) received half dose of vinpocetine (2.5mg/kg) and half dose of cilostazol(25mg/kg) orally for 14 day and in day 7 received glycerol 50% (10ml/kg) IM in hind limb. On day 14, labortary animals were euthanized under anesthesia, the administration of 100 mg/kg ketamine and 10 mg/kg xylazine intraperitoneally. Blood is collected from heart by direct cardiac puncture. samples were placed into gel tube and left for 10 min at room tempruture then centrifuged at 3000 rpm for 10 minutes at room temperature for serum separation. The serum was collected in eppendorf tubes and stored at -20C for the estimation of renal function parameters and for other studied biomarkers. Kidney tissue were immediately dissected out, cleaned from the adhering tissues, washed with distilled water

and weighed. Parts of the kidney from each group were taken to be preserved in Ten percent formalin, The fixed specimen of kidney, were processed overnight for dehydration, clearing, and impregnation using an automatic tissue processor. The mounted specimens were observed and were scored under light microscopy. For a semi-quantitative comparison of the structural changes, the abnormalities in the tissue sections were graded from 0 that have normal structure to 3 that have severe pathological changes.

Biochemicals analysis

1- Assessmentof renal proinflamatorymarkers

By measuring levels of (Kim-1, NGAL in serum and NF-kB in tissue homogenate) by using sandwich Elisakits to assess inflammationin accordance with the manufacturerprocedures. The Eliza plate has already been pre-coatedwith rat-specific anti-KIM-1, NGAL or NFkB antibodies. A sample and a standard were mixed together and incubatedto capture specific antigens by bonding antibodies were applied to the wells, the wellswere washed to remove any unbound antigen The anti-Kim-1, anti-NGAL or NF-kB biotinylated detectionAvidin-Horseradish conjugate and antibodiesPeroxidase (HRP) was added to each well, incubated then the unbound components were cleaned.Each well was then filled with substrate, and the bluecolor emerged and stop solution was included that stop the enzyme-substrate reaction, and the color yellow was formed. Color intensity can be adjusted at 450 nm measurement.

2- Assessment of renal antioxidant markers (MDA, GSH)

Oxidative stress assessed by estimation of (MDA,GSH) levels in tissue homogenate by ELISA kit (MyBioSource),After the enzyme conjugate has been properly rinsed out of the wells with PBS or TBS, the TMB substrate is used for color change, TMB reacts with peroxidase activity to form a blue product, which then turns yellow after the stop solution is added. The intensity of the color and the amount of target analyte in the sample are both positively correlated according to the manufacturer's instructions.

Statistical analysis

In the current study data was presented as mean with standard deviation (SD), and a one-way ANOVA test with a post-hoc test was used to determine the significance of differences between variant groups. Statistical results obtained through the use of social science statistical packages (SPSS). When the P-value was 0.05, the levels of significance were considered.

Results

1- Effects of vinpocetine and cilostazol on renal inflammatory markers.

A) kidney injury molecule-1 (Kim1)

Statistical results show that kim-1 levels significantly increased *(p<0.05)* in mean concentration of induction group (1244 ± 272) compaired with control group (225 ± 45.77), and when given vinpocetine reduce kim-1 levels (534 ± 198) significantly compared with induction group (1244 ± 272), vinpocetine groups show no significant differnces will all other group (control *p=0.1*, cilostazol *p=0.949,* combination *p= 0.274*). Cilostazol group show nearlly same results of vinpocetine group that reduce kim-1 levels (446 ± 297) significantly compared with induction group (1244 ± 272), cilostazol groups show no significant differnces will all other group *(control p=0.383, vinpocetine p=0.949, combination p= 0.681).* better result showen with combination group, that reduce kim-1 levels (286 ± 131) significantly and approach to control group (225 ± 45.77). as showen in table (1) and figure (1),(2),(3).

Table (1): Effect of vinpocetine and cilostazol on (Kim-1), (NGAL) and (NF-KB)

Study group	Kim1	NGAL	NF-kB
Control	225 ± 45.77 [a]	312 ± 32.4 [ab]	1.745 ± 0.415 [ac]

Induction	1244 ± 272 [b]	569 ± 190 [b]	4.315 ± 1.044 [b]
Vinpocetine	534 ± 198 [a]	296 ± 115 [ab]	2.188 ± 0.527 [a]
Cilostazol	446 ± 297 [a]	487 ± 199 [ab]	2.384 ± 0.763 [a]
Combination	286 ± 131 [a]	221 ± 28 [b]	1.040 ± 0.304 [a]

- *Each value represent mean ± SD*
- *Statistical analysis was done by using one-way ANOVA followed by the post hock test*
- *Different lower-case letters indicate significant differences between groups (p<0.05)*

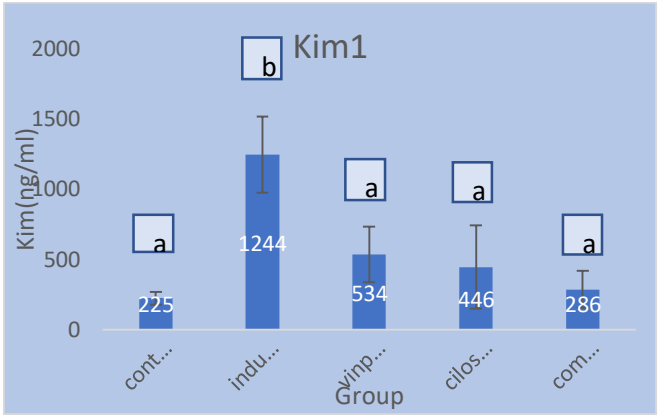

Figure (1): Effect of vinpocetine and cilostazol on (Kim-1) levels.

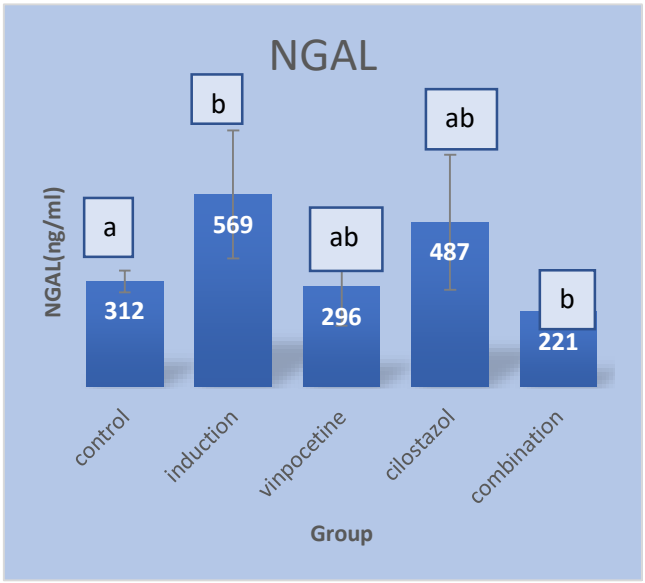

Figure (2): Effect of vinpocetine and cilostazol on NGAL levels.

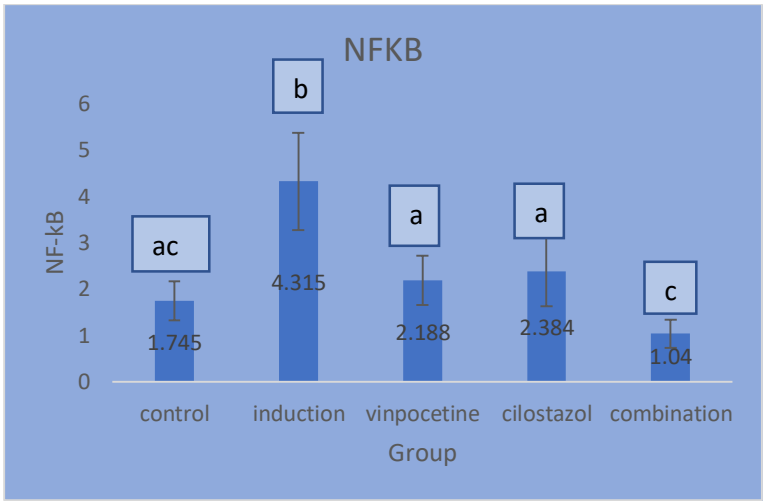

Figure (3): Effect of vinpocetine and cilostazol on NF-k B levels.

B) Neutrophil Gelatinase Associated Lipocalin (NGAL)

Statistical results show that NGAL levels significantly increased in mean concentration of

induction group (569 ± 190) compaired with control groups (312 ± 32.4), and when adminstration vinpocetine reduce NGAL levels significantly compared with induction group *(p<0.05)*, vinpocetine no significant reduction NGAL levels in mean concentration in compare with control group *(p=1.000)* and show no significant differnces with other group *(cilostazol p=0.160, combination p= 0.882).*Cilostazol group show no significant reduce NGAL levels (487 ± 199) compared with other groups except with combination group show significant differences *(p<0.05)*, better result showen with combination group, that significantly reduce (p<0.05) in mean concentration of NGAL levels (221 ± 28) in compaired with induction group, but show no significant differences with control group (p=0.792),as shown in table (1) and in figure (2).

C) Nuclear Factor Kappa B (NF-κB)

Regarding to NF-kB levels results show significantly increased (p<0.05) in mean concentration of NF-kB in compaired with induction group (4.315 ± 1.044), and adminstration vinpocetine significantly reduce *(p<0.05)* NF-kB levels in mean concentration (2.188 ± 0.527) compared with induction group (4.315 ± 1.044), vinpocetine groups show no significant differnce with control group p=0.778, and with cilostazol group p=0.986, but show significant differnces with combination group *(p<0.05).*Cilostazol group significantly reduce *(p<0.05)* NF-kB in mean concentration (2.484 ± 0.763) in compaired with induction group and with combination group *(p<0.05)*, but cilostazol give no significant differences with control group *(p=0.475)*, and with vinpocetine group (p=0.986). Statistical results showen with combination group, that significantly reduction *(p<0.05)* in NGAL levels (1.040 ± 0.304) in compaired with control group (1.745 ± 0.415), but show no significant differences with control group (p=0.377), As showen in table (1) and figure (3).

2- Effect of vinpocetine and cilostazol on oxidative stress markers.

A) glutamylcysteinylglycine (GSH)

Glutathione (GSH) levels significantly reduce *(p<0.05)* in mean concentration of induction group (9.116 ± 1.626) compaired with control group (18.676 ± 5.016). Adminstration of vinpocetine elevated GSH levels (11.678 ± 3.228) but remain lower in values than that of control group *(p=0.89)* and with induction group *(p=0.981)*, also vinpocetine show no significant differences *(p=0.981)* in mean GSH level in compaired with cilostazol but statistical result show significant differences with combination group *(p<0.05)*

cilostazol elevated GSH levels no significantly *(p=0.993)* in mean concentation (10.241 ± 2.776) compaired with induction group (9.116 ± 1.626), while cilostazol show significant differences *(p<0.05)* with control group, and cilostazol give significant differences*(p<0.05)* with combination group. combination group give better results that significantly elevated GSH levels (p<0.05) in mean concentration (22.962 ± 7.600) if we compaired it with induction group (9.116 ± 1.626), also combination group show no significant differences (p=0.493) in mean concentration if we compaired with control group, as shown in table (2) and in figure (4).

B) Malondialdehyde (MDA)

MDA levels significantly elevated *(p<0.05)* in mean concentration of of induction group (3.155 ± 0.784) compaired with control group (1.028 ± 0.342). Adminstration of vinpocetine significantly reduce MDA levels *(p<0.05)* in mean concentration (1.64 ± 0.293) if we compaired with induction group (3.155 ± 0.784), also vinpocetine drugs show no significant differences in mean concentration with control group *(p=0.416)*, and no significant differences with cilostazol *(p=0.660)*, and with combination group *(p=0.527)*. cilostazol significantly reduce MDA levels *(p<0.05)* in mean concentation (2.110 ± 0.905) but still lower than induction group (3.155 ± 0.784) and control group (1.028 ± 0.342) while cilostazol show no significant differences with combination group *(p=0.051)*. combination group significantly reduce in MDA levels

(p<0.05) in mean concentration (1.093 ± 0.408) if we compaired it with induction group (3.155 ± 0.784), also combination group show no significant differences in mean concentration if we compaired with control group (p=1.000). as shown in table (2) and in figure (5).

Study group	MDA(µmol/ml)	GSH (µg/ml)
Control	1.028 ± 0.342 [a]	18.676 ± 5.016 [ad]
Induction	3.155 ± 0.784 [b]	9.116 ± 1.626 [b]
Vinpocetine	1.64 ± 0.293 [ac]	11.678 ± 3.228 [ab]
Cilostazol	2.110 ± 0.905 [cd]	10.241 ±2.776 [b]
Combination	1.093 ± 0.408 [ad]	22.962 ±7.600 [d]

- *Each value represent mean ± SD*
- *Statistical analysis was done by using one-way ANOVA followed by the post hock test*
- *Different lower-case letters indicate significant differences between groups (p<0.05)*

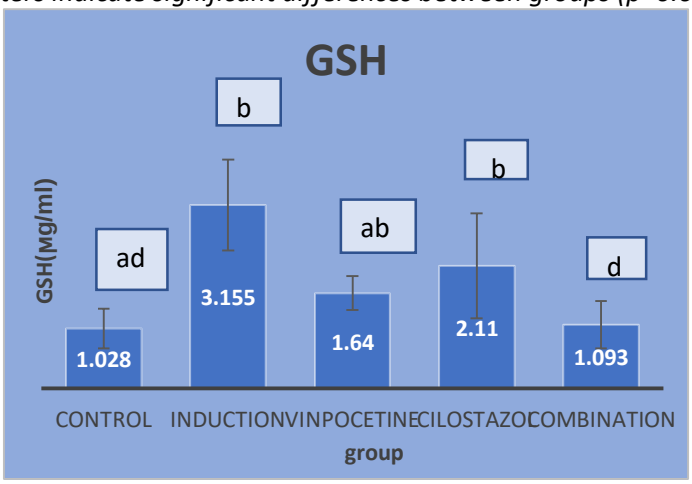

Figure (4): Effect of vinpocetine and cilostazol on oxidative stress markers (GSH) as mean and Std.deviation.

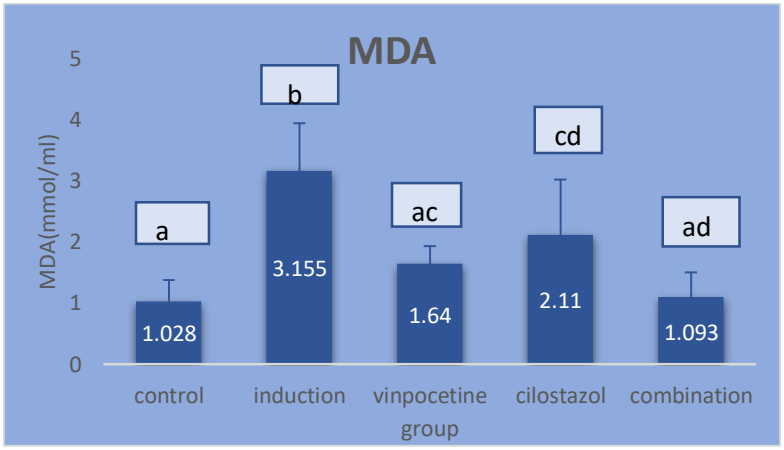

Figure (5): Effect of vinpocetine and cilostazol on oxidative stress markers (MDA) as mean and Std.deviation.

Disscusion

Acute kidney injury caused by glycerol has been showen to increase the production of reactive oxygen species (ROS) and/or deplete the antioxidant defense system due to myoglobin heme causes oxidative stress and lipid peroxidation in proximal tubular cells[14].In present study glycerol significantly increase MDA levels and decrease GSH levels *(p<0.05)* that agreement with previous studies that show glycerol induce ischemia and renal injury that linked to significantly increase in MDA levels, and decrease GSH levels, in addition those researchers attribute the cellular antioxidant defense system's diminished response to ROS overproduction, increased lipid peroxidation, and downregulation of associated gene expression, which was also demonstrated in previous studies [6][15]. Pretreatment with vinpocetine, cilostazol, and combination (vinpocetine + cilostazol) significantly reduce MDA levels, and elevation of GSH levels *(p<0.05)* to value near to control groups, these facts reinforces protective effect of these two agents on oxidative stress markers as in many previous studies that show GSH levels in renal tissue significantly elevated and MDA levels significantly reduced after adminstration of vinpocetine drugs, that found that vinpocetine inhibits reactive free radical generation, which aids in the reduction of high glucoseinduced oxidative damage [10][16]. Also this result agree with previous studies that found cilostazol significantly restored ROS balance by decreasing MDA levels while increasing GSH activity [17][18].In present study, using glycerol as models for induction of AKI that cause significant elevation *(p<0.05)*in inflammatory markers in serum (NGAL, Kim-1), and in tissue (NF-kB) this resultconsist with previous studies that found release myoglobin which is directly toxic to the proximal tubule, causing proximal epithelial cells to produce inflammatory cytokines. Myoglobin exposure induced the activity of the inflammasome component and proinflammatory factors. Myoglobin could directly stimulate pro-inflammatory cells once it enters the renal interstitium [14][19].In the current study, treatment with vinpocetine, cilostazol, andtherecombination (vinpocetine + cilostazol) recorded a significant decline *(p<0.05)* in these inflammatory markers (NGAL, Kim-, NF-kB),this results reinforce protective effect of vinpocetine and cilostazolon inflammatory markersthis results reinforce protective effect of vinpocetine and cilostazol on inflammatory markers.

conclusion

Vinpocetine, Cilostazol and combination (vinpocetine + cilostazol) protected rats from glycerol-induced nephrotoxicity. Renoprotective effect of these drugs could be partially mediated through its antioxidant and anti-inflammatory activity. also combination of vinpocetine plus cilostazol in half dose a for 14 days could be used safely in clinical practice.

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