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Repurposing of atorvastatin for its thrombocytopenia effect in experimentally induced animals

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ABSTRACT

Background & Objective: Repurposing atorvastatin for its thrombocytopenia effect in experimentally induced animals.

- To determine the platelet count.
- To report the repurposing activity of atorvastatin.

Methods: Healthy Albino Wistar rats weighing around 150-200 g were selected for studies. Animals were procured from a registered CPCSEA vendor. The temperature was maintained at 22-25℃ under 12 h light/dark conditions and the relative humidity of 41-45 % and provided with food and water ad libitum. The animals were housed in polypropylene cages and animals were divided into different groups. Group I: Normal group, Group II: Negative control group (Atorvastatin 40 mg/kg), Group III: Positive control group (Papaya Leaf Extract- 2000 mg/kg), Group Ⅳ: Treatment (Papaya leaf extract 2000 mg/kg + Atorvastatin 40 mg/kg). The experiment was carried out for a period of 15 days and the effect of atorvastatin on thrombocytosis was determined by platelet count.

Results: The effect of atorvastatin and papaya leaf extract on platelet count was determined. The positive control group (only Papaya leaf extract) shows the highest platelet count of 721.8 (10^3/mm^3) with a percentage reduction of -5.01%. The negative control group (only Atorvastatin) shows platelet count of 689.8 (10^3 /mm^3) with a percentage reduction of 1.14 %.

The normal control group (no drugs administered) which gives the normal platelet count in Albino Wistar rats which is 611.5 (10^3/mm^3) with a percentage reduction of (8.32) %.

The treatment group, where platelet count was boosted using papaya leaf extract followed by administration of atorvastatin, to lower the platelet count shows a platelet count of 616.6 (10^3/mm^3) with a percentage reduction of (5.56%). By comparing the values of the control and treatment groups, we found that the platelet count in treatment group was more than the normal group. From this we can conclude that atorvastatin probably reduces the platelet count to a little more than the normal platelet count in Wistar Albino rats induced with thrombocytosis.

Conclusion: It was observed that treatment with atorvastatin shows a significant ($p<0.01$) decrease in platelet count when compared to the thrombocytosis induced group.

Keywords: Repurposing; Atorvastatin; Papaya leaf extract; Thrombocytosis; Thrombocytopenia

I. INTRODUCTION

Drug repurposing (DR) is becoming more common as a method of developing novel treatments, also called Drug Repositioning. It is an operation that recognizes new therapeutic uses for previous, existent or available drugs. It is an effective strategy to discover or to develop drug molecules with new pharmacological indications [1].

Atorvastatin is a competitive antagonist which inhibits HMG-CoA reductase by preventing the conversion of HMG-CoA to mevalonate. Statin medications decrease cholesterol production in the liver. Atorvastatin is also said to increase the number of LDL receptors on the surface of hepatic cells [2].

In patients with homozygous or heterozygous familial hypercholesterolemia, mixed dyslipidemia, isolated hypertriglyceridemia, or nonfamilial hypercholesterolemia, atorvastatin exhibits a reduction in the overall cholesterol level, low-density lipoprotein cholesterol (LDL-C), apolipoprotein B (apo B), very-low-density lipoprotein (VLDL-C), and triglycerides (TGs) while promoting high-density lipoprotein cholesterol (HDL-C). In subjects with dysbetalipoproteinemia, Atorvastatin has been shown to reduce intermediate-density lipoprotein (IDL-C). Atorvastatin is approved by the FDA and it is used in combination with a proper diet to prevent cardiovascular events in patients with

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cardiac risk factors and in patients with abnormal lipid profiles [3].

This work is proposed for the repurposing of atorvastatin; whether it can be used in thrombocytosis in experimentally induced animals with papaya leaf extract. Thrombocytosis is a state in which there are an excessive number of platelets in the blood. Platelets are components of blood cells in plasma that stop bleeding by sticking together to form a clot [4]. Essential thrombocytosis is diagnosed when the platelet count is over 45,0000 per microliter of blood and there is either a Janus kinase 2 (JAK2), Calreticulin (CALR), or myeloproliferative leukaemia virus oncogene (MPL) mutation, with no pathogenic or reactive causes, according to the World Health Organization [5]. A wide range of diseases and conditions may cause a rise in platelets, which includes anaemia, loss of blood, cancer, chemotherapy, chronic myelogenous leukaemia, infection, Kawasaki disease, Myelodysplasia, and Polycythaemia Vera [6].

Papaya leaf extract, made from dried (or) fresh crushed leaves, contains a variety of chemicals, including flavonoids and other plant phenols, as well as alkaloids like carpaine, anthraquinone, saponins, and cardiac glycosides like carposide and tannins. Due to the presence of quercetin, one of the flavonoids present in papaya leaves, it has the ability to increase ALOX12 (arachidonate 12-lipoxygenase) or platelet type lipoxygenase by 15 times [7,8]. This enzyme increases the quantity and development of megakaryocytes, which enhances platelet formation. As a result, papaya leaf extract has the potential to function on platelet-activating factor receptor (PTAFR), a platelet-specific gene that boosts platelet count by 13.5-fold after oral treatment [8.9]. Hence, the present research project was to propose whether Carica papaya will be used to induce thrombocytosis in experimental animals based on the effect of atorvastatin.

II. METHODOLOGY

Preparation of extract:

Carica papaya leaves were washed and the stems were removed before use. The leaves were blended without adding water or other liquids. This mixture was filtered to obtain a pure extract of C. papaya leaves. Volume of the extract was measured and the extracts were stored at 4°C until use. Fresh extracts were prepared for each use [10]. The dose was selected with reference to the acute toxicity results of the plant Carica papaya and was kept at 2000 mg/kg [11].

Preparation of atorvastatin solution:

The dose of atorvastatin was kept at 40 mg/kg [12]. 40 mg of atorvastatin tablet was crushed using a mortar and a pestle to obtain atorvastatin powder followed by triturating it with acacia and small quantities of distilled water.

Experimental Design:

Wistar Albino rats weighing around 150-200 g were selected for the study. The Albino Wistar rats were anaesthetised using diethyl ether in a desiccator. Blood was withdrawn from the retro-orbital route. The platelet count was estimated using Celltac Haematology Analyser.

Table No. 1: Body weight and doses given

Group	Animal	Body Weight (g)	Dose (ml)
I.	Normal control group	160±8.94	-
I.	Positive control group (Papaya leaf extract- 2000 mg/kg)	170±11.25	0.68±0.04
I.	Negative control group (Atorvastatin-40 mg/kg)	156.67±6.15	0.62±0.02
V.	Treatment group– Atorvastatin (40 mg/kg) and Papaya leaf extract (2000 mg/kg)	176.67±14.98	0.70±0.05

Statistical Analysis

The platelet count was expressed as a Mean ±SEM (n=6). One-way ANOVA was used in the statistical analysis, followed by Dunnett's test. All groups were compared to the normal group, **p<0.01 (**)** was considered statistically significant. GraphPad prism 5 was used to do the statistical analysis.

III. RESULTS

The blood was withdrawn from the rats after anaesthetising from the retro-orbital route and

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platelet count was carried out using Celltac Hemoanalyser on Day 1, Day 7 & Day 15.

Table No.2: Platelet Count on Day 1, Day 7 & Day 15 and Mean ±SEM

Group	Groups	Results (10^3/ mm^3)			Percentage reduction (%)	Mean ± SEM
		Day 1	Day 7	Day 15		
I.	Normal Group (Saline) 1 ml/ 100g., p.o.	667	635.1	611.5	8.32	611.5 ± 4.3
I.	Negative Control Group- Atorvastatin 40 mg/kg., p.o.	697.8	713.6	689.8	1.14	689.8 ± 2.5
I.	Positive Control Group- Papaya Leaf extract- 2000 mg/kg., p.o.	687.3	699	721.8	-5.01	721.8 ± 11.8
'.	Treatment Group- 2000 mg/kg Papaya leaf extract+ 40 mg/kg Atorvastatin., p.o.	653	674.1	616.6	5.56	616.6 ± 13.5

Normal Group: Normal Saline 1 ml/100g., p.o., **Positive Control Group:** Papaya Leave Extract 2000 mg/Kg, **Negative Control Group:** Atorvastatin 40 mg/Kg, **Treatment Group:** Papaya Leaf Extract 2000 mg/Kg+ Atorvastatin 40 mg/Kg.

Two randomized groups i.e. positive and treatment were included along with one negative control and normal group to check the effect of atorvastatin in reducing platelet count in thrombocytosis condition. The normal group was not administered with any drugs and was taken as a standard. The platelet count of the animals were estimated by withdrawing the blood via retro-orbital route and was given for evaluation using Celltac Hemoanalyser. The increase in platelet count by administering Papaya leaf extract was found to be significant with a mean of **721.8 ± 11.8 (n=6)**.

Table No. 3: Comparison of Normal, Positive Control and Treatment Groups.

SL. NO	Comparison	Mean Difference	P value
1	Positive vs. Treatment	105.17	**P<0.01**
2	Positive vs. Normal	110.33	**P<0.01**

On comparing the data, we find that atorvastatin probably reduces the platelet count upon administration. The mean of the treatment was found to be **616.6 ± 13.5 (n=6)** which is nearly similar to the normal control group having a mean of **611.5 ± 4.3 (n=6)**. The mean difference of positive control group vs. treatment group was found to be **105.17 (p< 0.01).** The mean difference of positive control group vs. control was found to be **110.33 (p< 0.01).**The results for each group were expressed as Mean ±SEM (n=6). Data was analysed using one-way ANOVA followed by Dunnetts test. Values ranging from **p<0.01 (**)** were considered statistically significant compared to normal group. The statistical analysis was carried out with the help of GraphPad prism 5.

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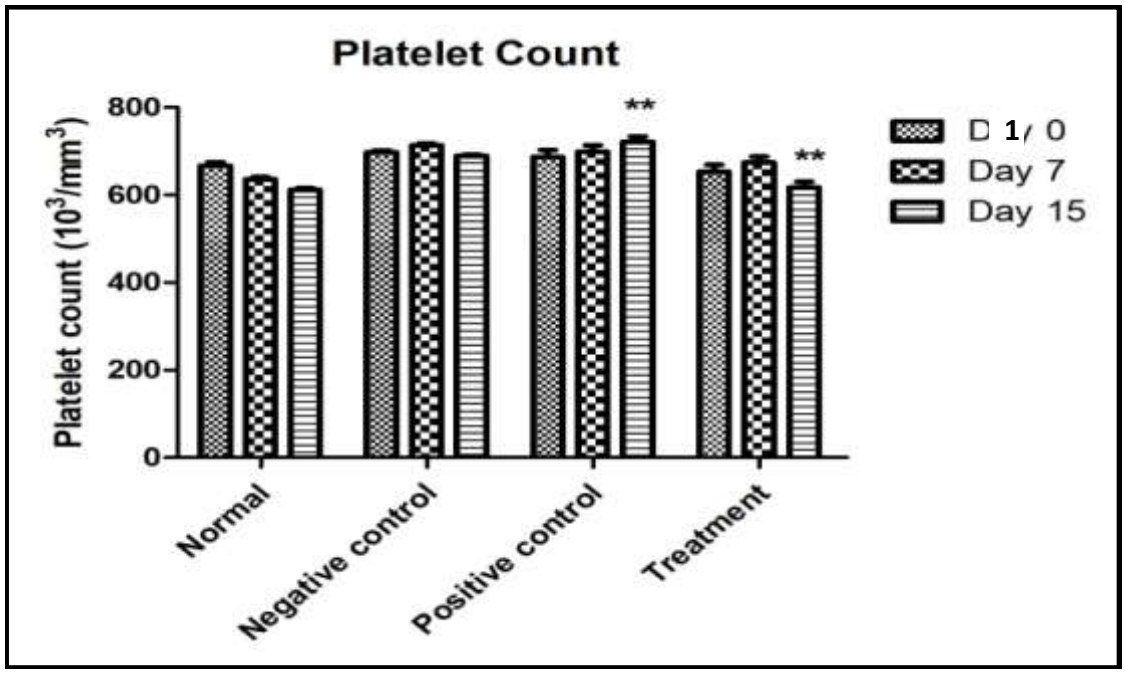

Figure No. 1: Effect of Atorvastatin on platelet count

From **figure no. 1,** the effect of atorvastatin and papaya leaf extract on platelet count was determined, where the positive control group (only Papaya leaf extract) shows the highest platelet count of **721.8 (10^3/mm^3)** with a percentage reduction of **-5.01%**, the negative control group (only atorvastatin) shows platelet count of **689.8 (10^3 /mm^3)** with a percentage reduction of **1.14%,** the normal control group (no drugs administered) shows platelet count of **611.5 (10^3/mm^3)** with a percentage reduction of **8.32%** and the treatment group, where platelet count was boosted using papaya leaf extract followed by administration of atorvastatin, the platelet count was lower and shows a platelet count of **616.6 (10^3/mm^3)** with a percentage reduction of **5.56%**.By comparing the values of the control and treatment groups, we found that the platelet count in treatment group is similar to that of normal group.From the above results we can conclude that atorvastatin probably reduces the platelet count slightly similar to that of normal group in experimentally induced thrombocytosis

IV. DISCUSSION

Drug repurposing is frequently a key component of a company's lifecycle management plan. It could also be in response to a clinical need that has gone unsatisfied [13]. It is extensively used since it is less harmful, offers lower expenses, and requires less time [14]. It is anticipated that repurposed pharmaceuticals will only take three to four years to allocate clinical trials [15]. Many drugs have been thoroughly evaluated for repurposing [16]. When treating rare, orphan, or neglected diseases [17], especially during pandemics, repurposing is frequently used [18].

Off-target activity is typically considered unfavourably when a medication demonstrates activity at many biological targets, resulting in unwanted molecular promiscuity and undesirable side effects [19]. However, this so-called "polypharmacology", which may offer treatment options for additional illnesses, especially if the disease pathophysiology is complicated [20].

Carica Papaya leaf extract was found to contain various phytochemicals among which quercetin is responsible for activating the genes of arachidonate 12-lipoxygenase (ALOX 12) and platelet-activating factor receptor (PTAFR), which impact platelet formation and aggregation [21]. Platelet activation is known to suppress cAMP synthesis and cyclic nucleotide-dependent protein kinase activity [22], and drugs that increase cAMP reverse platelet activation [23].

The synthetic coenzyme 3-hydroxy-3-methylglutaryl found in normocholesterolemic mice, the reductase inhibitor atorvastatin upregulates endothelial Nitric Oxide Synthase (eNOS) in thrombocytes, reduces platelet activation in-vivo, and protects against cerebral ischemia. Statins' antithrombotic and stroke-

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protective actions are partly mediated by eNOS overexpression. The findings imply that statins could be used as a unique preventative therapy technique that is not dependent on serum cholesterol levels [24].

CD40 ligand (CD40L) is a member of the tumor necrosis factor family and is a transmembrane protein that exists on immune system cells as well as endothelial cells, smooth muscle cells, macrophages and platelets. When interacting with its receptor CD40, CD40L exerts pro-inflammatory and pro-thrombotic activities, including increased expression of matrix metalloproteinases, chemokines, cytokines, and tissue factor (TF) [25].

CD40L is expressed by platelets when stimulated with common agonists [26]; then it is cleaved from the platelets within a few minutes to a few hours to form a soluble form (sCD40L) [27]. According to calculations,> 95% of circulating CD40L comes from platelet events in patients with acute coronary syndrome and patients with cardiovascular risk [28,29,30].

Patients with hypercholesterolemia are in a continuous pro-thrombotic state, which can be evidenced by increased TF expression and thrombin formation in the body [31].

It was observed by Tailor et al., 2004 that atorvastatin treatment to rats on a regular diet-maintained lowers plasma PF4 (platelet factor) levels. Pravastatin, simvastatin, and atorvastatin have all been found in humans to reduce platelet activation in response to hypercholesterolemia [32].

V. CONCLUSION

Thrombocytosis was induced in Albino Wistar rats by administering Papaya leaf extract.The experimental data exhibited an increase in platelet count on administering papaya leaf extract.It was observed that treatment with atorvastatin shows a significant decrease in platelet count when compared to thrombocytosis induced group.Remarkable decrease in platelet count was observed in the negative control group.Noteworthy increase in platelet count was observed in positive control group when compared to normal control group.Significant decrease in platelet count was observed in all the groups treated with atorvastatin.

Thrombocytopenia effect of atorvastatin was recorded during the study.We can conclude that changes in platelet CD40L expressions are leading to lower platelet CD40 expressions and plasma CD40 levels via a negative-feedback process.

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REFERENCES

[1]. Kasznicki J, Sliwinska A, Drzewoski J. Metformin in cancer prevention and therapy. Ann. Transl. Med. 2014; 2(6).
[2]. Feingold KR. Cholesterol lowering drugs. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K et al. editors. Endotext. South Dartmouth (MA): MDText.com; 2021.
[3]. McIver LA, Siddique MS. Atorvastatin. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2020.
[4]. Thrombocytosis [Internet]. Clevelandclinic.org. [cited 2021 Jul 31]. Available from: https://my.clevelandclinic.org/health/diseases/13350-thrombocytosis
[5]. Ashorobi D, Gohari P. Essential Thrombocytosis. StatPearls [Internet]. 2020 Jun 27.
[6]. Healthgrades.com. [cited 2021 Jul 31]. Available from: https://www.healthgrades.com/right-care/blood-conditions/high-platelets
[7]. Laufs U , Gertz K , Huang P , Nickenig G , Böhm M, Dirnagl U, Endres M. Atorvastatin upregulates type III nitric oxide synthase in thrombocytes, decreases platelet activation, and protects from cerebral ischemia in normocholesterolemic mice. Stroke. 2000; 31(10):2442-9.
[8]. Senthilvel P, Lavanya P, Kalavathi Murugan Kumar RS, Anitha P, Bag S, Sarveswari S, Vijayakumar V, Ramaiah S, Anbarasu A. Flavonoid from Carica papaya inhibits NS2B-NS3 protease and prevents Dengue 2 viral assembly. Bioinformation. 2013; 9(18):889.
[9]. Subenthiran S, Choon TC, Cheong KC, Thayan R, Teck MB, Muniandy PK et al. Carica papaya leaves juice significantly accelerates the rate of increase in platelet count among patients with dengue fever and dengue haemorrhagic fever. eCAM. 2013; 2013.

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[10]. Schönbeck U , Varo N, Libby P, Buring J, Ridker PM. Soluble CD40L and cardiovascular risk in women. Circulation. 2001; 104(19):2266-8.
[11]. Varo N, de Lemos JA, Libby P, Morrow DA, Murphy SA, Nuzzo R et al. Soluble CD40L: risk prediction after acute coronary syndromes. Circulation. 2003; 108(9):1049-52.
[12]. Heeschen C, Dimmeler S, Hamm CW, van den Brand MJ, Boersma E, Zeiher AM et al. Soluble CD40 ligand in acute coronary syndromes. NEJM. 2003; 348(12):1104-11.
[13]. Garlichs CD, John S, Schmeisser A, Eskafi S, Stumpf C, Karl M et al. Upregulation of CD40 and CD40 ligand (CD154) in patients with moderate hypercholesterolemia. Circulation. 2001; 104(20):2395-400.
[14]. Ferro D, Basili S, Alessandri C, Mantovani B, Cordova C, Violi F. Simvastatin reduces monocyte-tissue-factor expression type IIa hypercholesterolaemia. Lancet. 1997; 350(9086):1222.
[15]. Bavendiek U , Libby P , Kilbride M , Reynolds R , Mackman N , Schönbeck U . Induction of tissue factor expression in human endothelial cells by CD40 ligand is mediated via activator protein 1, nuclear factor κB, and Egr-1. J. Biol. Chem. 2002; 277(28):25032-9.
[16]. Pedersen TR, Kjekshus J, Pyörälä K, Olsson AG, Cook TJ, Musliner TA et al. Effect of simvastatin on ischemic signs and symptoms in the Scandinavian Simvastatin Survival Study (4S). Am. J. Cardiol. 1998; 81(3):333-5.
[17]. Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG et al. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. NEJM. 1996; 335(14):1001-9.
[18]. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, Macfarlane PW et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia.NEJM. 1995; 333(20):1301-8.
[19]. Sundarmurthy D, Jayanthi CR, Lakshmaiah KC. Effect of Carica papaya leaf extract on platelet count in chemotherapy-induced thrombocytopenic patients: A preliminary study. Natl J Physiol Pharm Pharmacol. 2017; 7(7):685.
[20]. Tailor A, Lefer DJ, Granger DN. HMG-CoA reductase inhibitor attenuates platelet adhesion in intestinal venules of hypercholesterolemic mice. Am. J. Physiol. Heart Circ. 2004; 286(4):H1402-7.
[21]. Gocmen AY, Ocak GA, Ozbilim G, Delibas N, Gumuslu S. Effect of atorvastatin on atherosclerotic plaque formation and platelet activation in hypercholesterolemic rats Can. J. Physiol. Pharmacol. 2013; 91(9):680-5.
[22]. Sundarmurthy D, Jayanthi CR, Lakshmaiah KC. Effect of Carica papaya leaf extract on platelet count in chemotherapy-induced thrombocytopenic patients: A preliminary study. Natl J Physiol Pharm Pharmacol. 2017; 7(7):685.
[23]. Li Z, Ajdic J, Eigenthaler M, Du X. A predominant role for cAMP-dependent protein kinase in the cGMP-induced phosphorylation of vasodilator-stimulated phosphoprotein and platelet inhibition in humans. Blood. 2003; 101(11):4423-9.
[24]. Oh WJ, Endale M, Park S-C, Cho JY, Rhee MH. Dual roles of quercetin in platelets: Phosphoinositide-3-kinase and MAP kinases inhibition, and cAMP-dependent vasodilator-stimulated phosphoprotein stimulation. eCAM. 2012; 2012:485262.
[25]. Nielson CP, Wingett D. Endothelial cell and cAMP regulation of T-cell CD40 ligand: relevance of calcium/calmodulin-dependent kinase IV signalling. Immunology. 2002; 105(4):430–40.
[26]. Van Riet-Nales DA, de Jager KE, Schobben AF, Egberts TC, Rademaker CM. The availability and age-appropriateness of medicines authorized for children in the Netherlands. Br. J. Clin. Pharmacol.. 2011; 72(3):465-73.
[27]. Fetro C, Scherman D. Drug repurposing in rare diseases: Myths and reality. Therapies. 2020; 75(2):157-60.
[28]. Ashburn TT, Thor KB. Drug repositioning: identifying and developing new uses for existing drugs. Nat. Rev. Drug Discov. 2004; 3(8):673-83.
[29]. Baker NC, Ekins S, Williams AJ, Tropsha A. A bibliometric review of drug repurposing. Drug Discov. Today.2018; 23(3):661-72.
[30]. Ismail HM, Dorchies OM, Scapozza L. The potential and benefits of repurposing

existing drugs to treat rare muscular dystrophies. Expert Opin. 2018; 6(4):259-71.

[31]. Liu C, Zhou Q, Li Y, Garner LV, Watkins SP, Carter LJ et al. Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases.

[32]. Rao MS, Gupta R, Liguori MJ, Hu M, Huang X, Mantena SR et al. Novel computational approach to predict off-target interactions for small molecules. Frontiers in big data. 2019; 2:25.