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Anti-Inflammatory Property of Metformin: An Animal Study.

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ABSTRACT

Inflammation is a part of the complex biological response of body tissues to harmful stimuli. Cytokines and other growth factors activate the Mitogen Activated Protein Kinase (MAP) pathway ultimately resulting in inflammation. Metformin inhibits the activation of MAP kinases and have anti-inflammatory properties. Hence with this background an attempt was made to confirm the anti-inflammatory property of metformin in adult albino rats. 18 adult albino rats were divided into 3 equal groups. After measuring the initial paw volume, by using a plethysmometer, Group I received 1ml of distilled water; Group II received T,Aspirin 300 mg/kg & Group III received T.Metformin 100 mg/kg orally. After 30 minutes of drug administration, 0.1ml of 1%carrageenan was injected into the left hind paw of each animal and the volume of rat hind paw up to the ankle joint was measured plethysmographically after 1,3,6 hours. Paw swelling due to inflammation was noticed in animals of control group and significantly reduced in test group. The results were analysed statistically using t test and p value was found to be statistically significant. Hence this study shows that metformin has got significant anti-inflammatory activity in animals which needs further clinical confirmation.

Keywords: MAPK, Metformin, Inflammation, Edema.

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INTRODUCTION

Inflammation is a part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells or irritants [1]. The cardinal sign of inflammation warmth, pain, swelling, and redness and hyperemia was described by Celsus 2,000 years ago [2]. Cytokines and chemokines promote the migration of neutrophils and macrophages to the site of inflammation [3, 4]. Cytokines and other growth factors activate the Mitogen Activated Protein Kinase (MAP) pathway ultimately resulting in inflammation [5].

Inflammation could be acute, sub-acute or chronic in nature. Inflammatory diseases include a variety of conditions ranging from rheumatoid arthritis, inflammatory bowel disease etc. Though inflammation is the unifying factor, therapy for each disease is unique. When the inflammation gets exaggerated and sustained without apparent benefit it produces severe adverse consequences, it should be intervened with proper anti-inflammatory drugs to prevent the complications

Currently many anti-inflammatory drugs are available of which commonly used ones include NSAID's & Corticosteroids. Most of the NSAID's are available even as over the counter drugs. Chronic use of these drugs have their own gastrointestinal renal and neurological adverse effects like which limits the chronic usage [6]. Hence there is always a surge for a drug with minimal side effects and additional therapeutic advantages. The association of inflammation with carbohydrate metabolism can actually be traced back to reports from the 1800s [7]. Research has established that the link between inflammation and insulin resistance resides at the level of the IκB kinase-β (IKKβ)/NFkB axis [7, 8].

Metformin is a biguanide group of oral hypoglycaemic drug which acts mainly suppression of gluconeogenesis in the liver, thereby decreasing the glucose production and increase the insulin mediated glucose uptake in muscle cells. These effects are probably accomplished by partial inhibition of the mitochondrial respiratory chain complex 1 with a subsequent increase in intracellular AMP levels and activation of AMP kinase [9].

Metformin have local and systemic anti-inflammatory effects and it produces anti-inflammatory effects by AMPK dependent and AMPK independent pathways. By activating AMP Kinase it causes inhibition of the TNF-α/NF-κB and mTOR signalling pathways and by increasing the expression of activated transcription factor-3 (ATF-3) it reduces IL-6 and TNF-α expression. It causes activation of Dicer, iPFK2 and miRNA leading to lower expression of several pro-inflammatory cytokines such as TNF-α and IL-6 by AMPK independent mechanism also [10, 11].

Hence with this background an attempt was made to confirm the anti-inflammatory property of metformin in adult albino rats.

MATERIALS AND METHODS

This study was conducted at the Central animal house, Government Vellore Medical College, Vellore after obtaining Clearance from Institutional Animal Ethical Committee at Vellore. 18 adult, male, Albino rats of weight 200 -220 gms were obtained from Central animal house, Government Vellore Medical College. All the animals were maintained under 12:12 hour light: dark cycle and were fed with standard laboratory chow and water ad libitum.

18 animals were divided into 3 equal groups. The animals in Group I (control) received 5ml of distilled water orally. Group II (standard) received T,Aspirin 300 mg/kg orally. Group III (Test group) received T.Metformin 100 mg/kg orally.

Groups		Drug Received
I	Control	Distilled water orally
II	Standard	T,Aspirin 300 mg/kg orally
III	Test	T.Metformin 100 mg/kg orally

6 Male Albino rats with a body weight between 200 and 250 g were used for control group animals, 5 ml of water was given by stomach tube. Tablet Metformin was obtained from Cipla Ltd and Tablet Aspirin from By Astra Zeneca Pharma India Ltd. Drugs were dissolved in normal saline and administered orally

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through the oral feeding tube to test group and standard group animals. Thirty minutes later, the rats are challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar side of the left hind paw according to the method described by Winter et al [12]. Carrageenan suspension was prepared as a homogenous 1.0% suspension of the powder in 0.9% sodium chloride solution (sterile normal saline). The animal paw is marked at the level of the lateral malleolus with ink and immersed in mercury up to this mark. The paw volume is measured plethysmographically immediately after injection, again1, 3 and 6 h, after challenge [13].

The increase of paw volume after1, 3 or 6 h is calculated and compared with the volume measured immediately after injection of the irritant for each animal .Effectively treated animals show much less edema. The average values difference between treated animals and control groups is calculated for each time interval and statistically evaluated. The difference at the various time intervals gives the duration of the anti-inflammatory effect of study drug.

RESULTS

All the animals in the control group developed edema, whereas in the standard group only minimal edema was produced. In test group, edema was produced in all the animals but it was less in amount compared to control group. Results were analysed statistically using t test. $P < 0.05$- considered as statistically significant.

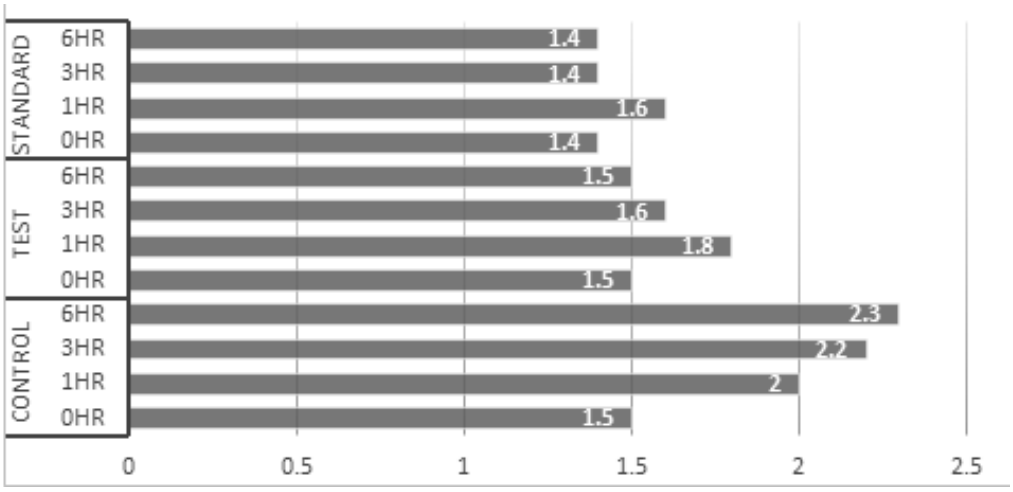
. The study showed the results of mean paw volume between control and test after1 hr was 2.0 $\pm$ 0.2 and 1.8 $\pm$0.1with the p-value of.038054.

The mean paw volume between control and test after 3 hr was of 2.2 $\pm$ 0.19, 1.6$\pm$0.1 respectively with p-value is .0056. The mean paw volume of control was 2.3$\pm$0.1 after 6 hrs and test was 1.5$\pm$0.1 with p-value of 0.0041. The results obtained are shown in Table 1 and Graph 1.

Table 1: Measurement of Paw Edema (In ml):

Animals	Control				Test				Standard			
	0hr	1hr	3hr	6hr	0hr	1hr	3hr	6hr	0hr	1hr	3hr	6hr
1	1.5	2.3	2.4	2.6	1.5	1.8	1.6	1.52	1.5	1.6	1.5	1.5
2	1.5	2.2	2.6	2.8	1.4	1.7	1.6	1.4	1.4	1.5	1.42	1.4
3	1.4	1.7	1.9	2.0	1.5	1.7	1.5	1.6	1.5	1.7	1.5	1.5
4	1.6	1.9	2.0	2.2	1.6	1.9	1.8	1.6	1.4	1.8	1.4	1.4
5	1.7	1.9	2.2	2.3	1.5	1.8	1.6	1.52	1.3	1.4	1.3	1.3
6	1.5	1.8	1.9	2.1	1.4	1.7	1.5	1.42	1.5	1.7	1.5	1.5
Mean	1.5	2.0	2.2	2.3	1.5	1.8	1.6	1.5	1.4	1.6	1.4	1.4
S.D	0.1	0.2	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Graph 1: Mean Paw Volume (ml)



DISCUSSION

Metformin is a commonly used first line Antidiabetic drug for Type 2 DM [14]. Apart from this anti hyperglycemic effects it shows various pleiotrophic effects like anti-inflammatory, antiaging, and anticancer and antioxidant effects [15].

During any harmful stimuli like irritant and pathogens body produces Cytokines and other growth factors which further activates the Mitogen Activated Protein Kinase (MAP) pathway ultimately resulting in inflammation. Metformin, a known anti-diabetic drug, inhibits the activation of MAP kinase and also activates the AMPK enzyme that in turn has various efficient impacts on various inflammatory cascade.

Carrageenan-induced rat paw edema test is the most sensitive and reproducible test for anti-inflammatory effects. The Carrageenan injection into rat paw produces an acute and local inflammatory response. It is associated with three distinct phases. The first phase is early mediated by mast cell degranulation and histamine and serotonin release (1 hr), the second phase (60 to 150 min) is characterized by bradykinin release and pain, and further eicosanoid production in the late phase (3-4hr) [16].

In this study, after 1hr of carrageen injection all the animal developed edema in paw and the mean Paw volume in control group was 2.0 $\pm$ 0.2, test group was 1.8 $\pm$ 0.1 and standard was 1.8 $\pm$ 0.1.

After 3hrs of carrageen injection and the test group shows less edema with mean value of 1.6 $\pm$ 0.1 compared to the control group with mean value of 2.2 $\pm$ 0.196 with P value 0.005.(Significant).

After 6hrs mean paw volume in control animals was 2.3 $\pm$ 0.1and test group mean volume was 1.5 $\pm$ 0.1 with P value of 0.00004 .In the standard group animals (aspirin) mean paw edema volume after 3hrs and 6hrs of carrageenan injection was 1.4$\pm$ 0.1 which was more less compared to control and test animals.

Carrageen is a substance with polysaccharide it produces acute inflammatory edema after subcutaneous injection by releasing chemical mediators. Metformin inhibits the development of this paw edema in late phase (3hr,6hr)compared to control group with significant p value of 0.005.This proves that metformin has anti-inflammatory property.

Similar to this study Researches done by Ajiboye et al [17]. Robin Kristofi et al [18] have also clearly shown that Metformin possesses anti-inflammatory activity. Study on Kim et al showed that Metformin reduces inflammation by attenuating macrophage activation [19]. According to Lin et al study in PTEN-knockdown murine macrophages, Metformin not only blocked reactive oxygen species (ROS) generation and Akt activation but also led to significant apoptosis causing growth inhibition in inflammatory cascade [20].

CONCLUSION

This study proves that anti-inflammatory effects of metformin by decreased paw edema volume after carrageen injection. It indicates that Metformin treatment in Diabetes patients not only helpful in lowering blood glucose level, it will also reduce the Diabetes related inflammatory complications. When combined with Metformin, the dose of NSAIDs could be reduced, thereby reducing the side effects of the latter. The limitation of this study is being done on a small number of rats and needs to be done on more numbers for greater validity.

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