Synthesis of Novel Indole Derivatives and Assessment of Their Anti-Inflammatory Activity in Experimental Animals

Arvind Kumar Srivastava*[1], Hriday Shankar Chaurasia[2], Amit Kumar Nigam[3], Suman Jaiswal[4], Surya Prabhakar Singh[5], Naveen Sharma[6], Prashant Kumar Singh[1], Neha Srivastava[7]

[1]Department of Pharmaceutical Chemistry United Institute of Pharmacy, Naini, Prayagraj, Affiliated to APJ Abdul Kalam Technical University, Lucknow India.

[2]Department of Pharmaceutical Chemistry, Jyotiraditya Institute of Pharmacy, JD Shikha Gram, Pratapgarh UP India, Affiliated to APJAKTU lucknow. UP India.

[3]Department of Pharmaceutical Chemistry, Deen Dayal Upadhyaya Gorakhpur University Gorakhpur, India.

[4]Department of Pharmacology, United Institute of Pharmacy, Naini, Prayagraj, Affiliated to APJ Abdul Kalam Technical University, Lucknow India.

[5]Department of Pharmaceutics United Institute of Pharmacy, Naini, Prayagraj, Affiliated to APJ Abdul Kalam Technical University, Lucknow India.

[6]Amity Institute of Pharmacy, Amity University Madhya Pradesh Gwalior Madhya Pradesh India.

[7]Department of Pharmaceutical Chemistry United Institute of Pharmacy, Naini, Prayagraj, Affiliated to APJ Abdul Kalam Technical University, Lucknow India.

[8]Department of Agriculture Studies, Sam Higginbottom University of Agriculture Technology and Science, Praygraj, India.

Corresponding Author:Arvind Kumar Srivastava*

Department of Pharmaceutical Chemistry United Institute of Pharmacy, Naini, Prayagraj, Affiliated to APJ Abdul Kalam Technical University, Lucknow India. Orcid: https://orcid.org/0000-0001-8036-5867

Abstract

Nimesulide and Rofecoxibs are only two examples of the many anti-inflammatory medications with an aryl-ether component that have been demonstrated to cause cell damage and cell death in the liver. We wanted to synthesise novel indole-substituted aryl ethers and evaluate their anti-inflammatory activities with minimal side effects, keeping this logic in mind. New aryl ethers that were substituted with indoles were synthesised.Inflammation is defense mechanism, through which body protects itself from foreign substances. But if human body is not able to deal with it, the secondary option is medications that is NSAIDs but limitations of these drugs are gastrointestinal lesions, nephrotoxicity, etc. To overcome this problem novel molecule has to be synthesised from time to time. Among all the newly synthesised chemicals, 3-methyl Indole derivatives were determined to have the most anti-inflammatory effects based on experimental results. Recent research has shown that 3-methyl Indole derivatives have promising therapeutic potential as an anti-inflammatory medication. The uniqueness of this study lies in its straightforward production process and its promising anti-inflammatory effects in live organisms. The viability of the synthesised chemicals as bioactive candidates with anti-inflammatory activity was further investigated using in-silico investigations. Using the carrageenan-induced rat paw oedema paradigm, the anti-inflammatory efficacy of the synthesised compounds was evaluated.

Keywords: NSAIDs, anti-inflammatory, COX enzymes, heterocyclic compounds, ect.

1. Introduction

It is the nonsteroidal anti-inflammatory drugs, also known as NSAIDs, that are the treatments that are utilized the most frequently in order to decrease fever, discomfort, and inflammation. Nonsteroidal anti-inflammatory medicines (NSAIDs), which are routinely used, are not particularly helpful at lowering pain or fever. This is due to the fact that they block both COX enzymes, COX-1 and COX-2. This is due to the fact that they have the potential to create adverse effects on the digestive system. The process of converting arachidonic acid into prostaglandin H2 is carried out by these COX enzymes, which are responsible for the conversion. The defense of cells in the gastrointestinal tract and the stomach is understood to be the responsibility of COX-1, according to the general consensus [1]. COX-2, on the other hand, is thought to be the factor that is responsible for the dispersion of inflammatory mediators [2]. Certain drugs, such as nimesulide and rofecoxib, amongst others, have been found to exhibit a large amount of anti-inflammatory activity. This is due to the fact that these medications act as specific inhibitors of the COX-2 enzyme. On the other hand, they have been linked to an increased potential for damage to the liver, in addition to the development of a variety of cardiovascular issues [3,4]. This was done solely due to the fact that they posed a greater risk of heart strokes and other cardiac issues [5]. Nimesulide, on the other hand, is not suggested in the event that the patient is experiencing hepatic and renal malfunctions because it has the potential to make the condition even more severe. Several fold a growth in the demand for novel anti-inflammatory bioactive chemicals that have a good effectiveness and an enhanced safety profile has been brought about as a result of these causes [6]. These rings are thought to be responsible for the biological activity of these compounds. The purpose of these rings is to provide places for substitution in order to affect the molecule conformation, solubility, and physicochemical properties. These adjustments are carried out in order to accomplish a sufficiently high level of the biological response that is required [7].

The compounds that include heterocyclic rings are essential for the process of chemical synthesis, as well as for the investigation of the numerous advanced therapeutic uses that can be found for these compounds. Several compounds that contain sulphur, nitrogen, and oxygen as heteroatoms have been shown to be extremely effective bioactive agents [8]. This

is a fact that cannot be denied. Among the many valuable structural motifs that may be found in natural molecules and bioactive chemicals, cyclic ethers and their derivatives are especially prevalent [9]. Due to the fact that it is of such profound significance in both the biological and synthetic sides of medicinal chemistry, the indole nucleus continues to receive a substantial amount of attention from medicinal chemists. This is the case among all heterocyclic compounds that are now in existence. Among the many biological effects of indole derivatives are their roles as microbicides [10], fungicides [11], analgesics [12], anti-inflammatorys [13,14], anti-tumors [15], anti-convulsants [16], anti-malarials [17], antioxidants [18], and tuberculosis prevention agents [19]. You can see this data in Figure 1.

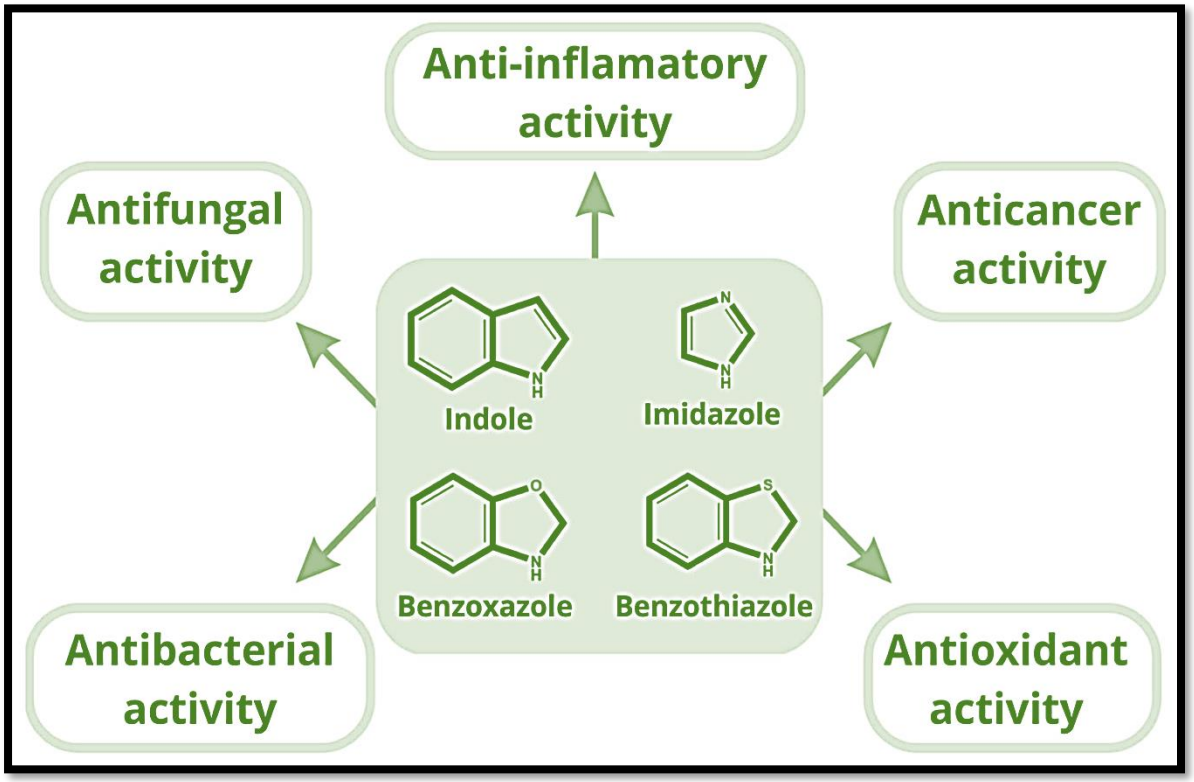


Fig.No.1 scaffolds based on organic chemistry

2.Heterocyclic Compound

The substantial applications of heterocyclic compounds are recognized in a variety of fields, but mainly in the fields of medicine and biology. Due to the extensive synthesis and functional applications that they possess, they are obtaining an increasing amount of prominence in the field of study.[20] This is a consequence of the fact that they have. Additionally, these components can be found in more than ninety percent of the products that

have been invented in recent times. After the indole nucleus was released into the environment, it was discovered that therapeutic substances exhibit a wide range of biological activity. This discovery was made possible by the introduction of the indole nucleus.

3. Indole Moiety

As a consequence of this mechanism's contribution to the formation of the material, a nitrogenous moiety is produced within the substance. This occurrence is brought about as a result of the benzene ring and the pyrrole nucleus being able to connect with one another at the two sites of the pyrrole ring. This is the cause of this occurrence. For the purpose of dissolving this aromatic molecule, a wide variety of solvents are considered to be suitable options. Solvents such as alcohol, benzene, ether, and other solvents are included in this category of substances. As a consequence of this mechanism's contribution to the formation of the material, a nitrogenous moiety is produced within the substance.[21]. This occurrence is brought about as a result of the benzene ring and the pyrrole nucleus being able to connect with one another at the two sites of the pyrrole ring. This is the cause of this occurrence. For the purpose of dissolving this aromatic molecule, a wide variety of solvents are considered to be suitable options. Solvents such as alcohol, benzene, ether, and other solvents are included in this category of substances.

4. Reactivity of Indole Moiety

The structure of indole is that of a heterocyclic molecule, which means that it is capable of performing chemical reactions with greater ease than other molecules. A comparison to pyrrole is something that may be made between the interaction sites. The process takes place at four distinct sites: carbon 3, nitrogen 1, the pi link between carbon and carbon, and the sigma bond between carbon and nitrogen. Strong acids, such as hydrochloric acid, are able to easily convert the C3 position of indole into a proton.[22]. On the other hand, the N atom requires a greater amount of effort in order to finish the protonation process. An additional demonstration of the cycloaddition reaction is provided by indole molecules, specifically compound C2-C3 and compound C2-N.

Approach that is Synthetic

Both the low yield and the high number of byproducts that are produced by the fischer indole synthesis are considered to be two of the most significant shortcomings of this method. The

indole moiety which shows alkaloidal property like indole -3-acetic acid which mainly regulates the plant growth. [23]

5. Indole molecule reactions and operations

In addition to the formation of metal complexes, it exhibits a wide range of reactions, including protonation, nitration, sulphonation, acylation, and halogenations. It is capable of producing nucleophilic as well as electrophilic reactions. An aromatic compound, it is a substance that can be found in nature quite frequently. Indiole nucleus is present in the amino acid known as tryptophan.[24].

Synthesis of 3-Methyl Indole derivaitves

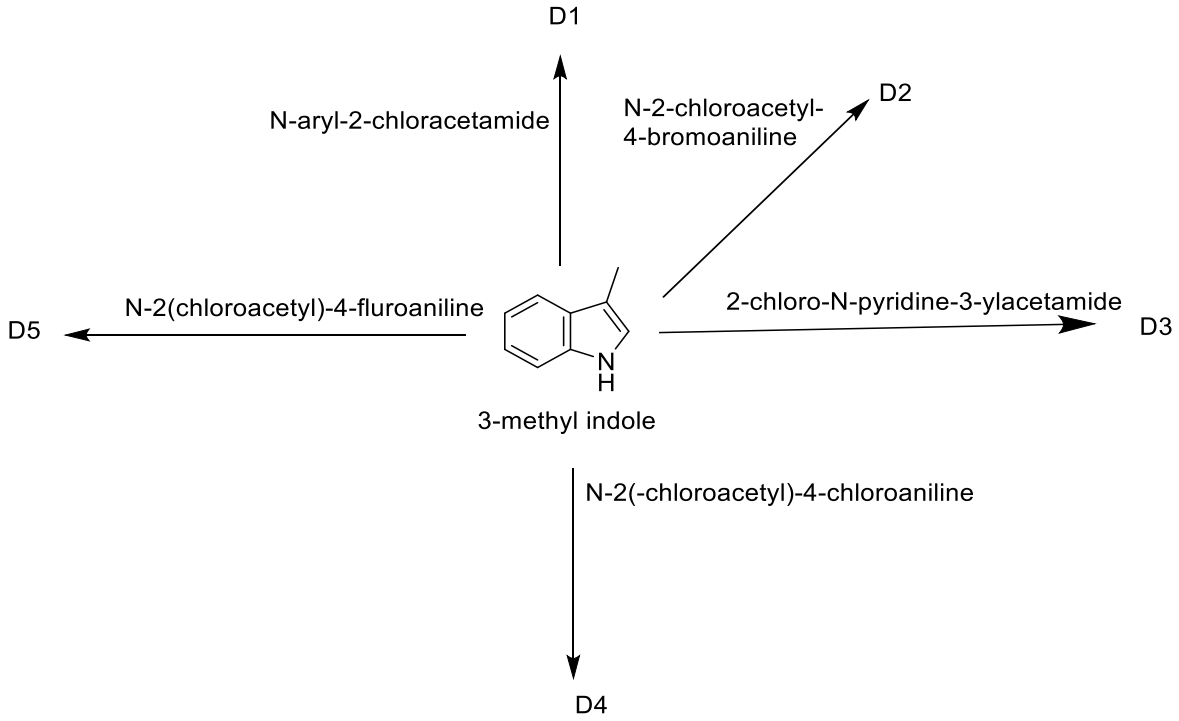


Fig.No.2 Synthesis of 3- methyl Indole Derivatives

D1- 2-(3-methyl -1-H-Indol-1-yl)-N phenyl acetamide

D2- 2-(3-methyl-1-H-indol-1-yl-N-(pyridine-2-yl) acetamide

D3- N-(4-bromophenyl)-2-(3-methyl-1H-indol-1-yl) acetamide

D4- N-(4-chlorophenyl)-2-(3-methyl-1H indol-1-yl) acetamide

D5- N-(4-Flurophenyl)-2-(3-methyl-1H indol-1-yl) acetamide

6. Pharmacological Activity

Experimental Animals

Albino wistar rat of both sexes (120-150) gm were obtained under the reference UIP/IAEC/Feb-2024/20, at the United Institute of Pharmacy Institutional Committee's CCSEA authorized the experiment's protocol.

Acute toxicity of drug

The animals were fasted overnight before the examination and OECD guidelines 423 was followed and for subacute toxicity OECD guidelines 402 is used.**[25]**

Experimental Design and Procedure

Group	Animal group	Drug treatment
I	Negative control	No treatment
II	Positive control	Inflammation will becaused
III	Standard drug (Diclofenac)	Inflamed group will be treated by standard drug 20 mg/kg
IV	D1	Received indole derivative 200 mg/kg
V	D2	Received indole derivative 200 mg/kg
VI	D3	Received indole derivative 200 mg/kg
VII	D4	Received indole derivative 200 mg/kg
VIII	D5	Received indole derivative 200 mg/kg

Paw thickness was measured, and the reading was recorded at 0,1,2,3, hrs

Swelling is expressed in terms of percentage**.**

$$\% \textbf{ Inhibition} = 1 - \frac{Vt}{Vc} * 100$$

V_T = edema volume in test group

V_C = edema volume in control group

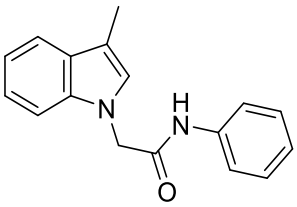
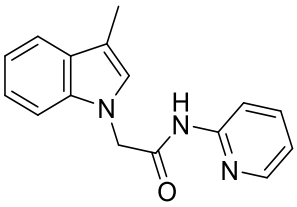
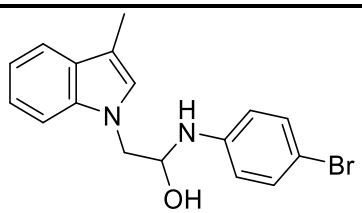
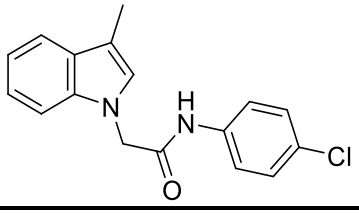
The mean and standard deviation ± were used to express the results.

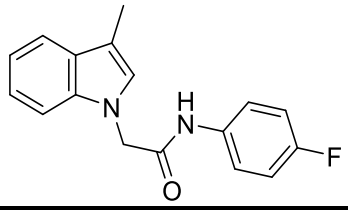
Standardised laboratory methods were used to evaluate the heamatological parameters such as red blood cell, WBC, heamoglobin. GraphPad Prsim 10 software was utilized to do statistical analysis on the data, which were provided as mean and standard deviation.

7. Result and Discussion

I. Structure and IUPAC name of 3-Methyl Indole Derivatives

Table No.1 Structure and IUPAC name of 3-methyl indole derivatives

Derivatives	Structures	IUPAC name
D1		2-(3-methyl -1-H-Indol-1-yl)-N phenyl acetamide
D2		2-(3-methyl-1-H-indol-1-yl-N-(pyridine-2-yl) acetamide
D3		N-(4-bromophenyl)-2-(3-methyl-1H-indol-1-yl) acetamide
D4		N-(4-chlorophenyl)-2-(3-methyl-1H indol-1-yl) acetamide

D5		N-(4-Flurophenyl)-2-(3-methyl-1H indol-1-yl) acetamide

II. Acute Toxicity Studies

The Albino wistar rats were used for the acute toxicity test. Oral gavage was used to administer each substance at doses of 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg. The animals were monitored for behavioural abnormalities or mortality in 50% of the group. Acute toxicity of all derivatives were checked and at 3rd position of 3-methyl indole shows maximum anti-inflammatory activity.

No. of animals	**Dose**	**No. of death of animals**
3	5mg/kg	0
3	50mg/kg	0
3	300mg/kg	0
3	2000mg/kg	0

Table No.2 Albino Wistar Rat Anti-Inflammatory Activity Screening

Comp. Code	**Inhibition of inflammation in ml**					**% Inhibition**			
	0 hr	1hr	2 hr	3hr	4hr	1hr	2hr	3hr	4hr
Positive Control	0.35±0.02	0.32±0.03	0.30±0.04	0.29±0.03	0.28±0.04				
Std (10 mg/kg, Diclofenac)	0.32±0.01	0.29±0.02	0.25±0.03	0.22±0.03 [a]	0.19±0.02 [a]	9.3	16	24.13	32.14
D1(200 mg/kg)	0.32±0.03	0.27±0.01	0.25±0.01	0.21±0.02[a, z]	0.17±0.03 [a, z]	18.5	16.6	27.5	39.28
D2(200 mg/kg)	**0.30±0.04**	**0.26±0.03**	**0.16±0.04**	**0.13±0.03 [a, b]**	**0.10±0.02 [a ,b]**	**18.75**	**46.66**	**55.17**	**64.28**
D3	0.33±0.04	0.29±0.0	0.20±0.0	0.16±0	0.20±0.03	9.37	33.3	44.	28.57

(200 mg/kg)		3	2	.03 [a, c]	[a, c]		3	82	
D4 (200 mg/kg)	0.31±0.02	0.30±0.01	0.20±0.02	0.13±0.01 [a, b]	0.12± 0.02 [a, b]	6.25	33.33	55.17	57
D5 (200 mg/kg)	0.31±0.03	0.31±0.04	0.24±0.03	0.14±0.02 [a,b]	0.12±0.01 [a ,b]	3.2	25	51.7	57.14

No toxicity was observed at 2000 mg/kg so 1/10th of original dose was taken as ED.

Mean ±SD method was used to represent the value. $^{a}p<0.0001$, when compared with positive treated group and $^{z}p>0.05$, $^{b}p<0.0001$,$^{c}p<0.001$ when compared with std group at the end of 3 hr and 4hr using turkey's multiple comparison test.

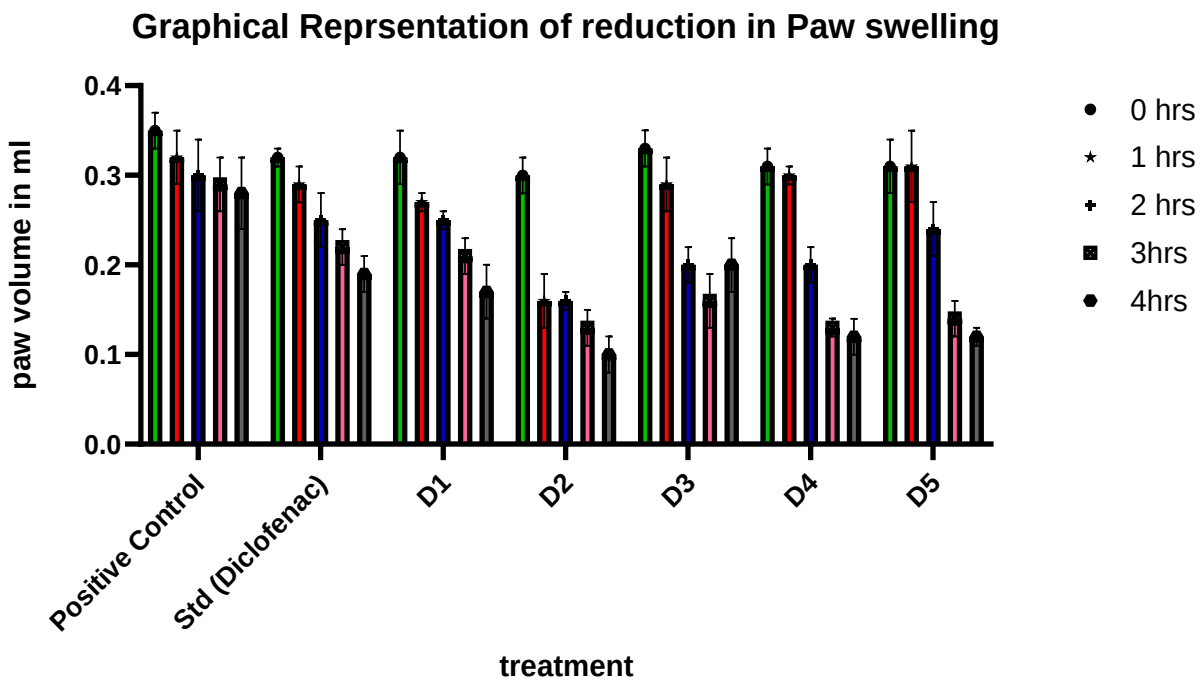


Fig.No.3 Graphical Representation of Reduction in Paw Swelling

III. Effect of Derivatives on Haematological Parameters

Table No.3 Effect of derivatives on Haematological Parameters

Group	RBC	HB	PLT	RDW
Control	6.17±0.38	13.11±0.80	2.78±0.02	15.17±0.01
Positive control	6.12± 0.37	13.00±0.78	2.76±0.03	14.17±0.02

Diclofenac(10mg/Kg)	6.35±0.02 [a]	13.15±0.02[a]	6.80±0.02[a]	16.17±0.02[a]
D1	6.34±0.02 [a]	12.41±0.82[a]	6.78±0.02[b]	17.7±0.94 [a]
D2	6.38± 0.03 [a,c]	13.3±0.81[a,c]	6.82±0.01[a,c]	18.60±0.84 [a,c]
D3	4.47±0.01 [b,c]	9.6±0.80[b,c]	5.61±0.03[d]	17.70±0.90 [a,c]
D4	5.38±0.02[b,c]	12.0±0.78[a]	6.50±0.02[d]	16.00±0.02 [a,d]
D5	4.38±0.01[b,z]	11.0±0.79[a]	5.50±0.01	14.14±0.91[a]

Mean ±SD method was used to represent the values. [a]p<0.0001, [b]p<0.001 versus positive treated group and [z]p>0.05 , [d]p<0.0001,[c]p<0.001 when compared with std group using turkeys's multiple comparison test.

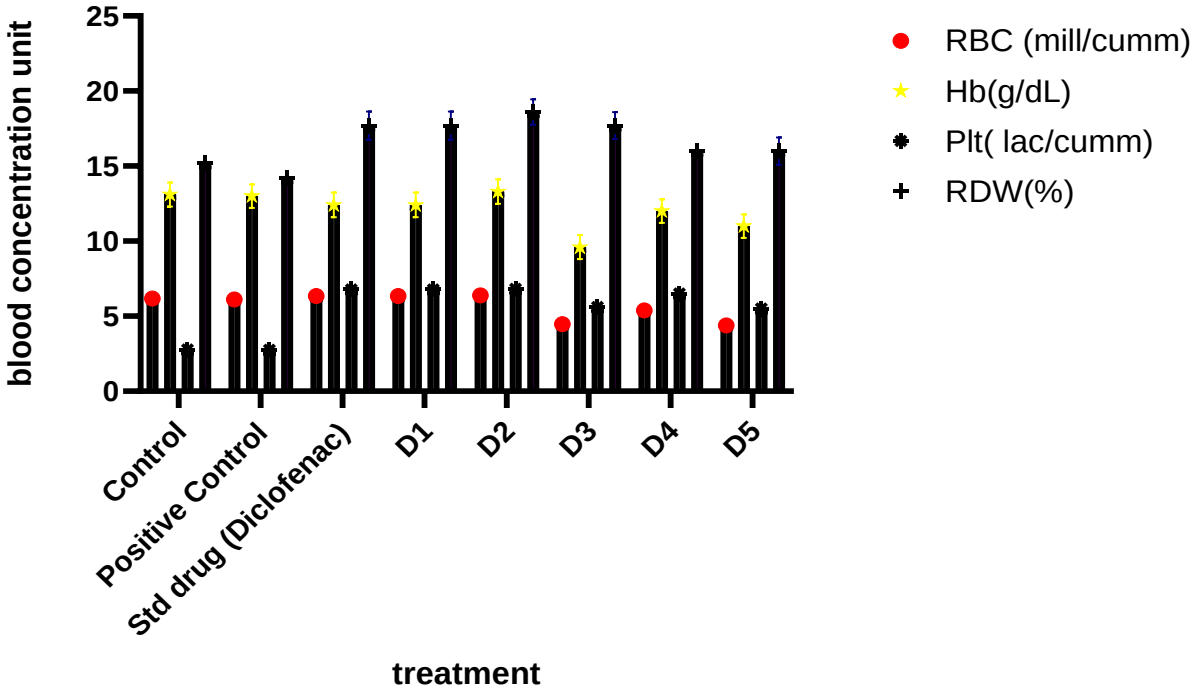


Fig.No.4 Graphical Representation of Haematological Parameters.

Result and Discussion

Some of the many biological actions that are carried out by the indole moiety include the inhibition of inflammatory, fungal, microbial, viral, anti-alzheimer's, antioxidant, and carcinogenic processes. These are only some of the many biological actions that are carried out. With the C3, N, C2-C3 pi link, and C2-N sigma bond being the most susceptible to being replaced, there are seven potential replacement sites that can be found within an indole molecule. According to the findings of research, the anti-inflammatory activities of medications are improved when the substitution occurs in the third position of the indole ring. This is found to be the case. The body's defence response against potentially harmful chemicals is inflammation, which is one of the most prevalent causes of disease. Inflammation is also one of the most common causes of disease.An immediate requirement is the synthesis of new compounds with the purpose of mitigating the severe side effects that are caused by nonsteroidal anti-inflammatory medications (NSAIDs). An example of one of these adverse effects is bleeding in the gastrointestinal system. Other adverse consequences include nephrotoxicity and cardiovascular toxicity.When it comes to achieving a reduction in

toxicity while simultaneously boosting selectivity, one of the most difficult goals for any new candidate to accomplish is to do this. The inquiry that we are currently conducting has led us to the conclusion that we should concentrate our efforts on 3-methyl indole. A method that is comprised of two stages has been developed, which has resulted in an improvement in the synthesis of five different 3-methyl indole derivatives. During the course of this inquiry, the N-aryl-2-chloracetamides were the primary intermediate that was created. They are manufactured using a method that is known as the chloroacetylation reaction, and they are utilised extensively in the synthesis of a wide variety of chemical compounds. This is due to the fact that they are versatile. With the use of the carrageenan inflammation in vivo model, the recently synthesised chemical was put through its paces in order to ascertain whether or not it contains anti-inflammatory capabilities. Due to the fact that the majority of nonsteroidal anti-inflammatory medicines (NSAIDS) have negative side effects, such as gastrointestinal bleeding and nephrotoxicity, there is a greater demand for the synthesis of new indole derivatives. Additionally, there is the chance that the newly synthesised candidate could be employed for the treatment of inflammation, and it is also possible to maximise its effectiveness to a certain extent.

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