ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com
Journal DOI: 10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Effect of Gastrointestinal Infection on the Tissue Distribution and Short Term Kinetics of Radio-iodine Labeled Metronidazole and 2Nitroimdazole in Male Albino Mice.

EL-Beih N.M. [1], Mohamed M.I. [2], Seddik U. [2], Hussien H. [2] and Essam H. M[2].

1. Faculty of Science ,Ein Shams University.
2. Nuclear Research Centre, Atomic Energy Authority, Cairo, Egypt.

Manuscript Info

Manuscript History:

Received: 11 May 2016
Final Accepted: 22 June 2016
Published Online: July 2016

Key words:

Corresponding Author

Hussien H.

Abstract

Specific pathological conditions affect the tissue distribution and kinetics of administered drugs; as they may alter the magnitude and duration of drug content in targeted organs Metronidazole (MET) and 2-Nitroimidazole (2-NIM) possess selective activity against many anaerobic gram-positive and Gram-negative bacteria and protozoa causing gastrointestinal infection. The main aim of the present study was to evaluate the gastrointestinal tissue (GIT) distribution of Metronidazole and 2-Nitroimidazole in Escherichia coli-induced gastrointestinal infection in mice. Sixty male albino mice were divided into four main groups; and each were subdivided into 3 sub-groups of five mice. Two main healthy groups were given i.v injection of (^{125}I-2-NIM or^{125}I-MET) and the other two groups were given 0.2 mL of Escherichia coli (E. coli) via oral route for induction of bacterial gastroenteritis 24 hr. before i.v. injection with (^{125}I-2-NIM or ^{125}I-MET). Each of the sub-groups was sacrificed by decapitation under chloroform anesthesia after i.v. injection of the tracer blood by 30 min., 60min., and 180min. Where blood, GIT, liver tissue samples and urine were collected at the time of decapitation; and the radioactivity was measured using a γ-counter. The uptake was expressed as the percent of injected dose per organ or body fluid. Blood was collected for determining complete blood picture (CBC), and Erythrocyte sedimentation rate (E.S.R) and sera from blood samples were separated for determining evidence of inflammatory response and for assessing the possible effects on hepato-renal function and lipid profile. Data from the present study showed also, that both MET and 2-NIM experienced high blood to tissue perfusion after only 30 min. They also showed that while (MET) continued to leak from the blood to the peripheral tissue over 180 min, (2-NIM), underwent a reversed tissue to blood mobilization after 60 min. MET in infected and non-infected mice experienced a similar but a paradoxical tissue to blood equilibration overtime while 2-NIM demonstrated an initial rise in blood and GIT after 30 min that was followed by a parallel leak from both tissues from 60 min. to180 min. in intact animals. GIT infection enhanced the local perfusion of both medications and this was more pronounced in 2-NIM treated animals. Data also demonstrated that more than 60% of injected 2- NIM tracers were excreted in urine after 180 min while only 20% of injected MET tracer was cleared in urine over the same period suggesting more tissue retention of MET than 2-NIM. In conclusion, the present study heralds' different tissue distribution kinetics between MET and 2-NIM with more affinity of MET to GIT tissue. They also infer that 2-NIM experiences a higher clearance rate from the body through the urine than MET. Despite MET was more concentrated in the GIT than 2-NIM, the later was more concentrated upon GIT infection than MET.

Introduction:-

Tissue drug distribution is nonhomogeneous and the tissue specificity manifested by impaired tissue drug penetration was best recognized for central nervous system (CNS) **(Kearney & Aweeka, 1999)**. The barrier mechanism in the CNS and other organs, such as the eye, is the presence of active transport pumps that lead to target site concentrations which, even at equilibrium, are lower than those in plasma **(Barza, 1993)**. This mechanism, which has been well described, both in vitro and in vivo is the reason for therapeutic failures **(Barza, 1993)**.

Physiological changes, such as fluid shifts, alterations in protein binding, and acid-base balance issues, may in turn alter a drug's distribution, potentially towards or away from its site of action **(Deitchman & Derendorf, 2014)**. In the meantime, analysis of drug and metabolite distribution is essential for understanding of the mechanisms underlying the pharmacological or toxicological effects **(Takai &Tanaka, 2015)**. In addition to the variation in tissue affinity for administered drugs, many pathological conditions affect the tissue distribution and kinetics of administered drugs; as they may alter the magnitude and duration of drug content in targeted organs. Disturbance in physiology may alter the pharmacokinetics or pharmacodynamics that determines drug dosing and effect **(Feghali *et al.*, 2015)**. The latest research on the pharmacokinetic of traditional Chinese medicine in the disease state such as diabetes, cerebral ischemia, liver injury, inflammatory disease, nervous system disorders and fever provided certain reference for clinicians designing reasonable administration dose **(Gong *et al*., 2015)**.

Metronidazole is one of the rare examples of a drug developed against a parasite which has since gained broad use as an antibacterial agent **(Freeman *et al.*, 1997)**. The antibacterial activity of metronidazole was discovered by accident in 1962 when metronidazole cured a patient of both trichomonad vaginitis and bacterial gingivitis **(Shinn, 1962).** However, it was not until the 1970s that metronidazole was popularized for treatment of infections caused by gram-negative anaerobes such as bacteroides or gram-positive anaerobes such as clostridia **(Lau *et al.*, 1992 and Freeman *et al.*, 1997).** 2- Nitroimidazole (Secnidazole) has been found to possess selective activity against many anaerobic Gram-positive and Gram-negative bacteria and protozoa **(khan *et al.*, 2015)**.

In general secnidazole and metronidazole were approximately equipotent in activity against Bacteroides fragilis, Trichomonas vaginalis and Entamoeba histolytica. In one study, secnidazole was more potent than metronidazole against Giardia lamblia (G. intestinalis, G. duodenalis. Secnidazole was as effective as metronidazole and tinidazole in eradicating G. lamblia, T. vaginalis and E. histolytica from the majority of infected patients. Bacterial or protozoal resistance develops rarely to the 5-nitroimidazole **(Gillis &Wiesman, 1996)**. Since the use of metronidazole and imidazole usually takes place in patients suffering from diarrhea associated with gastrointestinal infection, the main aim of the present study was to investigate the effect of gastrointestinal infection on the tissue distribution and kinetic of both drugs using the radio-tracer techniques in albino mice.

Methods:-

Mice assigned for infection study received 100µL of the E.coli suspension **(Carla *et al.*, 2011)**. After 24 hours, 0.1 ml of ^{125}I-MET or ^{125}I-2-NIM complex were injected intravenously into the tail vein. Samples of normal Blood, GIT and liver, infected blood; GIT and liver were measured using a γ-counter. The radioactivity ratio in the inflamed to normal (target to non-target, T/NT) was determined **(Douglas *et al.*, 2015)**.

Biochemical Investigations:-

Inflammatory markers in sera including complement 3(C3), complement 4 (C4), C-reactive protein (CRP) were determined by single radial immunodiffusion according (**Thompson *et al*., 1999)** and erythrocyte sedimentation rate ESR) was determined as described by **Van Kampen & Zijlstra, 1961.**

The contents of total bilirubin was measured according to **(Fevery, 2008),** aspartate aminotransferase (ASAT), alanine transaminase (ALAT), gamma-glutamyl-transferase (GGT) and alkaline phosphatase (ALP) were determined calorimetrically as described by **Mengel *et al*., 2005** using commercial kits (Stanbio lab. Inc., Texas, USA). Serum total protein, albumin, globulin and A/G ratio were measured and commercial kits were used

(Diamond Diagnostic and Stanbio lab. Inc., Texas, USA). Lipid profile including total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-cholesterol) and low density lipoprotein cholesterol (LDL-cholesterol) were determined utilizing kits **from (Spinreact)**.

Statistical analysis Student's t-test **(Raju, 2005)** and one way ANOVA **(Howell & David, 2002)** were carried out for the statistical analysis of the data; results are expressed as the mean ± S.D. and significant was shown as:
Insignificant at P (>0.05)
Significant at P (<0.05 - <0.0001)

Results and Discussion:-

The present study was conducted to investigate the possible change in drug tissue distribution of Metronidazole (MET) and 2-Nitroimidazole (2-NIM) induced by gastrointestinal infection utilizing the E.coli infection model in male albino mice. Data from present study showed that E.coli infection efficiently induced significant biological changes consistent with the ensue of bacterial-induced inflammatory response. This was evidenced by the significant rise in erythrocyte sedimentation rate (E.S.R), relative and absolute neutrophil counts and serum C - reactive protein, complement C3 and C4 levels in infected animals. Data also showed also that infection had almost no effect on the biological performance of the liver or the kidney apart from the rise of hepatic enzyme activity table (I). This later effect was most probably as a result of endo-toxemia associated with E.coli infection **(Chaipichit *et al*. 2015)**. It should be considered that hepatocellular damage is expected to be aggravated by local reabsorption of endotoxin through the entero-hepatic circulation **(Syer *et al*., 2015)**. The lipid metabolism was also resilient to the effect of infection as verified by unaltered serum lipid pattern table (I). Following intravenous injection (Dose =200 µl, 2-3 MBq), both compounds underwent high blood to tissue perfusion after only 30 min. 2-NIM, however, experienced higher blood to tissue penetration than MET (95.2%v versus 87.3%). Data suggested marked difference of blood-tissue kinetics between the two compounds. Metronidazole (MET) continued to leak from the blood to the peripheral tissue as supported by gradual decline of the serum level over the experimental period which was consistent with data from **Claude Martin *et al*., (1991)** study conducted in humans. 2-Nitroimidazole (2-NIM), however, underwent a reversed tissue to blood mobilization after 60 min. This seemed transient since after 180 min., blood 2-NIM level manifested a marked percentage decline. This paralled data from **Laughlin *et al*., (1996)** and suggested a different metabolic pathway of Metronidazole and 2-Nitroimidazole.

In Metronidazole injected mice, the GIT metronidazole content represented a true reflection of the drug deployment from the blood to tissue. It also indicated that the drug significantly targeted the GIT tissue which was in accordance with **Claude Martin *et al*., (1991)**. On the other hand, 2-Nitroimidazole GIT tissue content underwent the same blood level change pattern over time i.e initial rise followed by a falling off. Early targeting of GIT tissue following 2-NIM, i.v. infusion has been reported by **Laughlin *et al*., (1996)**. The latter also demonstrated a marked decline of the tissue content overtime. They also showed that the decline was more pronounced in the colonic tissue than in the oseophygeal tissue. Comparative analysis of the pharmacokinetics of MET and 2-NIM in non-infected animals has been previously studied **(videau *et al*., 1978)**. The present study emphasized this analysis in infected mice. Data showed that MET underwent the same blood to tissue mobilization pattern as in non-infected animal as illustrated in figures (1& 2). This was similar to **Lamp *et al*., 1999,** finding which showed that enteric infection did not alter the pharmacokinetic and pharmacodynamics of metronidazole. However, in E.coli infected mice, 2-NIM blood to tissue equilibration favored more blood to GIT mobilization than overtime clearance of both tissues as illustrated in figures (7& 8).This implicated a marked influence of infection on the drug blood-GIT kinetic in favor of the host. Tables (1& 2) discussed the bio-distributions of ^{125}I-MET and ^{125}I-2-NIM in normal and infected GIT (% ID) of male albino mice at 30, 60,180 minutes post injection.

In non-infected mice, MET underwent a more or less steady state concentration within he hepatic tissue. This was also observed in E. coli infected animals. In view of the gradual rise of MET content of the GIT tissue, it may be concluded that the entero-hepatic circulation had contributed in supporting the GIT increase in the drug content in the hepatic tissue. In GIT infected mice, liver metronidazole mounted marginally in comparison to the control level as shown in figures (3& 4). In non-infected mice, 2-NIM underwent steady decline in liver tissue which may suggest that it might had been be the source of the initial blood peak recorded after 30 min. In accordance with **Laughlin *et al*., (1996),** data from the present study supported the early liver targeting by 2-NIM when administered intravenously as illustrated in figures (9 & 10). Tables (3& 4) showed the bio-distributions of ^{125}I-MET and ^{125}I-NIM in normal and infected blood (% ID) of male albino mice at 30, 60,180 minutes post injection.

Recently, it has been demonstrated that 2-Nitroimidazole conjugation with the hepatocyte microsome is stable and does not alter its biological potentials **(Tasneen *et al*, 2015)**. This idealizes the drug fitness for treating amebic hepatitis. In addition, throughout the use of labeled 2-Nitroimidazole to identify hypoxic areas within the tumor tissue, it has been shown that the affinity of the hepatic tissue to 2-Nitroimidazole is much higher than tumor hypoxic tissue **(Engelhardt *et al*., 2002)**.

Unfortunately, data from the present study showed that GIT infection caused continued gradual clearance of 2-NIM from the hepatic tissue along with the blood decline displayed after 30 min as shown in figures (9 & 10). This may reduce the drug efficacy of preventing amebic hepatitis as a complication gastrointestinal amebiasis. Tables (5& 6) illustrated the bio-distributions of ^{125}I-MET and ^{125}I-2-NIM in normal and infected liver (% ID) of male albino mice at 30, 60,180 minutes post injection.

The present study demonstrated that MET excretion in urine parallel its clearance from the blood. Data from the present study suggested a marked difference in urinary clearance of 2-NIM in human and in mice. In a previous study, only 10 to 25 % of infused 2-NIM or its metabolite was recovered of 72 hr collected urine **(Gillis & Wiesman, 1996).** In the present study, more than 60% of injected 2-NIM tracers were excreted in urine after only 3 hrs. In the meantime, only 20% of injected metronidazole tracer was cleared in urine over the same period suggesting more tissue retention of metronidazole than 2-Nitroimidazole as illustrated in figures (5, 6, 11 & 12). Tables (7& 8) showed the Bio-distributions of ^{125}I-MET and ^{125}I-2-NIM in normal and infected urine (% ID) of male albino mice at 30, 60, and 180 minutes post injection.

The present study highlighted the fact that the pathological changes induced by bacterial infection diversely affected blood tissue equilibrium of metronidazole and 2-nitroimidazole. However, gastrointestinal infection with E.coli enhanced local concentration of both medications. In case of ^{125}I-2-NIM, the accumulated activity in the infected GIT at 3 hours post injection was higher by a factor of 2.3 and accumulated activity of ^{125}I-MET in the infected GIT at 3 hours post injection was the highest by a factor of 1.2 as illustrated in tables (1& 2). The increase in the tissue accumulation of both labeled drugs in the infected site versus normal one through the same time interval was consistent with data from a large number of studies emphasizing the role of different pathological conditions in the tissue distribution of drugs. Many situations in which changed drug penetration and blood-tissue in equilibrium have been reported. In particular, antibiotic failures have been attributed to impaired target site penetration in cases of soft tissue infections **(Rylander *et al*., 1979)**, osteomyelitis and orthopedic surgery **(McCullough & Gandsman, 1988)**, peridontitis and odontogenic infections, endocarditis (Mate et al., 1998), septic embolism, foreign body- and catheter-related infections **(Schierholz *et al*., 2000)**, gastric ulcer, hematomas **(Schierholz et al., 2000),** epidermal infections **(Mertin & Lippold, 1997)**, abscesses granuloma-inducing infections and tuberculosis **(Elliott *et al*., 1995)**, prostatitis **(Drach, 1975)**, eye infections **(Briggs *et al*., 1998)**, ear infections **(Ekedahl *et al*., 1978),** tonsillitis **(Savolainen *et al*., 1988)**, sinusitis, liver infections **(Schentag *et al*., 1983)**, urinary tract infections **(Naber, 1978)**, pelvic inflammatory disease **(Onsrud *et al*., 1982),** solid malignancies (Jain, 1998), respiratory tract infections (Rebuck & Braude, 1984), heart-lung bypass surgery, and septic shock **(Joukhadar *et al*., 2001)**. The increase in drug accumulation inside the gastro intestinal tract concomitantly with infection following intravenous administration emphasized their efficiency when given systemically.

However, in contrast to many models in which infection retarded drug tissue targeting , data from the present study demonstrated that GIT infection enhanced tissue uptake of both MET and 2-NIM medications. This was most probably due to accumulated blood in the inflammatory tissue **(Bruijnzeel *et al*., 2015)**. The higher ability of 2-NIM to penetrate the infected tissue than MET; may be explained by the fact that 2-NIM has immunologically identifiable side-chains that have been described to bind to hypoxic cells **(Hodgkiss, 1998)**.
It is well established that bacterial infection of the GIT leads to intestinal inflammation which is characterized by the occurrence of hypoxia of the mucosal surface **(Colgan & Eltzschig, 2012)** and alterations in metabolic supply and demand ratios, particularly for oxygen, leading to "inflammatory hypoxia" **(Eltzschig *et al*., 2012)**.

In conclusion, the present study emphasizes that 2-Nitroimidazole experience a different blood to tissue mobilization kinetics from Metronidazole. It also highlights the ability of Metronidazole and 2-Nitroimidazole to target the gastrointestinal tract when administered intravenously. This eases their uses in conditions in which oral administration is impossible like in patients with coma. Gastrointestinal infection significantly enhanced tissue accumulation of both medications. 2-Nitroimidzole was more efficiently accumulated in infected GIT tissue though it was more rapidly cleared from the body through the urine than Metronidazole.

Table 1:- Results of inflammatory markers (C3, C4, CRP, ESR), liver functions, hematological parameters, protein pattern and lipid profile in normal and infected male albino mice.

Parameters / Groups	Normal mice	Inflamed	t-value	p-value	Significantly
C3, mg dL^{-1}	78.8 ±4.4	152.4 ±1.8	-34.6	< 0.0001	Significant
C4, mg dL^{-1}	8.12 ±0.2	24.8 ±1.3	-28.7	< 0.0001	Significant
CRP, mg dL^{-1}	2.8 ±0.2	6.1 ±0.5	-12.73	< 0.0001	Significant
ESR(1^{st} hour)	4.4 ±0.5	12.4 ±0.5	-25.3	< 0.0001	Significant
Total billirubin	0.46 ±0.08	0.42 ±0.01	1.1094	>0.05	Insignificant
Direct billirubin	0.12 ±0.01	0.13 ±0.08	-0.2774	>0.05	Insignificant
Indirect billirubin	0.35 ±0.08	0.3 ±0.01	1.386	>0.05	Insignificant
ASAT, units L^{-1}	27.4 ±2.4	150 ±1.1	103.8	< 0.0001	Significant
ALAT, units L^{-1}	27 ±2	92.6 ±2.5	-45.8	< 0.0001	Significant
GGT, units L^{-1}	9 ±0.7	39.4 ±0.8	-63.9	< 0.0001	Significant
ALP, units L^{-1}	67 ±5.7	87.4 ±4.9	-6	< 0.01	Significant
Hb%	8.6 ±0.9	7.4 ±0.3	2.8	<0.05	Significant
Platelets count	262 ± 8.4	314 ± 12.9	-7.5534	<0.01	Significant
WBCs	6.6 ±0.7	15 ±1.6	-10.7	< 0.0001	Significant
Lymphocyte%	71.2 ±2.1	31.2 ±1.1	37.7	< 0.0001	Significant
Monocyte%	0.8 ±0.4	4.6 ±0.5	-13.27	< 0.0001	Significant
Neutrophil%	23.2 ±1.9	60.8 ±0.8	-40.7	< 0.0001	Significant
Abs.neutrophil count(x1000)	1.5 ±0.3	9.13 ±1	-16.3	< 0.0001	Significant
Eosinophil%	0.6 ± 0.5	0.4 ± 0.5	-16.34	< 0.0001	Significant
Total protein g/dL	6.2 ± 0.4	5.7 ± 0.3	2.23	>0.05	Insignificant
Albumin g/dL	2.7 ± 0.23	2.68 ± 0.08	0.18	>0.05	Insignificant
Globulin g/dL	2.2 ± 0.18	2.3 ± 0.12	-1.03	>0.05	Insignificant
A/G ratio	1.25 ± 0.16	1.2 ± 0.07	0.64	>0.05	Insignificant
Cholesterol (mg/dL)	134.8 ± 5.3	134.4 ± 5.7	0.11	>0.05	Insignificant
Triglycerides (mg/dL)	127.8 ± 2.1	127.2 ± 2.2	0.44	>0.05	Insignificant
HDL-cholesterol (mg/dL)	27.4 ± 1.8	26.4 ± 1.14	1.05	>0.05	Insignificant
LDL-cholesterol (mg/dL)	79 ± 4.4	81 ± 4.2	-0.58	>0.05	Insignificant

Table 2:- Bio-distribution of ^{125}I-MET in normal and infected GIT (% ID) of male albino mice at 30, 60,180 minutes post injection.

Organ or fluid	30minutes	60minutes	180minutes
Normal GIT	28.5 ± 0.5	33.1 ± 0.6	34 ± 0.6
Infected GIT	30.1±0.5	35 ± 0.6	41 ± 0.2
T_1/NT_1	1.05±0.0	1.05±0.1	**1.2± 0.1**
Significance	Significant at P<0.01	Significant at P <0.01	Significant at P<0.0001

Table 3:- Bio-distribution of ^{125}I-2NIM in normal and infected GIT (% ID) of male albino mice at 30, 60,180 minutes post injection.

Organ or fluid	30minutes	60minutes	180minutes
Normal GIT	8.1 ± 0.5	12.7 ± 2.3	11.5 ± 1.0
Infected GIT	8 ± 0.5	20.7 ± 2.3	23.4 ± 1.1
T_2/NT_2	0.98± 0.0	1.6 ± 0.1	**2.3 ± 0.1**
Significance	Insignificant, P >0.05	Significant, P <0.01	Significant, P< 0.0001

Table 4:- Bio-distribution of ^{125}I-MET in normal and infected blood (% ID) of male albino mice at 30, 60,180 minutes post injection.

Organ or fluid	30minutes	60minutes	180minutes
Normal blood	12.3± 2	7.6 ± 0.0	3.5 ± 0.1
Infected blood	10.4 ± 0.2	4.5± 0.2	3.8± 0.1
T_3/NT_3	0.8 ± 0.0	0.6± 0.1	1.1 ± 0.1
Significance	Insignificant, P >0.05	Significant, P < 0.0001	Significant < 0.0001

Table 5:- Bio-distribution of ^{125}I-2NIM in normal and infected Blood (% ID) of male albino mice at 30, 60,180 minutes post injection .

Organ or fluid	30minutes	60minutes	180minutes
Normal blood	4.8 ± 0.2	8.8 ± 0.8	6 ± 0.0
Inflamed blood	21 ± 0.2	17 ± 0.8	12.5 ± 0.0
T_4/NT_4	4.4 ± 0.0	2 ± 0.1	2.1 ± 0.1
Significance	Significant, P < 0.0001	Significant, P < 0.0001	Significant, P < 0.0001

Table 6:- Bio-distribution of ^{125}I-MET in normal and infected liver (% ID) of male albino mice at 30, 60,180 minutes post injection.

Organ or fluid	30minutes	60minutes	180minutes
Normal liver	9.8 ± 0.2	10.9 ± 0.2	11.7± 0.7
Infected liver	9.9 ± 0.2	10.2± 0.2	12.3 ± 0.2
T_5/NT_5	1.0± 0.0	-0.9 ± 0.1	1.1± 0.1
Significance	Insignificant, P >0.05	Significant, P <0.01	Significant < 0.0001

Table 7 :- (Bio-distribution of^{125} I-2NIM in normal and infected liver (% ID) of male albino mice at 30, 60, 180 minutes post injection..

Organ or fluid	30minutes	60minutes	180minutes
Normal liver	9.2 ± 0.2	8.8 ± 0.2	5.4± 0.1
Infected liver	8.5 ± 0.2	5.7 ± 0.2	5.5 ± 0.1
T_6/NT_6	-0.9± 0.0	-0.6 ± 0.1	1 ± 0.0
Significance	Significant, P <0.01	Significant, P< 0.0001	Significant, P< 0.0001

Table 8:- Bio-distribution of ^{125}I-MET in normal and infected urine (% ID) of male albino mice at 30, 60, 180 minutes post injection.

Organ or fluid	30minutes	60minutes	180minutes
Normal urine	12 ± 2.1	15.9 ± 0.2	20.6 ± 1
Infected urine	13.4 ± 0.8	21.5 ± 0.8	23.4 ± 0.2
T_7/NT_7	1.12 ± 0.0	1.4 ± 0.1	1.13 ± 0.1
Significance	Insignificant, P >0.05	Significant, P< 0.0001	Significant, P <0.01

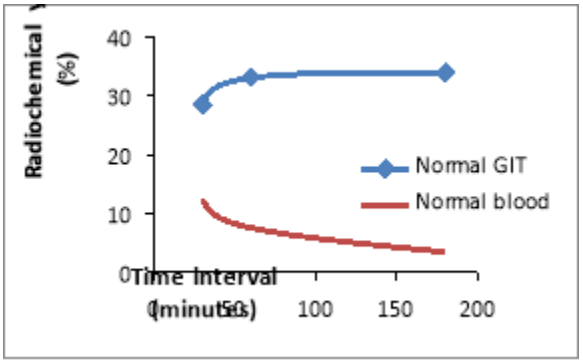
Table 9:- Bio-distribution of ^{125}I-NIM in normal and infected urine (% ID) of male albino mice at 30, 60, 180 minutes post injection.

Organ or fluid	30minutes	60minutes	180minutes
Normal urine	15.6± 0.6	36.2 ± 1.6	61.0± 0.6
Infected urine	5.6± 0.6	30.2± 1.6	44.1 ± 0.6
T_8/NT_8	0.4 ± 0.0	0.8 ± 0.2	0.7 ± 0.0
Significance	Significant, P < 0.0001	Significant, P <0.01	Significant, P< 0.0001

^{125}I-Metronidazole

^{125}I-2-Nitroimidazole

Figures (1- 6) **Figures (7- 12)**



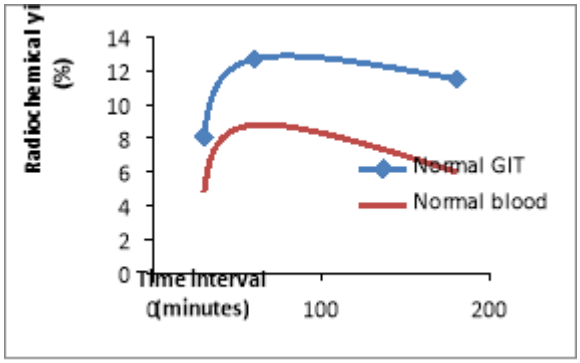
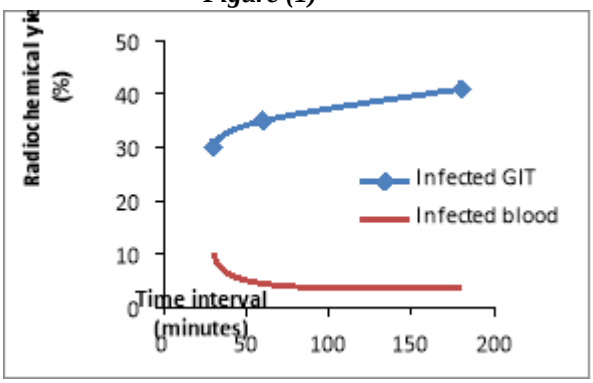


Figure (1) ↔ *Figure (7)*



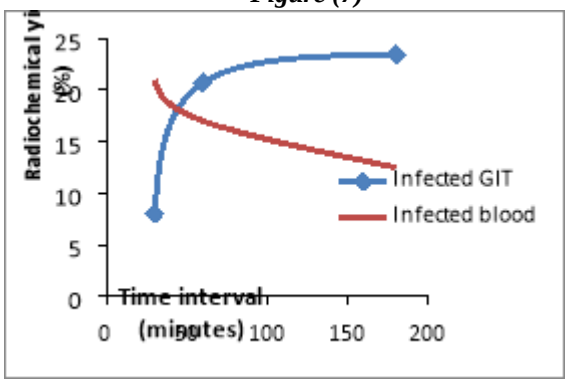
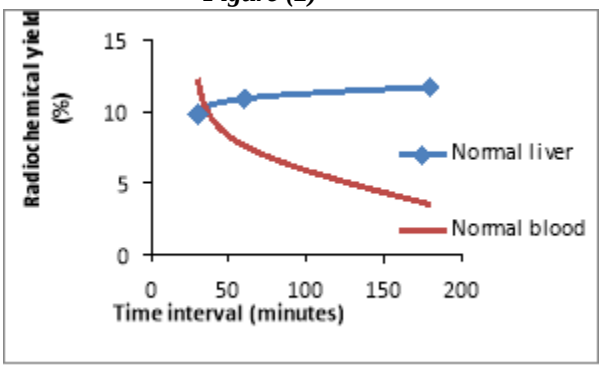


Figure (2) ↔ *Figure (8)*



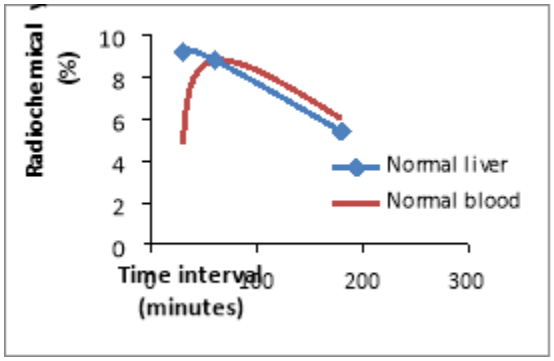


Figure (3) ↔ *Figure (9)*

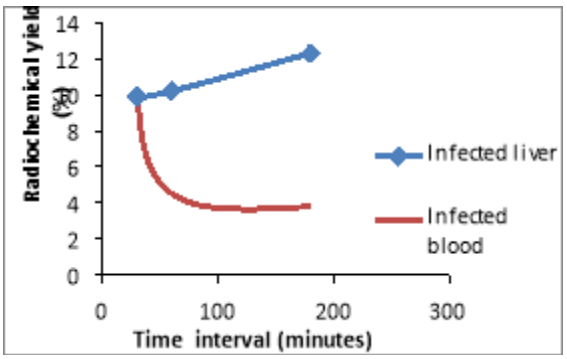


Figure (4)

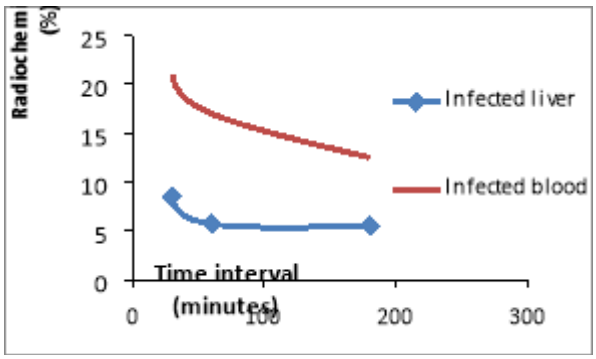


Figure (10)

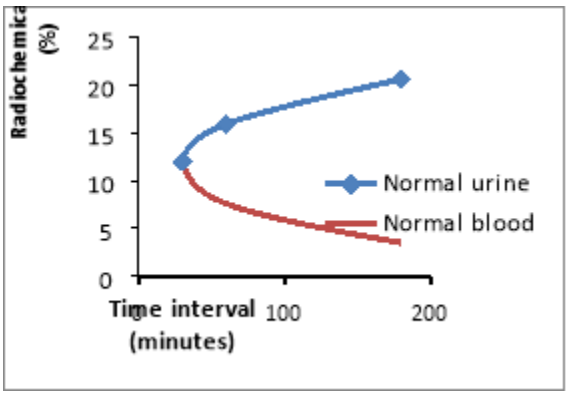


Figure (5)

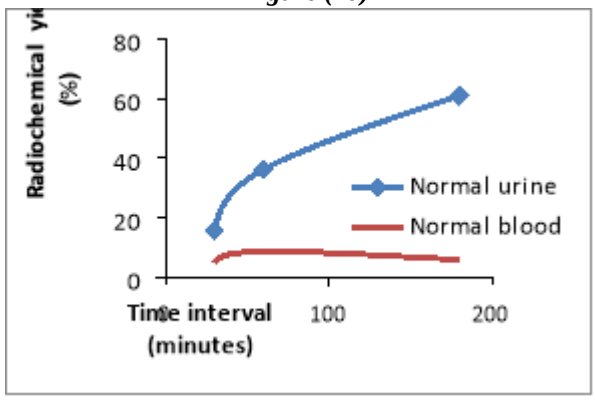


Figure (11)

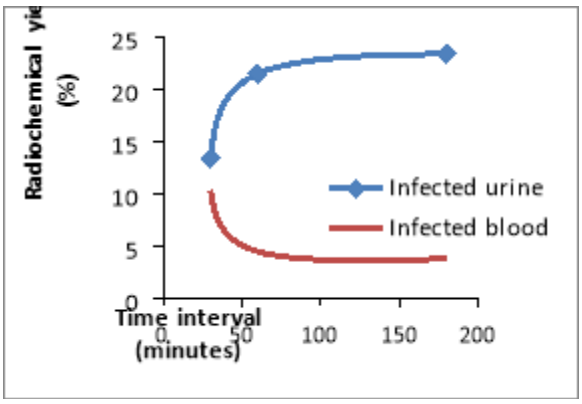


Figure (6)

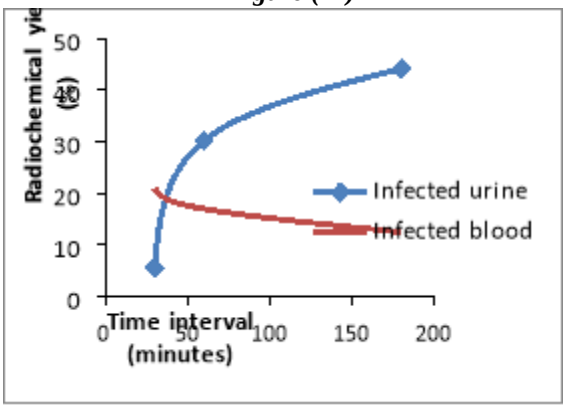


Figure (12)

Figures (1- 6) Blood-tissue kinetics of ^{125}I-MET in normal and infected male albino mice

Figures (7- 12) Blood-tissue kinetics of ^{125}I-2-NIM in normal and infected male albino mice

References:-

1. Barza, M. (1993). Anatomical barriers for antimicrobial agents. Eur. J. Clin. Microbiol. Infect. Dis. 12(Suppl. 1):S31-S35
2. Briggs, M. C., P. McDonald, R. Bourke, G. Smith, J. N. McGalliard, and D. Wong.(1998). Intravitreal penetration of teicoplanin. Eye 12:252-255.
3. Bruijnzeel PL[1], Uddin M[1], and Koenderman L[2] (2015).Targeting neutrophilic inflammation in severe neutrophilic asthma: can we target the disease-relevant neutrophil phenotype? J Leukoc Biol.pii: jlb.3VMR1214-600RR.
4. Carla Calderon Toledo, Ida Arvidsson, and Diana Karpman (2011) Cross-Reactive Protection against Enterohemorrhagic Escherichia coli Infection by Enteropathogenic E. coli in a Mouse Model ▽ Infect Immun. Jun; 79(6): 2224–2233.
5. Chaipichit N, Krska J, Pratipanawatr T and Jarernsiripornkul N (2015) Statin adverse effects: patients' experiences and laboratory monitoring of muscle and liver injuries. Int J Clin Pharm.45:115-23.
6. Claude Martin, Bernard Sastre, Marie Noelle Mallet, Bernard Bruguerolle, Jean Paul Brun, Philippe de Micco and Francois Gouin (1991). Antimicrobial agents and chemotherapy; Pharmacokinetics and Tissue Penetration of a Single 1,000-Milligram , Intravenous Dose of Metronidazole for Antibiotic Prophylaxis of Colorectal Surgery. *American Society for Microbiology.Vol.35(12): pp.2602-2605.*
7. Colgan SP and Eltzschig HK (2012) Adenosine and hypoxia-inducible factor signaling in intestinal injury and recovery. Annu Rev Physiol 74:153–175.
8. Deitchman AN and Derendorf H. (2014) Measuring drug distribution in the critically ill patient. Adv Drug Deliv Rev. 20; 77:22-26
9. Douglas R. Rice, Adam J. Plaunt, Serhan Turkyilmaz, Miles Smith, Yuzhen Wang, Mary Rusckowski, and Bradley D. Smith (2015) Evaluation of [^{111}In]-Labeled Zinc-Dipicolylamine Tracers for SPECT Imaging of Bacterial InfectionMol Imaging Biol. 17(2): 204–213.
10. Drach, G. W. (1975). Prostatitis. Man's hidden infection. Urol. Clin. North Am. 2:499-520.
11. Elliott, A. M., S. E. Berning, M. D. Iseman, and C. A. Peloquin. (1995). Failure of drug penetration and acquisition of drug resistance in chronic tuberculous empyema. Tuber. Lung Dis. 76:463-467
12. Eltzschig HK, Sitkovsky MV and Robson SC *(2012) Purinergic signaling during inflammation. N Engl J Med 367(24):2322–2333.*
13. Ekedahl, C., S. E. Holm, and A. M. Bergholm. (1978). Penetration of antibiotics into the normal and diseased maxillary sinus mucosa. Scand. J. Infect. Dis. Suppl. 14:279-284.
14. Engelhardt, E.L., Schneider, R.F., Seeholzer, S.H., Stobbe ,C.C., and Chapman ,J.D. (2002). The synthesis and radiolabeling of 2-nitroimidazole derivatives of cyclam and their preclinical evaluation as positive markers of tumor hypoxia. *J. Nucl. Med.43(6):837-850.*
15. *Feghali M, Venkataramanan R and Caritis S (2015) Pharmacokinetics of drugs in pregnancy. Semin Perinatol.; 39(7):512-519.*
16. *Fevery J. Bilirubin in clinical practice: a review. Liver Int. 2008; 28(5):592–605.*
17. *Freeman C D ,Klutman N E and Lamp K C (1997) .Metronidazole .A therapeutic review and update .Drugs ,54:679–708.*
18. *Gong ZP, Chen Y, Zhang RJ, Yang Q and Zhu XX.(2015) [Advances on pharmacokinetics of traditional Chinese medicine under disease states]; 40(2):169-173.*
19. *Gillis, J.C. and Wiseman, L.R. (1996) A Review of its Antimicrobial Activity, Pharmacokinetic properties and therapeutic use in the management of Protozoal Infections and Bacterial Vaginosis. Adis International limited, Drugs*; 51 (4): 621-663.
20. Hodgkiss RJ. (1998). Use of 2-nitroimidazoles as bioreductive markers for tumour hypoxia. Anticancer Drug Des.; 13(6):687-702.
21. *Howell, David (2002). Statistical Methods for Psychology. Duxbury. pp. 324–325.*
22. Jain, R. K. (1998). The next frontier of molecular medicine: delivery of therapeutics. Nat. Med. 4:655-657.
23. Joukhadar, C., M. Frossard, B. X. Mayer, M. Brunner, N. Klein, P. Siostrzonek, H. G. Eichler, and M. Müller. (2001). Impaired target site penetration of beta-lactams may account for therapeutic failure in patients with septic shock. Crit. Care Med. 29:385-391.
24. Kearney, B. P., and F. T. Aweeka.(1999). The penetration of anti-infectives into the central nervous system. Neurol. Clin. 17:883-900.
25. Khana, S, Haseeba, M, Baiga,H, Baggab, S, Siddiquib, M., Kamalc, D, and Khana, S(2015) Improved efficiency and stability of secnidazole – An ideal delivery system,Saudi Journalof Biological Science ,22 42-49.
26. Lamp KC, Freeman CD, Klutman NE and Lacy MK. (1999). Pharmacokinetics and pharmacodynamics of the nitroimidazole antimicrobials. *Clin Pharmacokinet. ; 36(5):353-373.*
27. *Lau AH, Lam NP, Piscitelli SC, Wilkes L. and Danziger LH(1992). Clinical pharmacokinetics of metronidazole and other nitroimidazole anti-infectives. Clin Pharmacokinet. 23(5):328-64.*
28. *Laughlin K.M., Evans, S. M., Jenkins,W. T., Tracy, M., Chan C.Y., Lord, E. M. and Koch, C. J. (1996) Biodistribution of the Nitroimidazole EF5 (2-[2-nitro-1 Himidazolyi]-N-(2,2,3,3,3- pentafluoropropyl) acetamide)in 1Mice Bearing Subcutaneous EMT6 Tumors1.The jounal of pharmacology and experimental therapeutics*(*JPET)* 277:1049-1057.
29. Mate, I., J. L. Larrea, A. Sanchez, J. L. Merino, J. M. Mesa, and J. A. Sobrino. (1998). Indications and surgical techniques in the acute phase of infective endocarditis. Rev. Esp. Cardiol. 51(Suppl. 2):86-91.
30. McCullough, R. W., and E. J. Gandsman. (1988). Pathophysiologic interpretation of time activity curves in dynamic bone imaging. Clin. Nucl. Med. 13:517-524.

31. Mertin, D., and B. C. Lippold. (1997). In-vitro permeability of the human nail and of a keratin membrane from bovine hooves: penetration of chloramphenicol from lipophilic vehicles and a nail lacquer. J. Pharm. Pharmacol. 49:241-245.
32. Mital A (2009). "Synthetic Nitroimidazoles: Biological Activities and Mutagenicity Relationships". *Sci Pharm* **77** (3): 497–520.
33. Mengel, Mark B.; Schwiebert, L. and Peter (2005). Family medicine: ambulatory care & prevention. McGraw-Hill Professional. pp. 268
34. Naber, K. G. (1978). Renal lymph concentrations of antibiotics. Scand. J. Infect. Dis. Suppl. 14:164-165.
35. Onsrud, M., T. Bergan, and H. Gjonnaess. (1982). Antibiotic levels in plasma and peritoneal fluid after amoxycillin, ampicillin, and bacampicillin treatment for pelvic inflammatory disease. Acta Obstet. Gynecol. Scand. 61:247-251
36. Raju, T. N. (2005). "William Sealy Gosset and William A. Silverman: Two "students" of science". Pediatrics 116 (3): 732–735
37. Rebuck, A. S., and A. C. Braude. (1984). Assessment of drug disposition in the lung. Drugs 28:544-553
38. Rylander, M., C. Mannheimer, and J. E. Brorson. (1979). Penetration of cephradine and cefazolin into ulcers of patients suffering from peripheral arterial circulatory insufficiency. Scand. J. Infect. Dis. 11:281-286.
39. Savolainen, S., P. T. Mannisto, A. Gordin, R. Antikainen, H. Haataja, R. K. Tuominen, and J. Ylikoski. (1988). Tonsillar penetration of erythromycin and its 2′-acetyl ester in patients with chronic tonsillitis. J. Antimicrob. Chemother. 21(Suppl. D):73-84.
40. Schentag, J. J., A. S. Heller, B. G. Hardy, and P. B. Wels. (1983). Antibiotic penetration in liver infection: a case of tobramycin failure responsive to moxalactam. Am. J. Gastroenterol. 78:641-644.
41. Schierholz, J. M., J. Beuth, and G. Pulverer. (2000). "Difficult to treat infections" pharmacokinetic and pharmacodynamic factors—a review. Acta Microbiol. Immunol. Hung. 47:1-8.
42. Shinn D L S. (1962) Metronidazole in acute ulcerative gingivitis. Lancet. ;i:1191.
43. Syer SD ,Blackler RW, Martin R, de Palma G, Rossi L ,Verdu E ,Bercik P ,Surette MG ,Aucouturier A ,Langella P, Wallace JL (2015 ;(J Gastroenterol.;50(4):387-93 .
44. Tasneen, R. ,Williams, K., Amoabeng ,O ., Minkowski, A., Upton, A.M. ,Nuermberger, E.L.(2015). Contribution of the nitroimidazoles PA-824 and TBA-354 to the activity of novel regimens in murine models of tuberculosis. *Antimicrob Agents Chemother*; 59(1):129-135.
45. Takai N, and Tanaka Y .(2015) Bioanalysis; Imaging of drug and metabolite distribution by MS: case studies. 7(20):2639-2648.
46. Thompson D, Pepys MB and Wood SP (1999). "The physiological structure of human C-reactive protein and its complex with phosphocholine". Structure 7 (2): 169–177.
47. Van Kampen ,E.J. and Zijlstra ,W.G. (1961 .(Standardization of haemoglobinometry .ll .The haemoglobin cyanide method . Act. 6: 38-44 .
48. Videau ,D ,.Niel ,G ,.Siboulet and Catalan ,F. (1978 .(Secnidazole :A 5-nitroimidazole derivative with a long half-life. British Journal of Venereal Diseases; 54, .80-77.