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***Protective Effect of Some Medicines on Red Blood Cells as
Anti-Inflammatory Activity***

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Dedication

We would like to present this writing to our families for giving us all the inspiration and support we need .

Also we would like to thank everyone was part of our education journey

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Abstract

The present research is objective to evaluate anti-inflammatory effect of gabapentin, metformin, furosemide, and atenolol in compare to celecoxib where The Human Red Blood Cell membrane standardizing method (HRBC) was selected to evaluate anti-inflammatory effect and estimate the Percentage of protection. drugs had different levels of protection celecoxib 71.077% While the other drugs were Gabapentin 80.32%, metformin 78.337 %, furosemide 58.899%, and atenolol 33.606%. Therefor our study detect the anti-inflammatory effect of four common drugs that considerably use by patient these days.

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Introduction

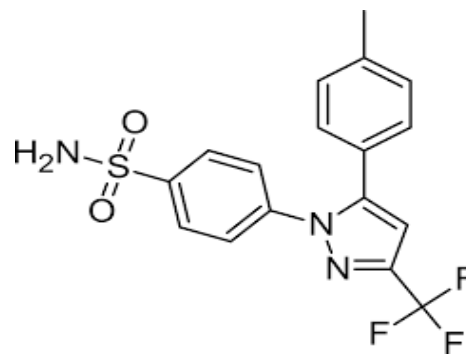
Inflammation is a complex biological response of vascular tissues to harmful stimuli. It is also a protective attempt by the organism to remove the injurious stimuli and initiate the healing process [1]. At the onset of an inflammation, the cells undergo activation and release inflammatory mediators. These mediators include histamine, serotonin, slow reacting substances of anaphylaxis (SRS-A), prostaglandins and some plasma enzyme systems such as the complement system, the clotting system, the fibrinolytic system and the kinin system [2.] inflammation can be Acute inflammation which is the initial response [4] and Chronic inflammation concedes a prolonged inflammatory response[5].

Non-steroidal anti-inflammatory drugs (NSAIDs) are a diverse group of compounds with similar biological capabilities: all NSAIDs reduce or eliminate the erythema, swelling, elevated temperature and pain caused by a variety of inflammatory stimuli. The mechanisms of action of NSAIDs have not yet been fully elucidated, but evidence suggests that their anti-inflammatory effects are primarily achieved through inhibiting prostaglandin production.

Drugs used in our research :

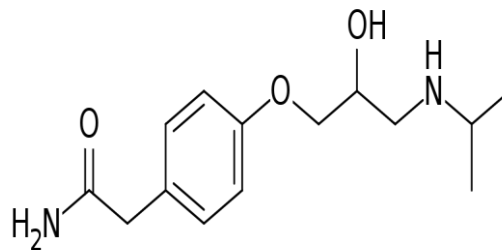
Celecoxib: is a COX-2 inhibitor and Non-steroidal anti-inflammatory drug. It is used to treat the pain and inflammation in osteoarthritis, acute pain in adults, rheumatoid arthritis, ankylosing spondylitis, painful menstruation, and juvenile rheumatoid arthritis.

Formula: $C_{17}H_{14}F_3N_3O_2S$



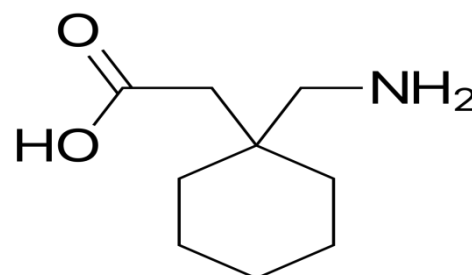
Atenolol: is a synthetic beta-1 selective blocker used in the management of hypertension and chronic angina, and to reduce mortality in known or suspected myocardial infarction in hemodynamically stable patients.

Formula: $C_{14}H_{22}N_2O_3$



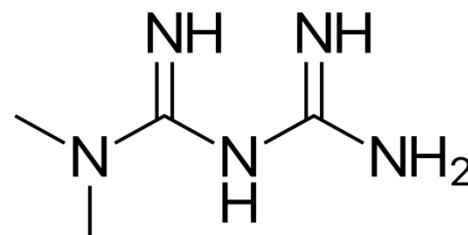
Gabapentin: is an anticonvulsant medication used in the management of peripheral neuropathic pains, post herpetic neuralgia, and partial-onset seizures.

Formula: $C_9H_{17}NO_2$



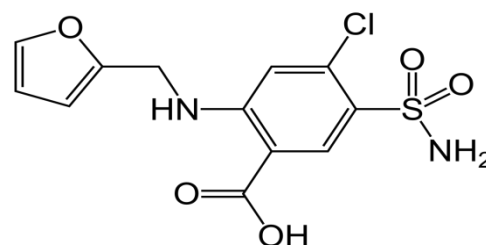
Metformin: is a biguanide drug used in conjunction with diet and exercise for glycemic control in type2 diabetes mellitus and used off-label for insulin resistance in polycystic ovary syndrome (PCOS).

Formula: $C_4H_{11}N_5$



Furosemide: is a loop diuretic used to treat hypertension and edema in congestive heart failure, liver cirrhosis, renal disease, and hypertension.

Formula: $C_{12}H_{11}ClN_2O_5S$



All these drug containing NH_2 groups in there structure .So base on that chooses them to study the anti-inflammatory effect on protection RBC.

Materials and Methods

Chemical

Ethanol, glucose, NaCl, Trisodium citrate, Citric acid, Distilled water, and Phosphate buffer (pH 7.4). All chemical were available in Basra university college laboratory.

Preparation of solution

As an initial step prepare Alsever's solution, Isosaline, and Hyposaline separately according to the concentration in (**Table1**).

Table 1. preparation of solution			
Solvent	Material	Concentration	Amount Of H2O
Alsever's solution	Glucose	1.025 g	50 ml
	NaCl	0.205 g	
	Trisodium Citrate	0.405	
	Citric acid	0.028 g	
Isosaline	NaCl	0.45 g	50 ml
Hyposaline	NaCl	0.35 g	50 ml

Preparation of drug

Preparation of drugs to be use in the research by dissolve each drug sheet in the suitable solvent according to (**Table2**) then filtrate and finally evaporate the solvent to get the drug without additive. the filtrate solutions were transformed to plate in order to evaporate.

Table 2. preparation of solution drug				
Drug	Dose	Number of tablet	Solvent	Amount of solvent
celecoxib (capsule) micro labs limited	200 mg	10	Ethanol	10 ml
Furosemide (tablet) Actavis	40 mg	10	Ethanol	10ml
Atenolol (tablet) Ajanta Pharma limited	50 mg	10	Ethanol	100 ml
Gabapentin (capsule) Hikma	100 mg	10	Water	10 ml
Metformin (tablet) Merck santé s.a.s.	500 mg	20	Water	10 ml

In vitro anti-inflammatory activity

The evolution of In vitro anti-inflammatory activity of selected drugs done by Human red blood cells (HRBC) membrane standardizing method. A (2.5 ml) of Blood was collected from the investigators themselves and mixed with sterilized Alsever's solution (2.5 ml) then take blood tube to be centrifuge at 4000 rpm for 12 min to obtain the serum and separate red blood cell followed by washing with isosaline solution 3ml two time. The HRBC suspension was prepared by packed cells, then the volume was complete to 10 ml with isosaline solution while 100mg of each drug in different tube with 10ml of distilled water. Finally prepare the test samples control (2ml of distilled water), standard (2ml of celecoxib), and Drug (2ml of Furosemide, Atenolol, Gabapentin, and Metformin) were separately mixed with 1 ml of phosphate buffer, 2 ml of hyposaline, and 0.5 ml of HRBC

suspension. The assay mixtures were left at room for 30 min. then they were centrifuged at 3000rpm for 10 min. The hemoglobin content in supernatant was picked and with spectrophotometer at 560 nm wavelength absorbance obtained. The percentages of HRBC member stabilization was calculated by the following equation

$$\text{Percentage of protection} = 100 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Results

After recording the absorbance of drugs we start to estimation Percentage of protection (**Table3**),(**Figure1**) Show comparison between celecoxib (anti-inflammatory drug as standard) Percentage of protection and (Gabapentin, atenolol, furosemide, and metformin) Percentage of protection .

1. celecoxib (standard)

$$\begin{aligned} \text{Percentage of protection} &= 100 - \frac{0.247}{0.854} \times 100 \\ &= 71.077\% \end{aligned}$$

2. Furosemide

$$\begin{aligned} \text{Percentage of protection} &= 100 - \frac{0.351}{0.854} \times 100 \\ &= 58.899\% \end{aligned}$$

3. Atenolol

$$\begin{aligned} \text{Percentage of protection} &= 100 - \frac{0.567}{0.854} \times 100 \\ &= 33.606\% \end{aligned}$$

4. Gabapentin

$$\begin{aligned} \text{Percentage of protection} &= 100 - \frac{0.168}{0.854} \times 100 \\ &= 80.327\% \end{aligned}$$

5. Metformin

$$\begin{aligned} \text{Percentage of protection} &= 100 - \frac{0.185}{0.854} \times 100 \\ &= 78.337\% \end{aligned}$$

Table 3. Result – Absorbance and Percentage of protection

Sample	Concentration	Absorbance	Percentage Of Protection
Celecoxib	100mg	0.247	71.08%
Furosemide	100 mg	0.351	58.90%
Atenolol	100mg	0.567	33.61%
Gabapentin	100 mg	0.168	80.33%
Metformin	100mg	0.185	78.34%

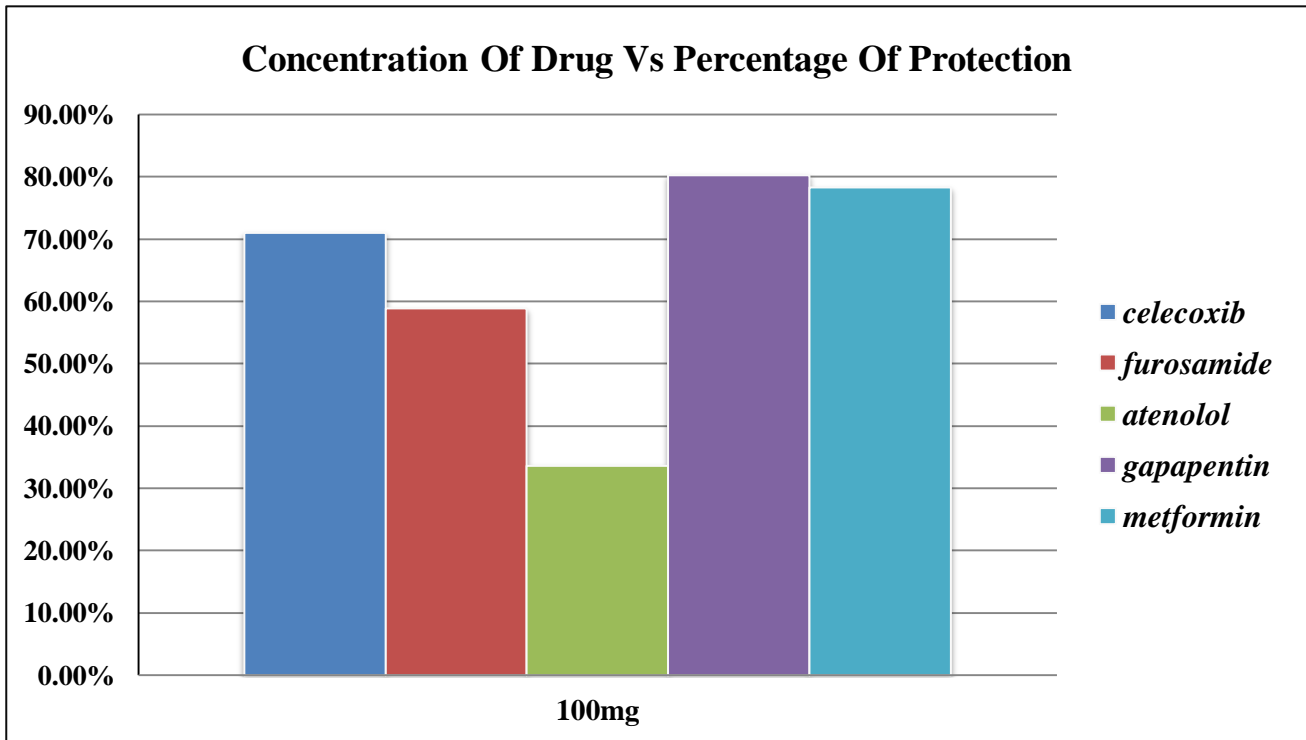


Figure.1 Percentage of protection for Celecoxib, Furosemide, Atenolol, Gabapentin, Metformin

Discussion

Today a lot of people take a long course of medication also chronic disease drugs are long life drugs that in the other hand inflammation is a common phenomenon cause of injury need NSAIDs to inhibit COX, an enzyme that converts Arachidonic acid to prostaglandins, as a result patient will be upset of multidrug use. the estimation of anti-inflammatory of selected drugs (gabapentin, metformin, furosemide, and atenolol) can be beneficial if these drugs have anti-inflammatory effect then patients will take less medication and less side effect will be obtained.

Human Red Blood Cell membrane standardizing method (HRBC) depend on Stabilization of Human red blood cell membrane which is analogous to the lysosomal membrane by hypotonic solution that induced membrane lysis can be taken as an in vitro measure of anti-inflammatory activity (either stop or inhibit the conversion or protect the lysosomal membrane to inhibit inflammation). the percent of protection for RBC membrane give an idea about anti-inflammatory activity of gabapentin, metformin, furosemide. and atenolol but in different level. when compare these drugs with standard celecoxib, gabapentin, and metformin have evidence higher protection to the membrane. for gabapentin, its able to reduce pro-inflammatory mediators (e.g., TNF- α , IL-1 β , and IL-6) and up-regulates anti-inflammatory cytokine IL-10[12] while metformin significantly sup-pressed NF- κ B DNA-binding activity and I κ B phosphorylation, resulting in the down regulation of IL-1 α and IL-8 expression in cells stimulated with TNF- α [11]. even Furosemide have anti-inflammatory by production and release of cytokines interleukin (IL)-6, IL-8, and tumor necrosis factor-alpha from peripheral mononuclear cells[13]but its activity conceder less effective than other while atenolol is the weakest of them all.

Conclusion

As we see from our research result the percentage of protection for Red blood cells was good, that's mean we can use some drug as anti-inflammatory. Now days a lot of people use anti-inflammatory broadly, so we can use our research as standard for detection the protection properties for drug have same functional group or same chemical properties. We went use different concentration from our drug (celecoxib, furosemide, metformin, gabapentin, atenolol) but because of the current situation (COVID_19)we don't have enough time to detect that.

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