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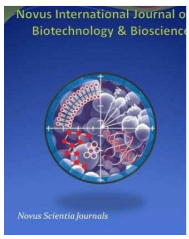

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An open label, multicenter, controlled clinical trial to evaluate efficacy and safety of DNT -53 in patients with low platelet count

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ABSTRACT

A normal platelet count in a healthy individual is between 1, 50,000 and 4, 50,000 per µL of blood. 95% of healthy people will have platelet counts in this range. Some will have statistically abnormal platelet counts while having no demonstrable abnormality. Management of platelet count effectively has been a distant reality. DNT-53, a propitiatory blend of herbs has shown to increase the plate count effectively as studied in the ancient literatures and research publication. The aim of the study is to evaluate the safety and efficacy of "DNT-53" in patients with low platelet count, associated with co-morbid conditions. An open labeled nonrandomized proof of concept study enrolled 72 low platelet count patients (06 groups- Group A-Alcoholic & Spleen Disorders - 12 Subjects, Group B- Vitamin B12 Deficiency - 12 Subjects, Group C- Malaria-12 Subjects, Group D-Viral Infections-12 Subjects Group E- Drug Induced-12 Subjects ,Group F- Bacterial Infections-12 Subjects) who met the selection criteria. The efficacy was assessed by measuring the Platelet count at baseline and on day 3, 5,7,9,11,13 and 28 and followed up for 56 days. The effect of DNT-53 on other co-morbid conditions was also evaluated. Planned student't' test was applied. Mean platelet count on Screening (day-0), day 11 and Day 56 was found to be 127131.94, 207708.33, 270680.56 Lakhs/cumm. However statistical increase of 38.79% and 53.03% in Platelet Count was found on day 11 and Day 56 when compared from Screening Platelet count.

From the data obtained, it was found that the investigational product DNT-53 was showing significant percentage increase in Platelet count throughout the study duration when compared to screening platelet level in all enrolled subjects, which was considered as an important parameter in this study.During the study we were not found any safety issues related to the Investigational Product. "HDNT-53" is effective in treating the low platelet count associated with co-morbid conditions with positive outcome on the quality of life.

KEY WORDS: DNT-53, Low platelet count, Blood, co-morbid conditions.

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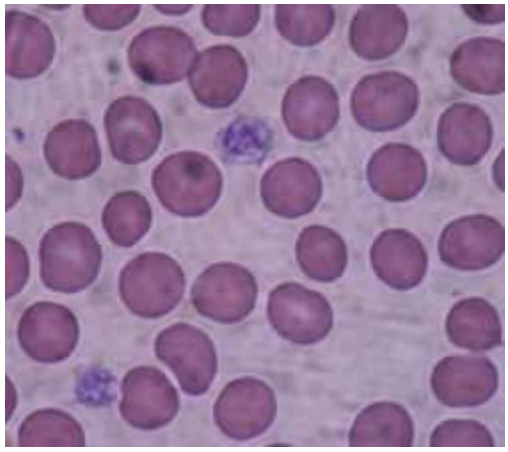
INTRODUCTION

Platelets, or **Thrombocytes**, are small; disk shaped clear cell fragments i.e. cells that do not have a nucleus, 2–3 µm in diameter, which are derived from fragmentation of precursor mega karyocytes. The average lifespan of a platelet is normally just 5 to 9 days. Platelets are a

natural source of growth factors. They circulate in the blood of mammals and are involved in homeostasis, leading to the formation of blood clots.

If the number of platelets is too low, excessive bleeding can occur. However, if the number of platelets is too high, blood clots can form (thrombosis), which may obstruct blood vessels and result in such events as a stroke, myocardial infarction, pulmonary embolism or the blockage of blood vessels to other parts of the body, such as the extremities of the arms or legs. An abnormality or disease of the platelets is called a thrombocytopathy, which could be either a low number of platelets (thrombocytopenia), a decrease in function of platelets, or an increase in the number of platelets. There are disorders that reduce the number of platelets, such as heparin-induced thrombocytopenia (HIT) or thrombotic thrombocytopenic Purpura (TTP) that typically cause thromboses, or clots, instead of bleeding.



Platelets release a multitude of growth factors including platelet-derived growth factor (PDGF), a potent chemo tactic agent, and TGF beta, which stimulates the deposition of extracellular matrix. Both of these growth factors have been shown to play a significant role in the repair and regeneration of connective tissues. Other healing-associated growth factors produced by platelets include basic fibroblast growth factor, insulin-like growth factor 1, platelet-derived epidermal growth factor, and vascular endothelial growth factor. Local application of these factors in increased concentrations through Platelet-rich plasma (PRP) has been used as an adjunct to wound healing for several decades.

A normal platelet count in a healthy individual is between 1,50,000 and 4,50,000 per μL of blood. 95% of healthy people will have platelet counts in this range. Some will have statistically abnormal platelet counts while having no demonstrable abnormality. However, if it is either very low or very high, the likelihood of an abnormality being present is higher.

Both thrombocytopenia and thrombocytosis may present with coagulation problems. In general, low platelet counts increase bleeding risks; however there are exceptions. High counts may lead to thrombosis, although this is mainly when the elevated count is due to myeloproliferative disorder.

Cause of Abnormal Platelet Count

There are many reasons of having abnormal platelet count. Abnormal Platelet Count consist of either Low Platelet count i.e Platelet falls below the Normal level of 150,000 per ml of blood and High Platelet count i.e increase of Platelets in the blood above 450,000 per ml of blood.There are various reasons for these abnormalities; mainly few of them are listed below.

Cause of Low Platelet count.

Low platelets count is observed when there is less number of platelet circulating in the blood. If this happens then, there are possibilities of bleeding from gums and nose. This happens only when the platelet count drops below 10000 per ml of blood. One cause of a low platelet count is decreased platelet production by the bone marrow. A number of conditions can cause the bone marrow to stop producing enough platelets. Certain types of cancers such as leukemia or cancers involving the lymph system can do it. More commonly, a viral infection can interfere with platelet production by the bone marrow. Some medications, vitamins deficiencies (B12

and folate), and even excessive alcohol intake can temporarily suppress the bone marrow causing a low platelet count. In rare cases, it can be due to a genetic condition or associated with kidney failure.

Objectives:

Primary objectives:
To evaluate the efficacy of DNT-53 for treating Low Platelet Count
Secondary objectives:
To evaluate the safety of DNT-53 in subjects with Low Platelet Count

MATERIAL AND METHODS

Patients both male and female aged 18-60 years and those willing to give written informed consent were selected. They were with low platelet count.

Inclusion criteria

Patients with low Platelet count ,suffering from diseases like Malaria, Vitamin B12 (or) folate deficiency, certain viral & bacterial infections, female subjects who confirmed non-pregnant status and agreed to comply with proper contraception throughout the study duration and patients willing and able to comply with all trial requirements were included in the study. The subjects who were enrolled in the study were having the low platelet count from several days and were on treatment therapy to control the platelet count. The medical history of the 72 patients reported that the mean platelet count of 127131.9 lakhs/cumm.

Exclusion criteria

Patients having the platelet count less than 80,000 per micro liter, significant renal insufficiency, history of cerebro-vascular disease, HIV infection, AIDS, hepatitis B or C, or other immunosuppressive disorders, drug abuse within past 2 years, pregnancy and breast feeding women were excluded from the study.

Study was conducted by non- randomized, open labeled, interventional study by ICBio clinical research. It involved the clinical attendance of the subjects on recruitment and on follow up. Subjects enrolled in the study received study drug (From Baseline Visit to 07 Days - Daily 4 sachets will be taken orally at a definite interval of 4 hrs. (1 sachets at a time),morning 8 AM, 12 PM (afternoon), 4 PM (Evening) and 8 PM (Night),at 08th day – 03 Doses will be taken orally (1 sachets at a time) morning 8 AM, 12 PM (afternoon) and 8 PM (Night),at 09th day - 02 Doses will be taken orally (1 sachets at a time)morning 8 AM and 8 PM (Night),at 10th day - 01 Dose will be taken orally (1 sachet) 8 PM (Night).The powder should be swallowed using spoon along with water) during each visit. Study drug would be the alternative or in addition to the therapies already being used.

The safety and efficacy parameters were compared with baseline and follow-up data with laboratory investigations, demographics were analyzed in the study. Adverse events/ side effects were noted for each follow up visit.

Ethics Committee approval

All study related documents Protocol, CRF, Dairy Card, Investigator Brochure and ICF (English and Kannada versions). Written informed consent was obtained from the subject(s) before the start of the trial and after due approval from IEC/IRB. Ethics Committee notifications as per the GCP guidelines issued by Central Drugs Standard Control Organization and ethical guidelines for biomedical research on human subjects issued by Indian Council of Medical Research has been followed during the conduct of the study

[Clinical IEC (Independent Ethics Committee for Ethics in Research and approved on 16th may 2012)].

Study outcomes

Primary outcomes

- Increase in the Platelet count after the drug intervention
- Efficacy of the drug.

Secondary outcomes

- Any changes observed in the Platelet count level.
- Safety concern of the drug.

Visit details

The patients were screened and enrolled. The enrollment day was considered as the baseline data and the patient were asked to visit on: Day3, Day5, Day7, Day9, Day11, Day13,Day 28 and Day56.

Statistical analysis

Data analysis was carried out using Statistical Analysis System. Student‘t’ test for independent samples was used to compare group mean baseline values and response differences (outcomes minus baselines) between the groups. Planned student‘t’ test for paired values was used to compare outcome versus baseline values with in groups. Significant differences between mean data were determined using $P < 0.05$.

RESULTS

Demographic and other baseline characteristics

In the study around 132 patients were screened and out of them 72 patients were selected. The other 60 patients were considered as screen failure as they did not meet the inclusion criteria. The enrolled subjects consisted of 45 Males and 27 females (Table 1).

Table: 1 Demographics of the Study Patients

Gender	Male	45
	Female	27
Age (years)	n	72
	Mean	37.04
	Min	18
	Max	60
Height (m)	n	72
	Mean	168.13
	Min	145.00
	Max	185.00
Weight (kg)	n	72
	Mean	63.28
	Min	40.00
	Max	86.00
BMI (kg/m^2)	n	72
	Mean	22.37
	Min	16.20
	Max	28.30

Efficacy Analyses

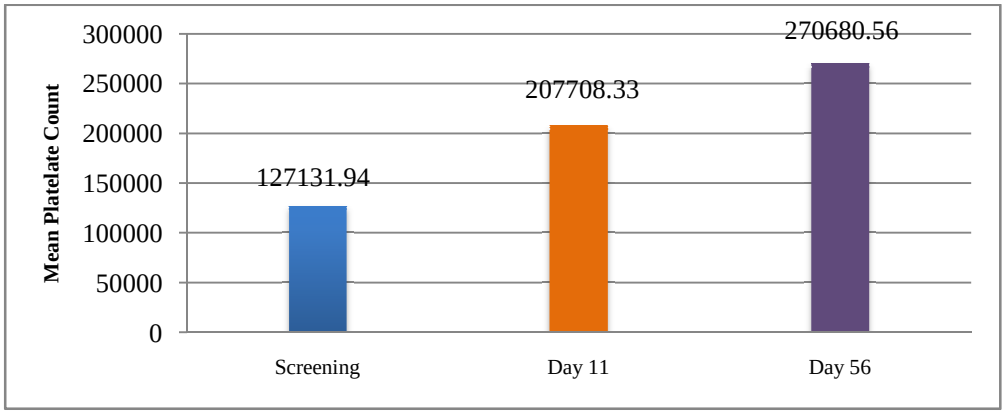
Totally 72 subjects were enrolled in to the study and all 72 subjects completed the study the efficacy was analyzed by considering the screening, Day 11 and Day 56 platelet count.

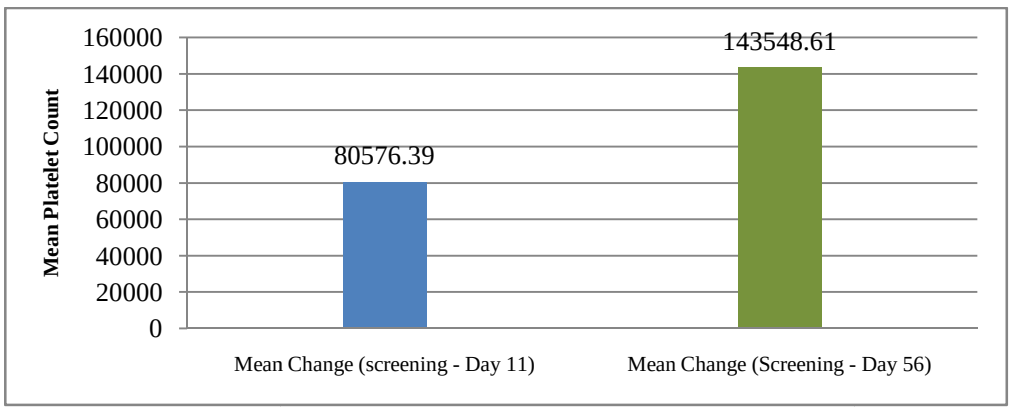
The overall Mean Platelet count of 72 subjects (n=72)

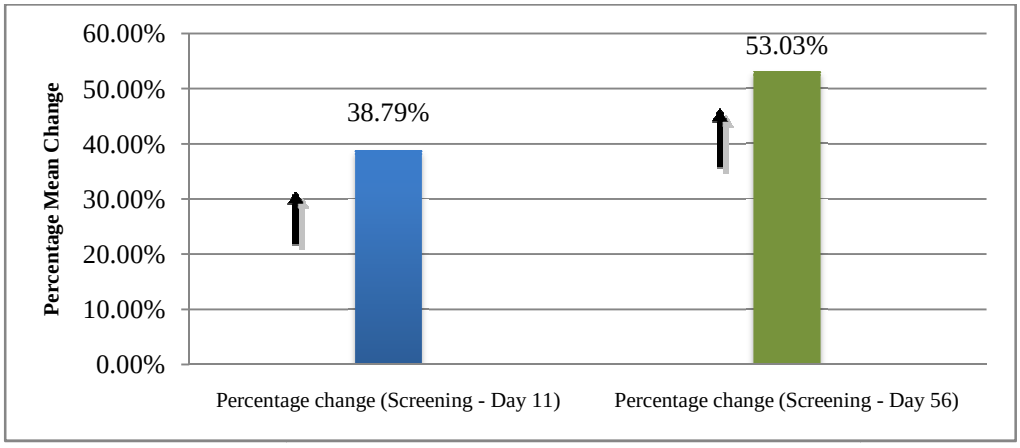
Mean platelet count on Screening (day-0), day 11 and Day 56 was found to be 127131.94, 207708.33, 270680.56 Lakhs/cumm. However statistical increase of 38.79% and 53.03% in Platelet Count was found on day 11 and Day 56 when compared from Screening Platelet count.

	Screening (Lakhs/cumm)	Day 11 (Lakhs/cumm)	Mean change (Lakhs/cumm)	Percentage Increased
Mean Platelet Count	127131.94	207708.33	80576.39	38.79%

	Screening (Lakhs/cumm)	Day 56 (Lakhs/cumm)	Mean change (Lakhs/cumm)	Percentage Increased
Mean Platelet Count	127131.94	270680.56	143548.61	53.03%





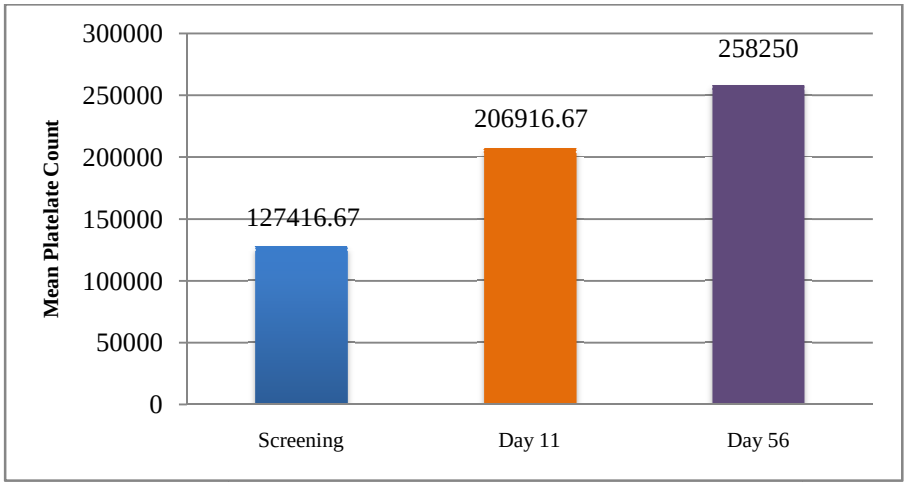


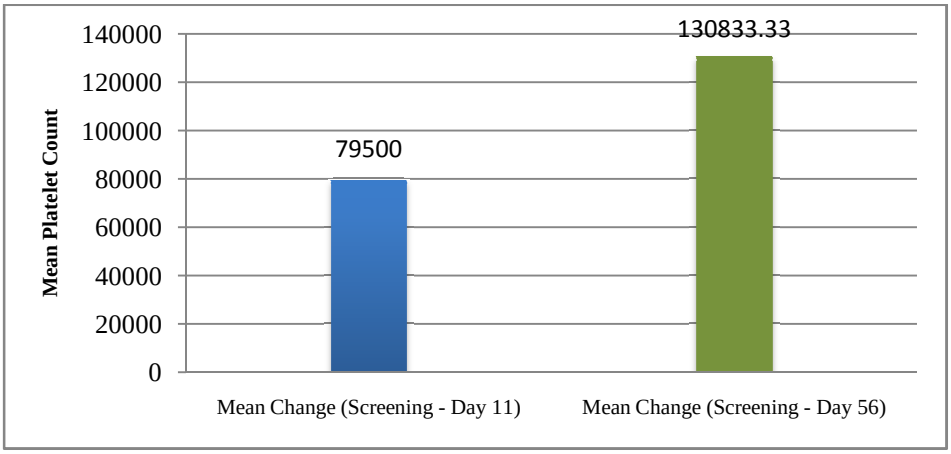
Mean Platelet Count; Mean Change and Mean percentage change group wise
Mean Platelet count of Alcoholic & Spleen Disorders Group Subjects (n=12)

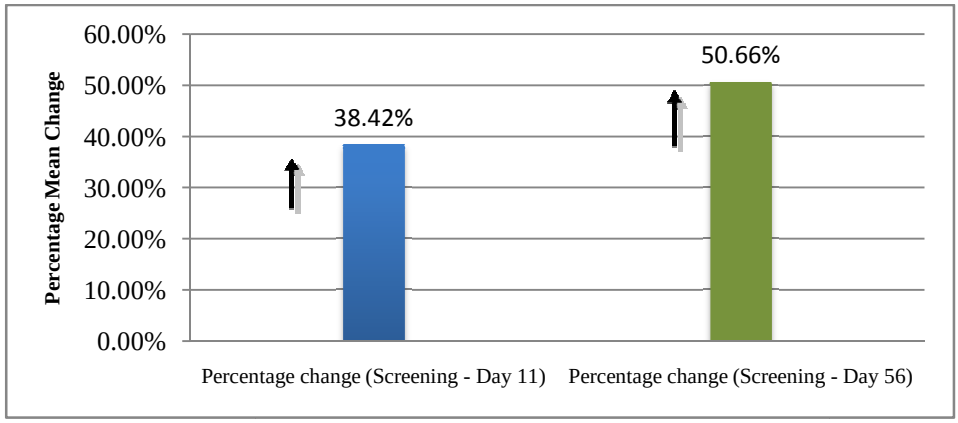
In this Group total 12 subjects were completed the study and Mean platelet count on Screening (day-0), Day 11 and Day 56 were found to be 127416.67, 206916.67, 258250 Lakhs/cumm. However statistical increase of 38.42 % and 50.66% in Platelet Count were found on day 11 and Day 56 when compared from Screening Platelet count.

	Screening (Lakhs/cumm)	Day 11 (Lakhs/cumm)	Mean change (Lakhs/cumm)	Percentage Increased
Mean Platelet Count	127416.67	206916.67	79500	38.42 %

	Screening (Lakhs/cumm)	Day 56 (Lakhs/cumm)	Mean change (Lakhs/cumm)	Percentage Increased
Mean Platelet Count	127416.67	258250	130833.33	50.66 %





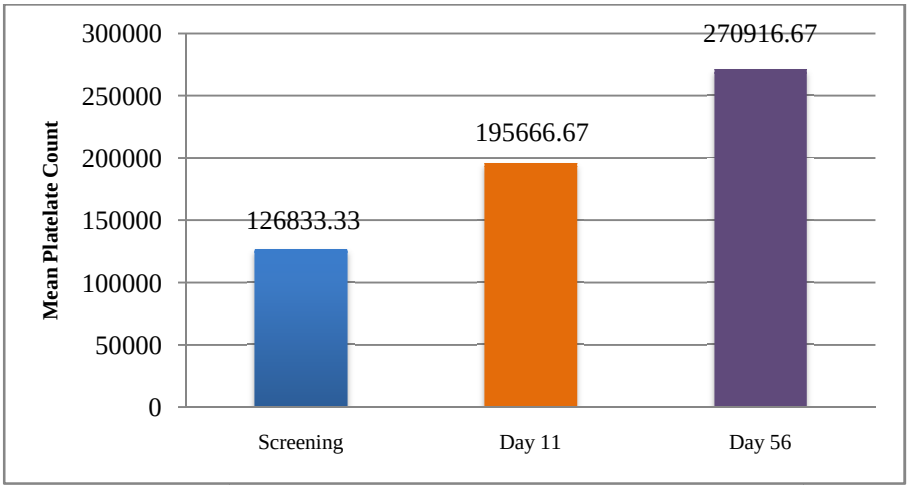


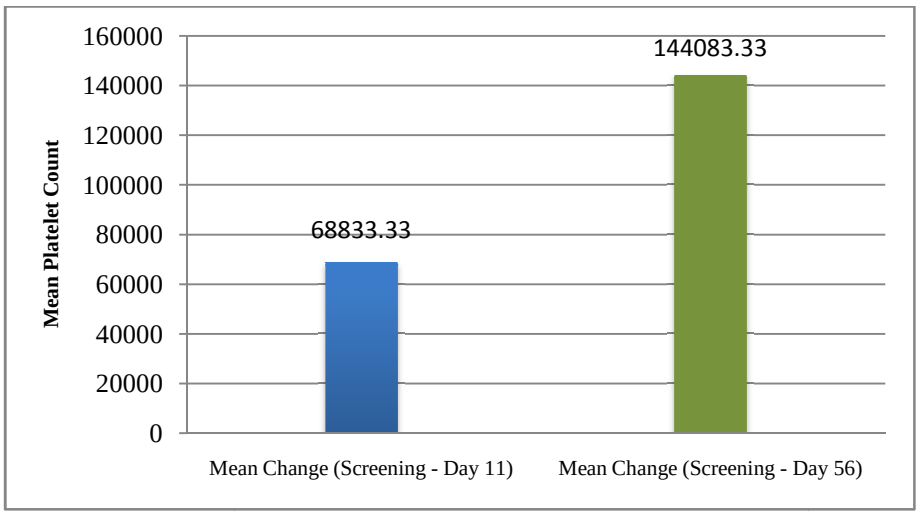
Mean Platelet count of Vitamin B12 Deficiency Group Subjects (n=12)

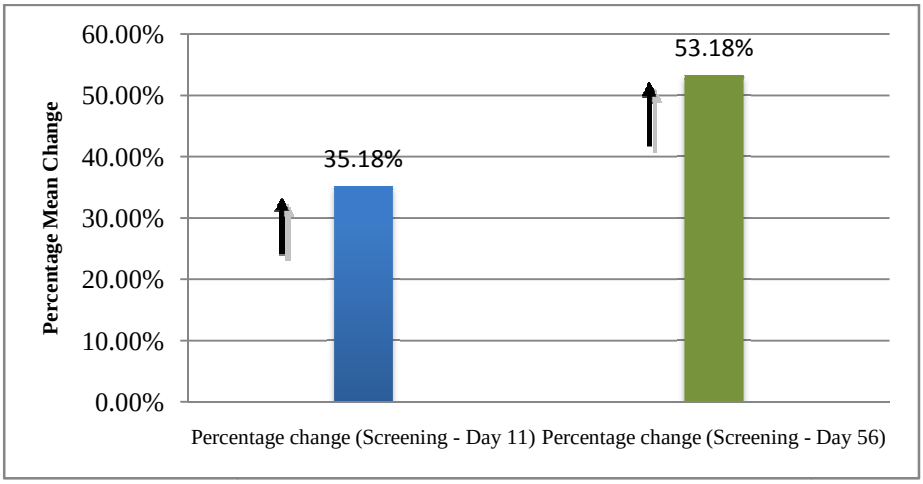
In this Group total 12 subjects were completed the study and Mean platelet count on Screening (day-0), Day 11 and Day 56 were found to be 126833.33, 195666.7, 270916.67 Lakhs/cumm. However statistical increase of 35.18 % and 53.18% in Platelet Count were found on day 11 and Day 56 when compared from Screening Platelet count.

	Screening (Lakhs/cumm)	**Day 11 (Lakhs/cumm)**	**Mean change (Lakhs/cumm)**	**Percentage Increased**
Mean Platelet Count	126833.33	195666.7	68833.33	35.18 %

	Screening (Lakhs/cumm)	**Day 56 (Lakhs/cumm)**	**Mean change (Lakhs/cumm)**	**Percentage Increased**
Mean Platelet Count	126833.33	270916.67	144083.33	53.18 %







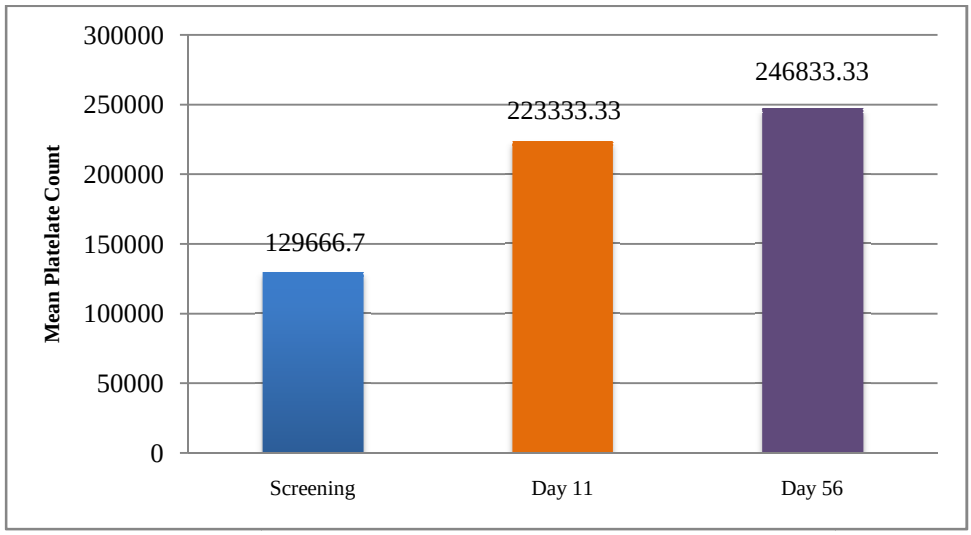
Mean Platelet count of Viral Infection Group Subjects (n=12)

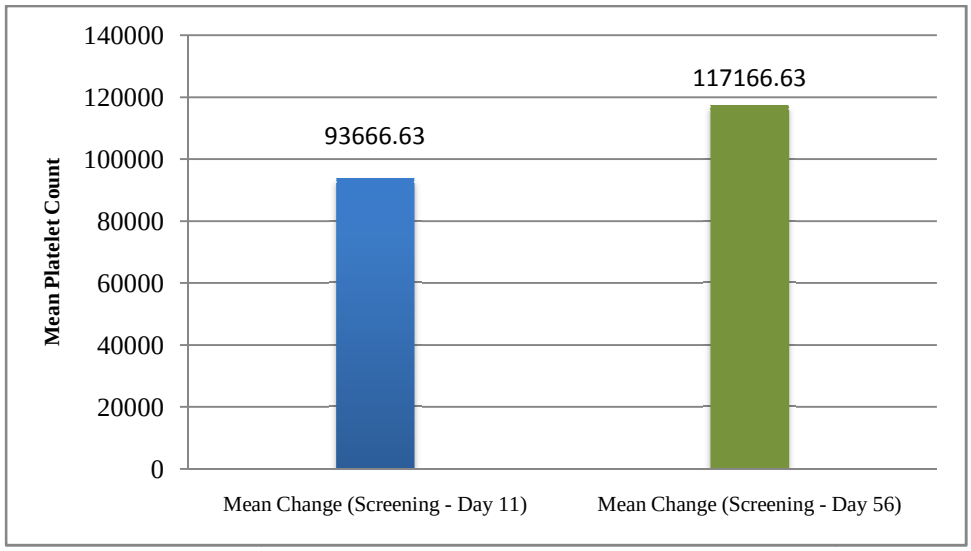
In this Group total 12 subjects were completed the study and Mean platelet count on Screening (day-0), Day 11 and Day 56 were found to be 129666.7, 223333.33, 246833.33

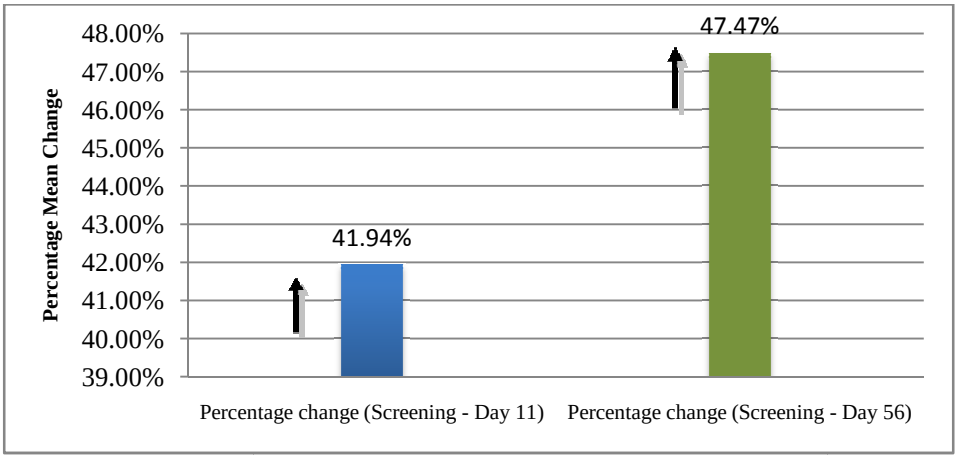
Lakhs/cumm. However statistical increase of 41.94 % and 4747% in Platelet Count were found on day 11 and Day 56 when compared from Screening Platelet count.

	Screening (Lakhs/cumm)	Day 11 (Lakhs/cumm)	Mean change (Lakhs/cumm)	Percentage Increased
Mean Platelet Count	129666.7	223333.33	93666.63	41.94 %

	Screening (Lakhs/cumm)	Day 56 (Lakhs/cumm)	Mean change (Lakhs/cumm)	Percentage Increased
Mean Platelet Count	129666.7	246833.33	117166.63	47.47 %





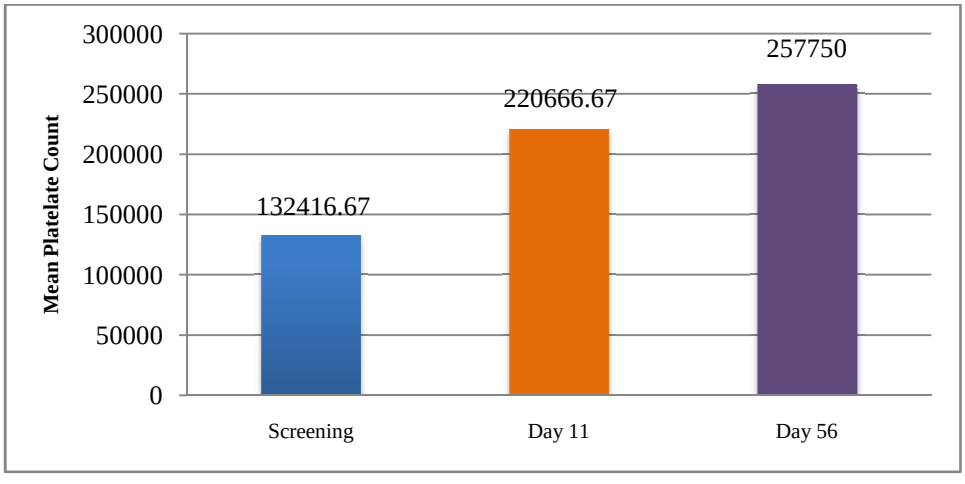


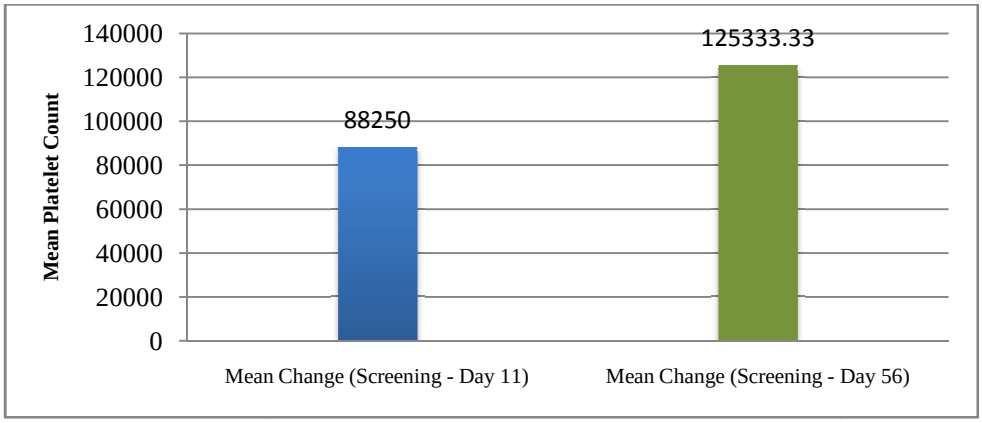
Mean Platelet count of Drug Induced Group Subjects (n=12)

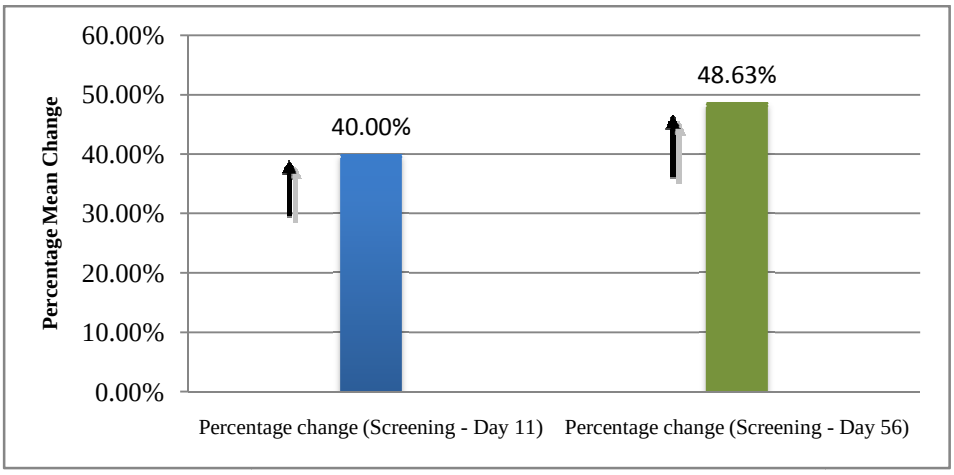
In this Group total 12 subjects were completed the study and Mean platelet count on Screening (day-0), Day 11 and Day 56 were found to be 132416.67, 220666.67, 257750 Lakhs/cumm. However statistical increase of 40.0% and 48.63% in Platelet Count were found on day 11 and Day 56 when compared from Screening Platelet count.

	Screening (Lakhs/cumm)	Day 11 (Lakhs/cumm)	Mean change (Lakhs/cumm)	Percentage Increased
Mean Platelet Count	132416.67	220666.67	88250	40.0 %

	Screening (Lakhs/cumm)	Day 56 (Lakhs/cumm)	Mean change (Lakhs/cumm)	Percentage Increased
Mean Platelet Count	132416.67	257750	125333.33	48.63%





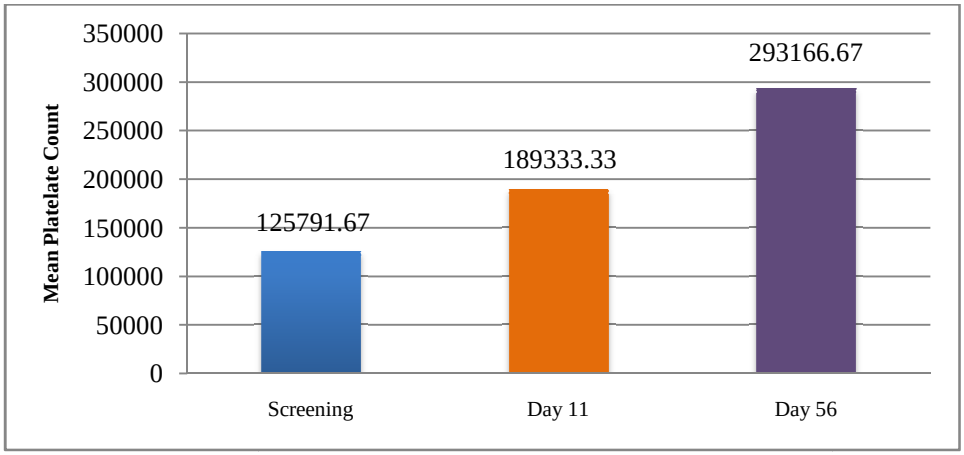


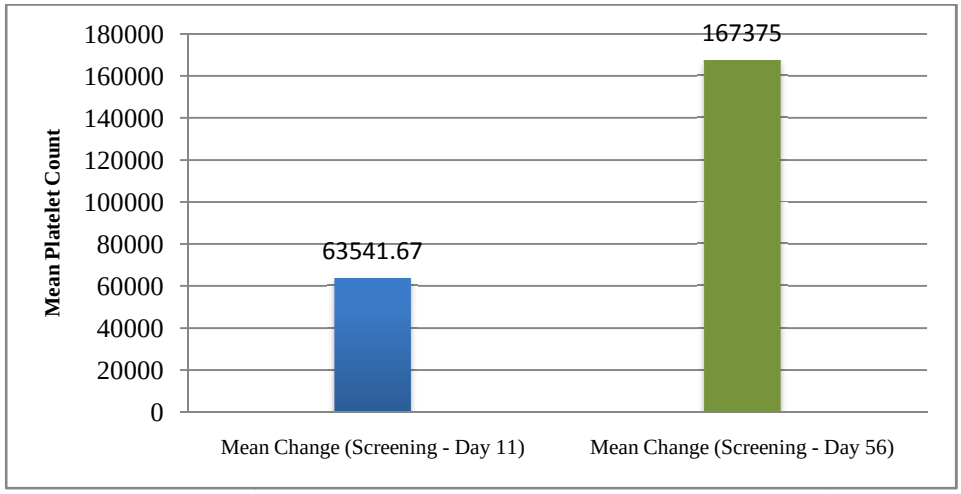
Mean Platelet count of Malarial Group Subjects (n=12)

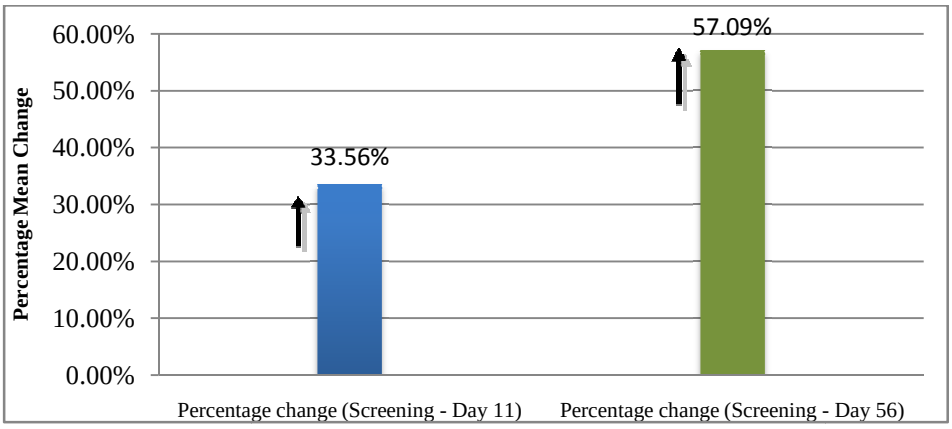
In this Group total 12 subjects were completed the study and Mean platelet count on Screening (day-0), Day 11 and Day 56 were found to be 125791.67, 189333.33, 293166.67 Lakhs/cumm. However statistical increase of 33.56 % and 57.09 % in Platelet Count were found on day 11 and Day 56 when compared from Screening Platelet count.

	Screening (Lakhs/cumm)	Day 11 (Lakhs/cumm)	Mean change (Lakhs/cumm)	Percentage Increased
Mean Platelet Count	125791.67	189333.33	63541.67	33.56 %

	Screening (Lakhs/cumm)	Day 56 (Lakhs/cumm)	Mean change (Lakhs/cumm)	Percentage Increased
Mean Platelet Count	125791.67	293166.67	167375	57.09 %





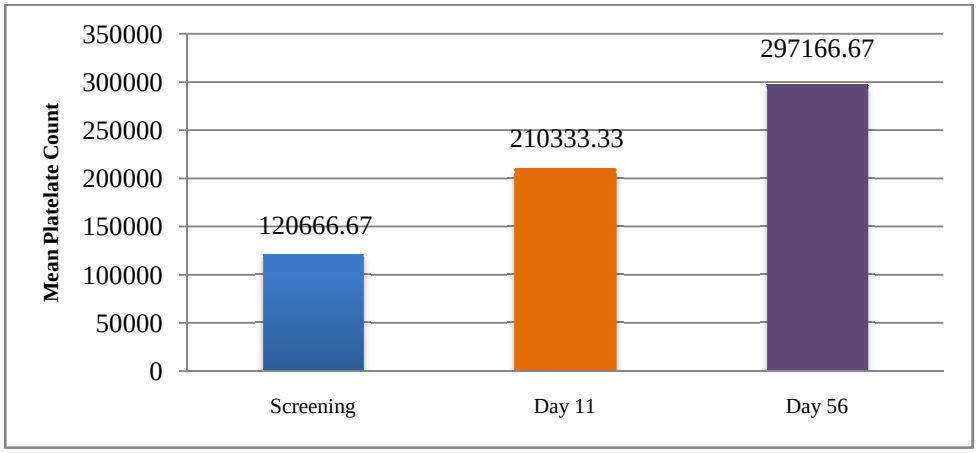


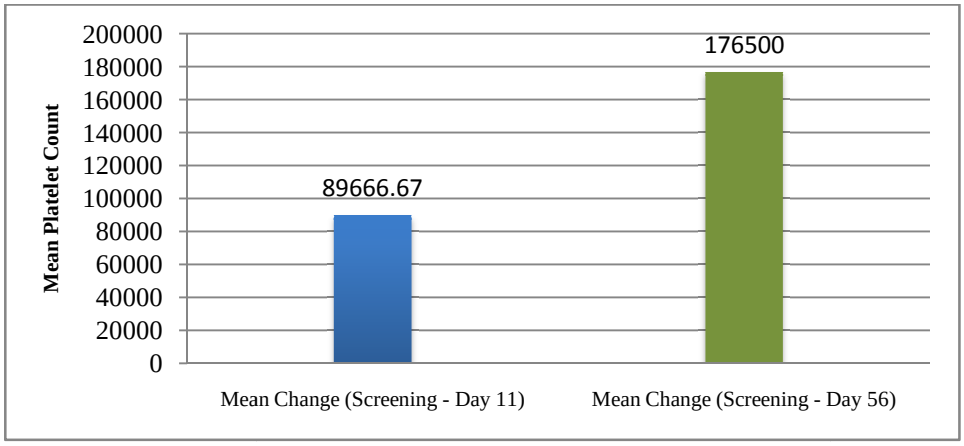
Mean Platelet count of Bacterial Infection Group Subjects (n=12)

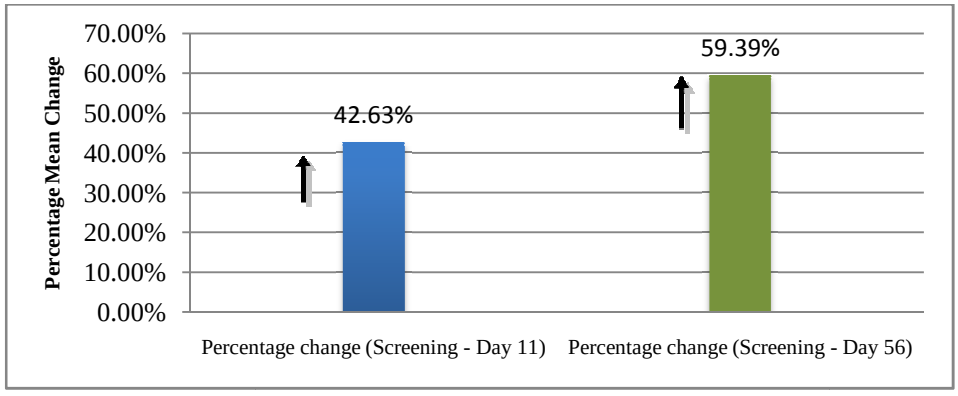
In this Group total 12 subjects were completed the study and Mean platelet count on Screening (day-0), Day 11 and Day 56 were found to be 120666.67, 210333.33, 297166.67 Lakhs/cumm. However statistical increase of 42.63 % and 59.39 % in Platelet Count were found on day 11 and Day 56 when compared from Screening Platelet count.

	Screening (Lakhs/cumm)	Day 11 (Lakhs/cumm)	Mean change (Lakhs/cumm)	Percentage Increased
Mean Platelet Count	120666.67	210333.33	89666.67	42.63 %

	Screening (Lakhs/cumm)	Day 56 (Lakhs/cumm)	Mean change (Lakhs/cumm)	Percentage Increased
Mean Platelet Count	120666.67	297166.67	176500	59.39 %







DISCUSSION

During baseline the subjects were screened according to the inclusion- exclusion criteria's and their platelet count was taken down. The Mean platelet count on Screening (day-0), day 11

and Day 56 was found to be 127131.94, 207708.33, 270680.56 Lakhs/cumm. However statistical increase of 38.79% and 53.03% in Platelet Count was found on day 11 and Day 56 when compared from Screening Platelet count.

The patients were then asked to follow the drug schedule and to come for follow up regularly on Day 3 (Visit 2), Day 5 (Visit 3), Day 7 (visit 4), Day 9 (Visit 5), Day 11 (Visit 6), Day 13 (visit 7) , Day 28 (Visit 8)and Day 56 (Visit 9) from the baseline visit. During these visits the subject's vitals and other physical examinations, lab investigations were conducted. During the last visit that is at Day 56 (Visit 9) the subject's platelet count showed a significant change from the baseline.

Further, when these changes were compared with each visit statistically the p value was < 0.005; hence we can conclude that DNT-53 is a potent herbal combination in increasing platelet count.
DNT-53 can be taken safely along with other drugs.
Finally, it can be concluded from the above study that DNT-53 is an effective to increase the platelet count and also is safe to be taken along with other prescribed medication which helps in reducing other illness associated with low platelet count.

CONCLUSION

DNT-53, herbal combination is effective in achieving the normal platelet count when administered with other platelet increasing drugs. DNT-53 is safe and effective in treating low platelet countwith added effect on the associated co-morbid conditions.

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