The Influences of Aminophylline and Indomethacin in Glycerol-Induced Acute Renal Failure in Rats

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Abstract

Background: Adenosine mediates homodynamic changes and resulted in the production of acute renal failure (ARF) in female Albino-Wister rats, therefore, adenosine level increases highly in ARF.

Objective: This experiment was designed to investigate the effect of the adenosine antagonist aminophylline and the adenosine agonist indom-ethacin on glycerol-induced ARF.

Method: Glycerol induced ARF was produced by a single dose (10ml/kg, 50%v/v with distilled water i.m) in rats, which were restricted to drinking water. Aminophylline was used in our study in a dose of 25mg/kg, i.p) while the dose of indomethacin was 10mg/kg, i.p), assessment of renal function was done by measuring blood urea nitrogen (BUN), serum creatinine (Scr), and creatinine clearance (Ccr)

Results: Aminophylline exert its protective effect against glycerol induced-ARF by lowering the elevation in concentrations of BUN, Scr, and mortality rate with a markedly attenuation of the sever impairment Ccr. On the other hand, indomethacin potentiates glycerol induced-ARF by significantly increase the elevation in concentrations of BUN, Scr and mortality rate with severely suppressed the decrement in Ccr.

Conclusion: Aminophylline has ameliorating effect on glycerol induced ARF while the prostaglandin inhibitor indomethacin has reversed effect.

Key words: ARF, glycerol, adenosine, aminophylline and indomethacin.

Introduction

Acute renal failure (ARF) is defined as a syndrome of diverse origin characterized by the abrupt reduction of renal function. Impairment in renal function was demonstrated by the significant elevation in concentration of blood urea nitrogen (BUN), serum creatinine(Scr) and markedly increment in mortality rate associated with significant decrease in creatinine clearance(Ccr).[(1)]

Intramuscular glycerol injection causes muscle cell necrosis and haemoglobinuria and provokes local fluid accumulation. After glycerol injection into muscle, virtually all rats deprived of water for 24hr.s will develop ARF.

The earliest changes in renal function include a pronounced fall in renal blood flow and fall in glomerular function. These changes are reversible if volume expansion is produced within 6hr.s of glycerol administration.[(2)] However, when the same volume load is given (18-20hr.s) after glycerol injection or during maintenance phase of glycerol-induced ARF, the fall in GFR doesn't improve even though the renal blood flow is returned to normal.[(3)]

A marked increased in both BUN and Scr was seen in rats injected with glycerol and this effect was reproducible and associated with no mortality within 24hr.s.[(4)]

Numerous investigators have docum-ented that renal ischemia is commonly observed early in the course of glycerol -induced ARF and this ischemia has been implicated as responsible for the cellular insult. Renal ischemia results in rapid decrease in tissue ATP and a rises in the ATP degradation products: adenosine, inosine and hypoxanthine.[(5)]

Most organs exhibit a post hypoxic vasodilatation and increased blood flow due to adenosine accumulation but the kidney frequently exhibit post hypoxic vasoconstriction and there is good evidence that this is also mediated by adenosine release and accumulation during the hypoxic period.[(2)]

Method

Fifty seven Albino-Wister female rats were used in this study the animals' age was ranged from 10-15weeks with a corresponding body weight of 100-200gm for each. The animals were divided into four groups:

First group- control group.

Second and third groups-drug group and drug treated glycerol group.

Fourth group-glycerol group.

First group-control group: composed of ten rats, the animals were received normal saline (10ml/kg i.m.).

Second and third groups- drug group and drug treated glycerol group:

1.Indomethacin group (adenosine agonist):

Seventeen rats were used in this experiment, among them twelve rats were subjected to the induction of ARF by glycerol (10ml/kg, 50% v/v

with distilled water, i.m) with restriction of drinking water for 24hrs. All rats were received indomethacin (10mg/kg i.p) in two doses.
The first dose was administered just before the induction of ARF by glycerol while the second dose was administered 24hrs.later.
2. Aminophylline group (adenosine antagonist):
Twelve rats were received aminophylline (25mg/kg i.p), among them seven rats were subjected to the induction phase of ARF by glycerol after 40-60min. of aminophylline administration.
Fourth group- glycerol induced acute renal failure group:
ARF was produced in eighteen rats by an intramuscular injection of glycerol (10ml/kg 50% v/v distilled water) as a single dose under light ether ansthesia.The administrated dose was divided in two portions each one was administrated in a hind limb, the blood samples were collected after cutting the tip of the rat tail and renal function was assessed by measuring blood BUN, Scr and Ccr in collecting blood samples

Results

Effect of adenosine antagonist (Aminophylline) on glycerol induced-acute renal failure:
Figures (1, 2 and 3) show changes of Scr, BUN, and Ccr respectively on 1st, 3rd and 8th days after glycerol administration.
On the 1st day in aminophylline glycerol treated group, the concen-tration of Scr and BUN decreased markedly to 1.47± 0.046 mg/dl ($p<0.001$) and 65.039± 0.972 mg/dl ($p<0.01$) respectively as compared with 3.025± 0.036mg/dl and 73.825± 1.293mg/dl in glycerol treated group respectively (Fig.1 and 2), Ccr was increased markedly from 0.601± 0.007 ml/min/100gm in glycerol treated group to 3.447± 0.117ml/min/ 100gm ($p<0.001$), (Fig. 3).
On 3rd day, the concentration of Scr and BUN were lowered to 1.237± 0.047mg/dl ($p<0.01$) and 47.398± 1.422mg/dl and ($p<0.001$) respectively as compared with 3.22± 0.062mg/dl and 105.89± 0.926mg/dl in glycerol treated group (Fig.1 and 2), Ccr was reduced on 1st day but it was remained higher than in glycerol treated group 0.524± 0.021 ($p<0.05$) versus 0.257± 0.11ml/min/100gm in glycerol treated group (Fig .3).
On the 8th day, Scr and BUN were decreased markedly to subnormal values as 0.709± 0.038mg/dl ($p<0.01$) and 23.966± 0.726mg/dl ($p<0.001$) respectively compared with 1.309± 0.007mg/dl and 55.84± 1.53mg/dl in glycerol treated group respectively (Fig.1 and 2). Ccr was 1.635± 0.049ml /min/100gm ($p<0.001$) as compared with 0.169± 0.009ml/min/ 100 mg in glycerol treated group (Fig .3).
At the end of the experiment, no death in rats was recorded (0% mortality rate) as compared with glycerol alone (50% mortality rate).
Effect of adenosine agonist (Indomethacin) on glycerol -induced acute renal failure:
Figures below show changes of Scr and BUN on the 1st, 3rd and on 8th day after ARF induced by glycerol.
In the indomethacin treated glycerol group, the elevation of those parameters was higher than those in glycerol group.
On the 1st day the concentration of Scr and BUN in indomethacin group were increased markedly to 1.341± 0.08mg/dl ($p<0.01$) and 38.632± 1.378mg/dl ($p<0.001$) respectively as compared with 0.381± 0.065mg/dl and 15.85 ± 0.802 mg/dl in control group respectively (Fig.4 and 5), Ccr was reduced markedly to 0.038± 0.002ml /min/100gm ($p<0.001$) as compared with 4.077± 0.25ml/min /100gm in control group (Fig.6)(Table 2), also the concentration of Scr and BUN in indomethacin treated glycerol group were increased markedly to 3.467± 0.047ml/ min/ 100gm ($p<0.05$) and 159. 59± 1.375 ml/dl ($p<0.001$) respectively as compared with 3.011± 0.037mg/dl and 77.94 ± 1.09 mg/dl in glycerol group respectively (Fig.4 and 5), Ccr was reduced significantly to 0.125±0.002 ml/ min/ 100gm ($p<0.01$) as compared with 0.698±0.0085 ml/min/100gm in glycerol group (Fig.6) (Table2).
On 3rd day Scr and BUN in indomethacin group were increased markedly to 2.301± 0.057mg/dl ($p<0.001$) and 58.14± 1.106mg/dl ($p<0.001$) respectively as compared with 0.42± 0.03mg/dl and 17.117 ± 0.672 mg in control group respectively (Fig.4and5), Ccr was reduced markedly to 0.084± 0.002 ml/min/100gm ($p<0.001$) as compared with 3.611± 0.25ml/min/100gm in control group (Fig.6) (Table 2).
In the indomethacin treated glycerol group, the Scr and BUN were increased markedly to 4.062± 0.043mg/dl ($p<0.05$) and 108.521± 1.341mg/dl ($p<0.05$) respectively as compared with 3.214± 0.044mg/dl and 99.997 ± 2.99 mg/dl in glycerol group respectively (Fig.4 and 5) Ccr was highly reduced to 0.003±0.00004ml/min/100gm ($p<0.001$) as compared with 0.047± 0.0006ml/min/100gm in glycerol group (Fig.6)(Table 2).
On the 8th day of experiment the Scr in indomethacin group had no detrimental effect with control group but BUN was increased

markedly to 39.103± 0.671mg/dl ($p<0.001$) as compared with 17.665± 0.663mg/dl in control group respectively (Fig.4 and 5), Ccr was still reduced significantly to 0.389± 0.028ml/min/100gm ($p<0.001$) as compared with 3.506± 0.235ml /min/100gm in control group (Fig.6) (Table 2).

In the indomethacin treated glycerol group, the Scr had no detrimental effect with that in glycerol, but BUN increased to 77.777± 1.216mg/dl ($p<0.05$) as compared with 61.022± 1.014mg/dl in glycerol group respectively (Fig.4 and 5), Ccr was reduced markedly to 0.05± 0.002 ml/min/100gm ($p<0.001$) as compared with 0.173± 0.0052 ml/min/100gm in glycerol group respectively (Fig.6) (Table 2).

One rat was died in indomethacin group so the mortality rate was 20% ($p<0.01$) as compared with 0% in control group while there are 9 rats were died in Indomethacin treated glycerol group, so the mortality rate was 75% ($p<0.00001$) as compared with 0% in control group or with 50 % ($p<0.0001$) as compared with glycerol group.

Table (1) the effect of Aminophylline on creatinine clearance, blood urea nitrogen and serum creattinine on 1st, 3rd and on 8th day in glycerol induced – ARF

GROUP		CREATININE CLEARANCE ML/MIN./100G	BLOOD UREA NITROGEN MG/DL	SERUM CREATININE MG/DL
1st day	Control	3.537±0.209	16.208±0.843	0.318±0.02
	Aminophylline	1.969±0.114	14.69±0.742	0.549±0.031
	Glycerol	0.601±0.007	73.825±1.923	3.025±0.036
	Aminophylline+Glycerol	3.447±0.117**	65.039±0.972*	1.47±0.046**
3rd day	Control	3.575±0.245	17.950±0.576	0.389±0.028
	Aminophylline	1.635±0.104	26.704±1.004	0.539±0.033
	Glycerol	0.089±0.025	105.89±0.926	3.22±±0.062
	Aminophylline+Glycerol	0.524±0.021**	47.398±1.422**	1.237±0.047*
8th day	Control	3.726±0.279	17.34±0.709	0.441±0.033
	Aminophylline	2.123±0.142	16.435±0.845	0.549±0.036
	Glycerol	1.169±0.009	55.840±1.53	1.309±0.067
	Aminophylline+Glycerol	1.635±0.094**	23.699±0.726**	0.709±0.038

The values represent mean ± S.E.M

***: P <0.01, **: P <0.001 as compared with control group**

Table (2) the effect of Indomethacin on creatinine clearance, blood urea nitrogen and serum creattinin on 1st, 3rd and on 8th day in glycerol induced – ARF

GROUP		URINE VOL. ML/24H/100G	CREATININE CLEARANCE ML/MIN./100 G	BLOOD UREA NITROGEN MG/DL	SERUM CREATININE MG/DL
1st day	Control	5.9+0.3	5.414±0.319	14.84±0.715	0.381±0.195
	Indomethacin	4.8+0.3	0.038±0.002	38.63±1.378 ##	1.341±0.079#
	Glycerol	1.7+0.1	0.698±0.008	77.94±1.09	3.011±0.037
	Indomethacin +Glycerol	1.1+0.1	0.125±0.001***	159.59±1.375***	3.467±0.047***
3rd day	Control	5.8+0.2	3.834±0.0231	16.20±0.631	0.393±0.023
	Indomethacin	4.4+0.2	0.084±0.002 ##	58.14±1.106 ##	2.301±0.057 ##
	Glycerol	1.7+0.1	0.047±0.006	99.99±2.99	3.214±0.044
	Indomethacin +Glycerol	1.0+0.1	0.003±0.004***	108.52±1.341 *	4.062±0.043*
8th day	Control	5.7+0.3	4.707±0.284	18.17±0.492	0.375±0.018
	Indomethacin	5.0+0.3	0.379±0.028 ##	39.10±0.671 ##	0.593±0.04 NS
	Glycerol	1.8+0.3	0.173 ±0.005	61.02±1.014	1.361±0.041
	Indomethacin +Glycerol	0.7+0.1	0.050±0.001 ***	77.77±1.216 *	1.388±0.041 NS

The values represent mean ± S.E.M
#: P <0.01, ##: P <0.001 as compared with control group
***: P <0.05, **: P <0.001, ***: P<0.001 as compared with control group**
NS: Not significant

Table (3): Mortality rate at the end of the 8th day after injecting distilled water in control, glycerol, drugs and drugs with glycerol.

GROUP	MORTALITY RATE	
	No.	Ratio
Control	0/10	0%
Glycerol	9/18	50%
Aminophylline	0/5	0%
Aminophylline +Glycerol	0/7	0%
Indomethacin	1/5	20%
Indomethacin +Glycerol	9/12	75%

The values represent number and ratio.
P <0.01( ), P <0.0001( ) and P<0.00001 ( ) as compared with control group.

Figure (1): Effect of Aminophyline on serum creatinine.

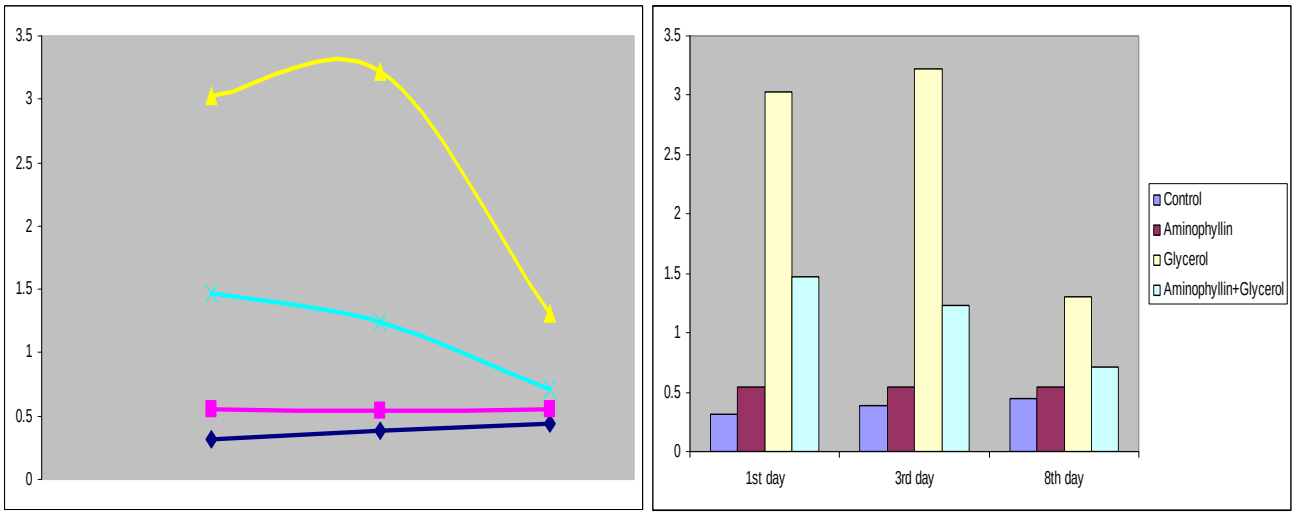


Figure (2): Effect of Aminophyline on blood urea nitrogen.

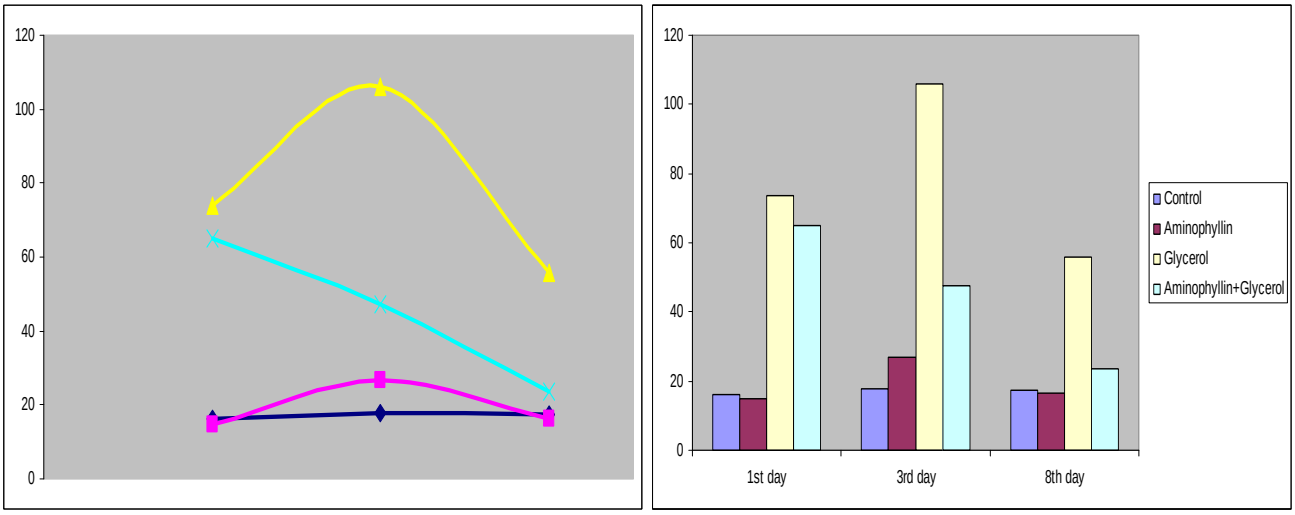


Figure (3): Effect of Aminophyline on creatinine clearance.

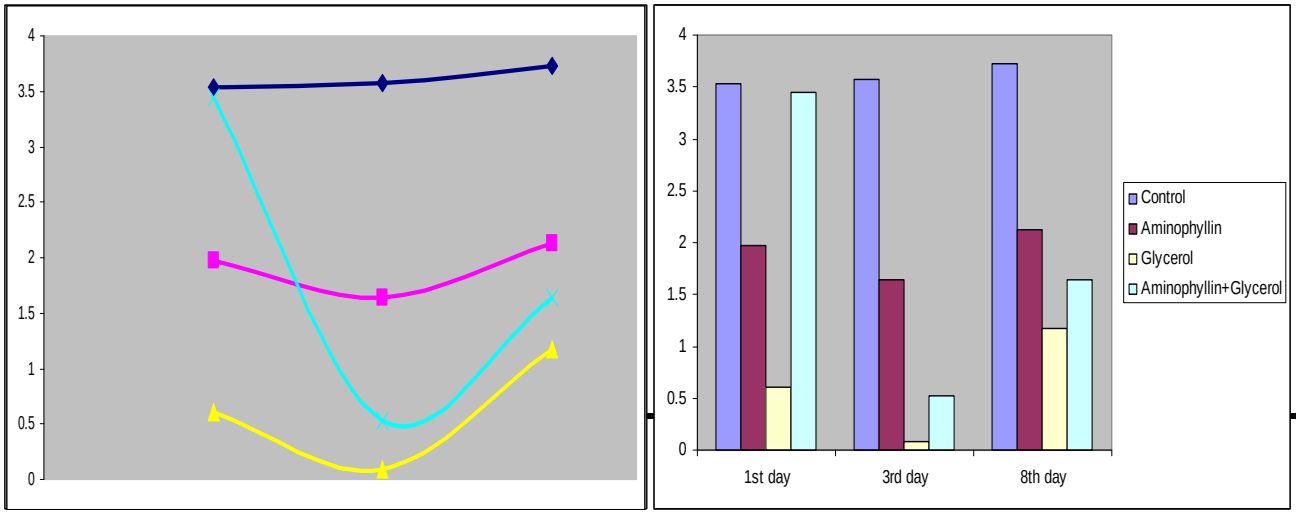


Figure (4): Effect of Indomethacin on serum creatinine.

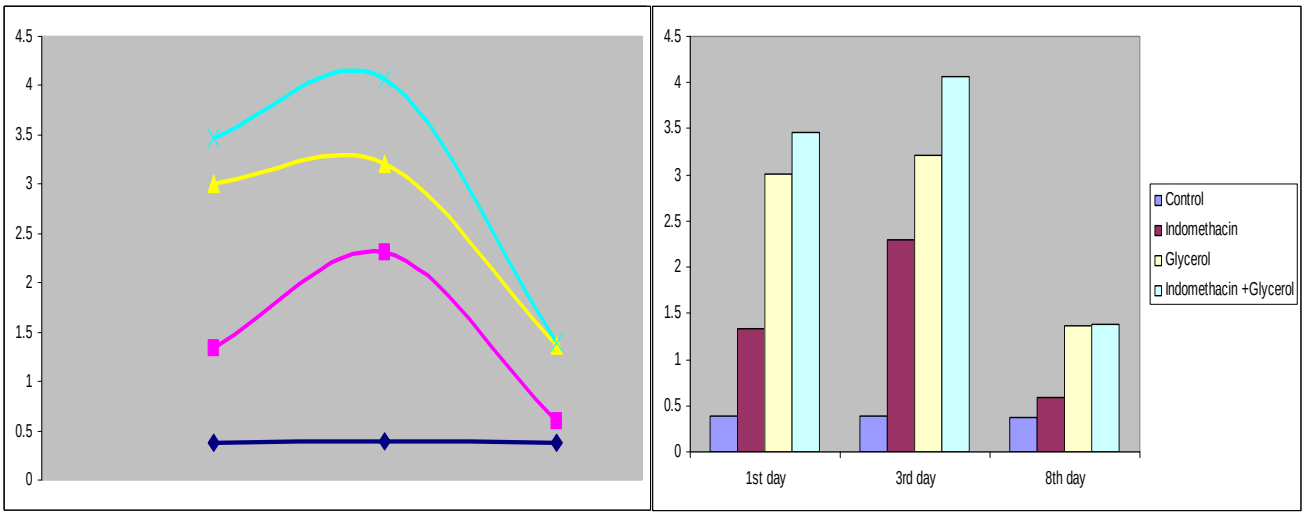


Figure (5): Effect of Indomethacin on blood urea nitrogen.

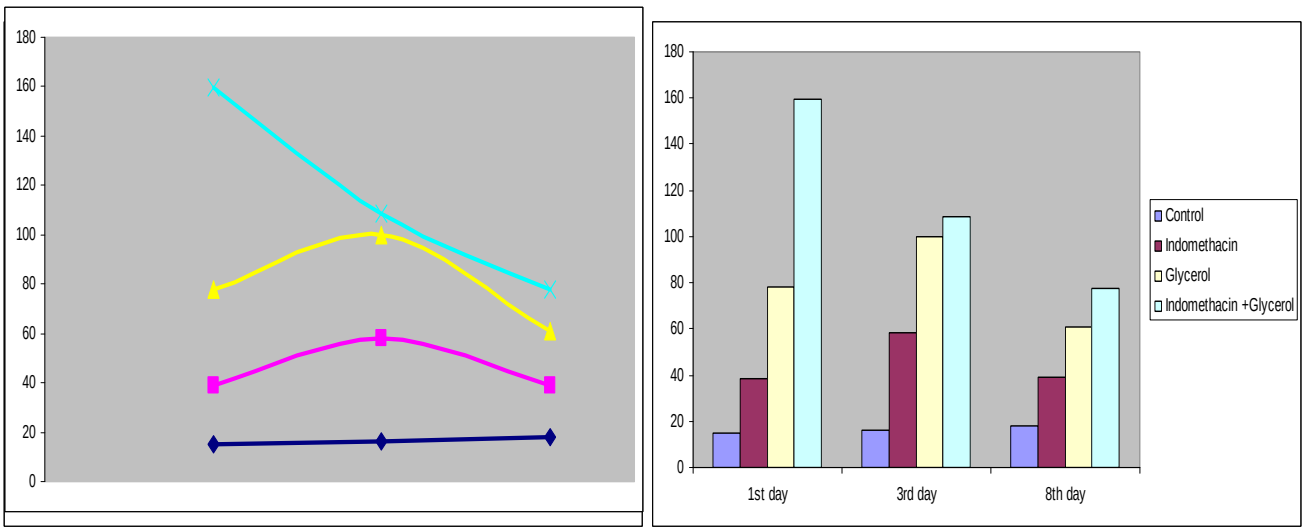
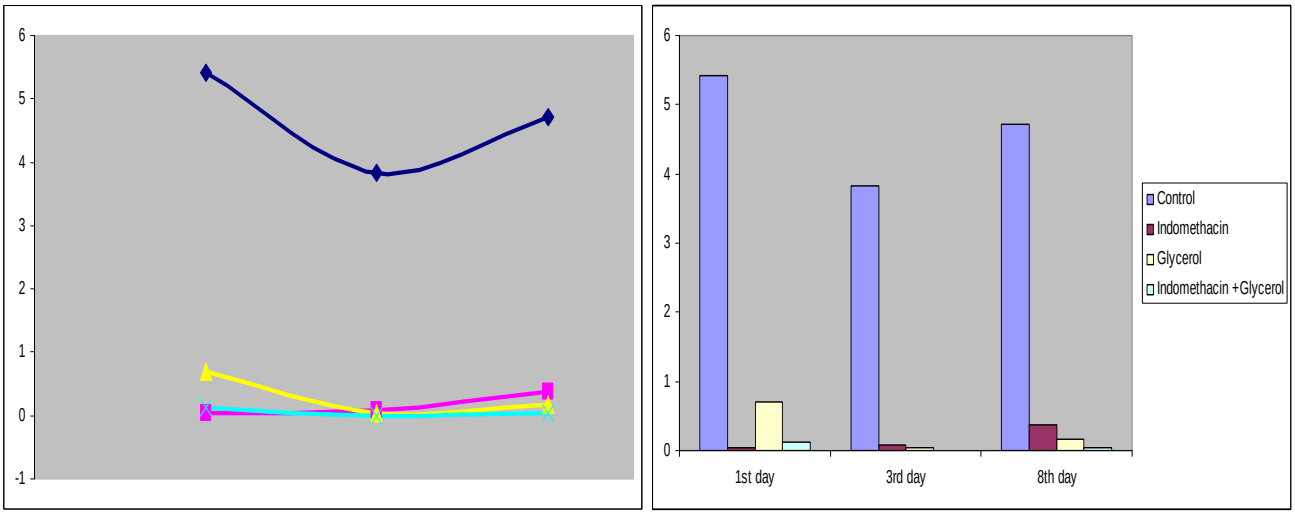


Figure (6): Effect of Indomethacin on creatinine clearance.



Discussion

In the present study aminophylline was effective in ameliorating some of biochemical and functional correlate of glycerol induced-ARF in rats. The result of our study demonstrated that after injection of glycerol Ccr decreased markedly Scr and BUN increased markedly Aminophylline was showed a marked and significant amelioration of the severity of glycerol induced-ARF on 1st, 3rd and on 8th day after induction by glycerol. Also there was no death in rats of this group; there was significant difference with glycerol treated rats.[6, 7]

It is postulated that adenosine has a deleterious effect by increasing afferent arteriolar resistance, probably via an action at the adenosine receptors [8, 9]. Thus, it seems that aminophylline exerted renal protective effects by inhibiting the decrease in renal blood flow caused by adenosine receptor activation.

These findings suggest that the induction of glycerol induced-ARF is largely due to the effect of adenosine released from the glycerol-injected muscle via hemolysis or rhabdo-myolysis.[10, 11]

We also studied the role of indomethacin (adenosine agonist) which prevents or blocks cellular adenosine uptake, thereby increasing the concentration and potentiating the effect of extracellular adenosine.

Scr and BUN concentrations were increased markedly in indomethacin with glycerol treated rats than rats treated with glycerol alone while Ccr was severely decreased on 1st, 3rd and 8th day after induction of ARF by glycerol except Scr; it was not significantly different from glycerol treated rats on 8th day. The mortality rate was highly raised from 50% in glycerol treated rats to 75% in indomethacin with glycerol treated rats. On other hand, the rats treated with indomethacin alone were shown detrimental effect, there was elevation in Scr and BUN markedly, and meanwhile, Ccr was decreased in all days of measurements. Mortality rate was 20% which is significantly differing from normal rats.

Many substances known to antagonize adenosine uptake by some types of cells (dipyridamole, papaverins, sodium diclofenac, meclofenamate) have adenosine like reno-vascular effects, whatever their effects on renal blood flow, they tend to reduce GFR and filtration fraction (that explain why Ccr was severely decreased) [12, 13].

These results showed that indomethacin aggravates the homodynamic changes in ischemic ARF induced by glycerol. These observations provide further evidence for the hypothesis that adenosine mediates homodynamic changes in ischemic ARF and that homodynamic changes account at least partially for the reduced GFR . [14 ,15]

Conclusion

Aminophylline exerts its protective effect against glycerol induced-ARF, by lowering the elevation in concentrations of BUN, Scr and mortality rate with a marked attenuation of the severe impairment of Ccr, while indomethacin potentiates glycerol induced-ARF by significantly increasing the elevation in concentrations of BUN, Scr and mortality rate with severely suppressing the decrement in Ccr.

References

1. Bellomo,R., Ronco,C., Kellum, JA., Mehta, RL; and Palevsky,P. (2004) Acute renal failure-definition, outcome measures, animal models, fluid, therapy and information, technology needs. The second international consensus conference of Acute Dialysis Quality Initiative (ADQI) Group. Crit. Care 8:R204-R212.

2. Mehta, RL. Kellum, JA. And shah, SV. (2007) Acute Kidney Injury Network (AKIN): report pf an initiative to improve outcomes acute kidney injury. Critical care; 11, R31.

3. Molitoris, BA. Levin, A. and Warnock, D.(2007) Improving outcomes of acute kidney injury: report pf an initiative. Nat. Clin. Pract. Neohrol; 3(8): 439-442.

4. Nash, K., Hafeez, A. and Hou, S. (2002). Hospital- acquired renal insufficiency. Am.J.Kidney Dis; 39:930-936.

5. Richard, D. Howland, and Mary J.Mycek (2006) .Anti- inflammatory Drugs and autacoids. Lippincotts Illustrate reviews: Pharmacology. 3rd edition.P495-514.

6. Kellett, R, Bowmer, C.J., Collis, M.G. and Yates, M.S. (1989). Amelioration of glycerol –induced acute renal failure in the rats with 8-cyclopentyl-1, 3- dipropylx-anthine. Br. J. Pharmacology. 98:1047-1066.

7. Mizumoto, H., Karasawa, A. and Kubok. (1993). the diuretic and renal protective effects of 8-roradmantan-3yl-1, 3 dipropylx-anbthine (Kw-3902), a novel adenosine A1- receptor antagonist via pertussis toxin insensitive mechanism. J. Pharmacology Exp. Ther. 266(1):200-206.

8. Rossi, N.F. and Churchill. P.C. (1998). Mechanism of adenosine receptor induced renal vasoco-nstriction in the rat .Am. J. physiol. 255: H 885 – H 890.

9. Hegarty .J. Middleton, R. and Krebs, M. (2005) > sever acute renal failure. Place of

care, incidence and outcomes. QJM, 98:661-666.

10. Ishikawa, I., Naoto, S., Kerichi, T. and Yasushi, S. (1993).Changes of adenosine levels in the carotid artery, renal vein and interior vena cava after glycerol or mercury injection in the rat. Nephron. 64:605-608.

11. Rincon – Sanchez, AR., Covarrubias, A., Rivas – Estilla, AM., pedraza-Chaverri, J., Cruz, C., Islas-Carbajal, MC. And Panduro, A.(2005). PGE.2 alleviates kidney and liver damage, decreases plasma rennin activity and acute phase response in cirrhotic rats with acute liver damage. Exp. Tox. Pathol. 56:291-303.

12. Meunier, F.M. and Morel, N. (1978). Adenosine uptake by cholinergic synaptosomes from torpedo electric organ. J. Neurochemistry. 31: 845.

13. Fernando, JJ. Maria Luisa, RV., Ana Rosa, RS. Maria C.M, et al.(2008). Acute renal failure induced by carbon tetrachloride in rats with hepatic cirrhosis. Annals of Hepatology. 7 (4): 331338.

14. Philips, J.W. (1981) Indome-thacin, ibuprofen and meclofen-amate inhibit adenosine uptake by rat brain synaptosomes. Eur. J. Pharmacy. 72: 139.

15. Sang Kyung, J., Su Young, Y., Kyung Hyun, C., Dac Ryong, C., Won Yong, C., and Hyoung Kyu, K. and Nam Hee, W. (2001) & -MSH decreases apoptosis in ischemic acute renal failure in rats: possible mechanism of this beneficial effect. Nephrol. Dial .transplant.16 : 1583-1591