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ANTIVIRAL POTENTIAL OF DESMODIUM GANGETICUM (L.) DC. EXTRACT: DEVELOPMENT OF ANTIVIRAL PHYTOMEDICINE (GANJHUVIR^{R}) FOR THE TREATMENT OF DENGUE FEVER ASSOCIATED WITH THROMBOCYTOPENIA

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BSTRACT: Natural products can be the alternative of synthetic medicine and antiviral drugs can be potential from natural products. An attempt was made to evaluate the antiviral activity from the extract of *Desmodium gangeticum* (L.) DC. as a phytomedicine (GanjhuVir^{R}) for the treatment of dengue fever associated with thrombocytopenia. The present study was evaluated acute and subacute toxicity in rat as well as clinical trial in treated and without treated patients who admitted in hospital due to dengue fever associated with thrombocytopenia. The rats were grouped into group 1 (control) and 2, 3 and 4 (treated) for toxicity test while 51 patients were included in the clinical trial and 25 were treated and 26 provided only standard management as control. The parameters *viz*. platelet count, fever profile, average hospitalization period, clinical profiles and viral load reduction were tested for both the group. All the data related to morbidity, mortality and behavioural features were observed similar between exposed and control group. The haematological and biochemicals findings were comparable between the group. The platelet counts were significantly ($P<0.001$) increased and body temperature and hospital stay were significantly ($P<0.001$) decreased in the treated group than control group. Moreover, GanjhuVir^{R} is a phytomedicine extracted from studied plant and capable to treat viral activity especially for Dengue virus. This is non-toxic as per animal study and is safe without any adverse events and normalize the clinical findings among patients. Future research is suggested to know the efficacy of mild to moderate Covid-19 Patients.

INTRODUCTION: In recent research, the status of phytomedicine and their therapeutic actions has found high opportunity and has attracted great interest worldwide [1, 2]. As per traditional knowledge, the people of several countries depend on herbal medicines for the therapy of many health disorders [2, 3].

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During SARS-COV-2 scenario, researchers have been shown interest to develop herbal formulations for the improvement of the immune system to prevent viral infections in people [2, 4, 5]. On the other hand, different forms of preparation from *Carica papaya* leaves extract have long been used traditionally for treating dengue fever [6].

Among several viruses, these two have most prevalent in India and other parts of the globe. Till now, the treatment of the viral activities by using synthetic drugs is inconclusive. Besides several medicinal plants, *Desmodium gangeticum* (L.) DC. commonly called Shalparni belongs to the family Leguminosae (Fabaceae), subfamily Papilli-

onaceae. Trout [7] reported that genus *Desmodium* contains 170 tropical and subtropical species and this is distributed throughout the warmer parts of India. According to Toyigbénan *et al.* [8], several species under this genus have been used as traditional medicines.

The potent phytochemicals such as of isoflavones, isoflavanones, C-glycosyl flavonoids, pterocarpans, coumaronochromones, kaempferol-3-O-rutinoside, methyl salicylate β-D-glucopyranoside, leonuriside A, syringaresinol - 4' – O – β - D-glucopyranoside, (17Z, 20Z) - hexacosa-17, 20-dien-9-one and gangenoid have investigated in national and international studies [9, 10, 11].

For a drug discovery process, toxicity test in animals to know the safety profile of drug candidate followed by a clinical trial in human is a necessary step [12, 13, 14]. Moreover, only leaf extract of *C. papaya* enhanced platelet counts during dengue fever as per clinical trial [15], but antiviral potential of GanjhuVir^R developed from *Desmodium gangeticum* (L.) DC. extract especially for the prevention of Dengue fever associated with thrombocytopenia is lacking. The present study was attempted to evaluate the safety profile especially oral toxicity test in rat and clinical trial in human by using GanjhuVir^R developed from *Desmodium gangeticum* (L.) DC. extract especially for the treatment of Dengue fever associated with thrombocytopenia.

MATERIALS AND METHODS:

Plant Collection and Processing: The medicinal plant *Desmodium gangeticum* (L.) DC. was collected from the Khunti District of Jharkhand, India, during the period of 2019. The plant was identified by Botanical survey of India (Central National Herbarium), Howrah, West Bengal, India (voucher number – CNH/Tech IV/Repository/2015/17).

Freshly collected plant material was cleaned to remove adhering dust and then dried under shade. The dried samples were powdered and stored at room temperature (25°C) for further studies. Prior to test of acute and sub-acute toxicity as well as Dengue clinical trial, the powder was extracted with saline and further sufficient quantities of excipients were added to obtain GanjhuVir^R Syrup, which was further utilized for safety and clinical trial study.

Oral acute and Subacute Toxicity Test in Rat:

Acclimatization and Feeding of Rat: For both the test, the acclimatization was performed for Albino Wister rat (age = 4-6 weeks and body weight 60-90 gm) in the designated animal house. The temperature was maintained 22^{o}C (+3^{o}C), the relative humidity had been at least 30% and preferably not exceeded 70% and the lighting was maintained artificially in which photoperiod was 12 h light and 12 h dark.

For feeding conventional laboratory diets was used with continuous supply of drinking water. All the animals were caged as per the dosing group such as Group 1 (Vehicle control), Group 2 (Therapeutic dose as TD = 1.8 ml/Kg), Group 3 (Average dose as TD x 5 = 9 ml/Kg) and group 4 (Highest dose as TD x 10 = 18 ml/Kg), respectively.

Administration of Doses for Acute Toxicity Test in Rat: The test compound (GanjhuVir^R) was administered in a single dose by gavage using oral feeding needle. All animals were fasted prior to dosing. Following the period of fasting, all the animals weighted and then test compound administered and their food was withheld for further 3-4hrs in test animals. In each group, 10 animals (5 males and 5 females) were kept for experiments. Mortality was observed for 24 h and 48hrs duration and different clinical signs such as convulsions, lethargy, sleep, coma, salivation, diarrhoea, skin colour, fur, eyes and mucus membrane were monitored, and data were recorded for death of animals

Administration of Doses for Subacute Toxicity Test in Rat: The test compound (GanjhuVir^R) was administered in a single dose as mentioned earlier by gavage using oral feeding needle. In each group, 12 animals (6 males and 6 females) were kept for experiments. The duration of experiments was followed 15 days (TC = test compound experiment), 50% of 15th day (Imm. Exp. = Immediate as 48 h after last exposure – instant effect) and 50% of 30th day (Post. Exp. = Post exposure as 15 days after last exposure – reversibility of toxicity, if any).

Study of Haematological and Biochemical Features of Rat: During subacute toxicity test, different haematological such as Hb, RBC, WBC, platelet count, differential count and biochemical blood glucose, total protein, serum creatinine, SGOT and SGPT were estimated as per standard protocol for all groups twice at 15th day and 30th day of exposure.

Clinical Trial for Dengue Fever and Thrombocytopenia in Patients: A total 54 patients who suffered from Dengue fever associated with thrombocytopenia were recruited in the present study. Among 54 patients, 3 patients were rejected because they did not fulfil inclusion criteria.

Finally, 51 patients were included in the study and 25 were treated with GanjhuVir^{R} and standard management while 26 provided only standard management without GanjhuVir^{R} as control.

The parameters *viz.* platelet count, fever profile, average hospitalization period, clinical profiles, viral load and viremia clearance were tested both the group.

RESULTS AND DISCUSSION:

Evaluation of Oral Acute and Subacute Toxicity in Rat: Table 1 describes oral acute toxicity study in rats for different groups for four different durations.

All the data related to morbidity, mortality and behavioural features were observed similar when exposed to GanghuVir^{R} compared to control group. Table 2 describes oral subacute toxicity study in rats for different groups for four different durations.

All the data related to morbidity, mortality and behavioural features were observed similar when exposed to GanjhuVir^{R} compared to control group. **Table 3** evaluates haematological and biochemical parameters in rats for different groups for two durations. All the average data were comparable between GanjhuVir^{R} treated groups untreated (control) group.

TABLE 1: ORAL ACUTE TOXICITY STUDYIN RATS EXPOSED TO GANGHUVI R^{R} FOR DIFFERENT GROUPS

Parameters	30 min	4 h	24 h	48 h
Skin and Fur	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal
Lethargy	Nil	Nil	Nil	Nil
Sleep	Normal	Normal	Normal	Normal
Coma	Nil	Nil	Nil	Nil
Convulsions	Nil	Nil	Nil	Nil
Tremors	Nil	Nil	Nil	Nil
Diarrhoea	Nil	Nil	Nil	Nil
Morbidity	Normal	Normal	Normal	Normal
Mortality	Nil	Nil	Nil	Nil

TABLE 2: ORAL SUBACUTE TOXICITY STUDY IN RATS EXPOSED TO GANJHUVIR R FOR DIFFERENT GROUPS

Parameters	1st week	2nd week	3rd week	4th week
Skin and Fur	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal
Lethargy	Nil	Nil	Nil	Nil
Sleep	Normal	Normal	Normal	Normal
Coma	Nil	Nil	Nil	Nil
Convulsions	Nil	Nil	Nil	Nil
Tremors	Nil	Nil	Nil	Nil
Diarrhoea	Nil	Nil	Nil	Nil
Morbidity	Normal	Normal	Normal	Normal
Mortality	Nil	Nil	Nil	Nil

TABLE 3: HAEMATOLOGICAL AND BIOCHEMICAL STUDY FOR ALL GROUPSOF RATS EXPOSED TO GANJHUVIRR (MEAN ± SD; N = 12)

Parameters	Group 1		Group 2		Group 3		Group 4	
	15th day	30th day	15th day	30th day	15th day	30th day	15th day	30th day
Haemoglobin (11.5-16.1 gm/dl)	12.30	11.65	13.25	12.37	11.89	13.21	13.78	13.21
WBC (6.6-12.6x10^3/mm^3)	7.65	6.78	10.34	9.32	8.42	7.91	8.73	7.89
RBC (6.76-9.75x10^3/mm^3)	6.98	7.21	8.43	7.31	7.41	8.72	7.64	8.76
Neutrophils (1.77-3.38/mm^3)	2.45	2.17	2.81	2.38	2.57	1.98	2.46	2.76
Lymphocytes (4.78-9.12x10^3/mm^3)	6.52	5.64	5.38	6.43	8.62	7.64	5.67	6.45
Eosinophils (0.03-0.08x10^3/mm^3)	0.06	0.07	0.07	0.06	0.04	0.07	0.06	0.06
Monocytes (0.01-0.04x10^3/mm^3)	0.03	0.02	0.04	0.05	0.03	0.03	0.04	0.02
Basophils (0.00-0.03x10^3/mm^3)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Platelets (150460 x 10^3/mL)	325	316	328	412	298	312	369	417
Blood glucose (50-130 mg/dl)	121	115	109	114	118	125	109	113
Total protein (5.6-7.6 gm/dl)	6.54	7.12	5.69	6.53	6.43	7.26	5.78	6.15
Serum creatinine (0.2-0.8 mg/dl)	0.5	0.6	0.4	0.4	0.4	0.6	0.5	0.6
SGOT (>32 I U/L)	14.31	16.54	24.73	28.43	26.45	26.53	24.76	25.68
SGPT (>32 I U/L)	21.34	19.47	27.54	31.23	27.32	29.78	34.86	31.78

Clinical Trial Among Patients: Fig. 1 represents the comparative analysis of platelet count (thousands) in GanjhuVirR treated group compared to control group.

On the baseline, an increased value found in the treated group (71.90 ±17.70) in comparison with control group (61.06 ± 20.03) while in day 1, day 3 and day 5 significantly (P<0.001) increased in treated group (78.00 ± 13.18, 128.19 ± 11.32 and 159.19 ± 18.22) when compared to untreated (control) group (60.88 ±18.19, 88.08 ± 20.22 and 102.10 ± 13.19).

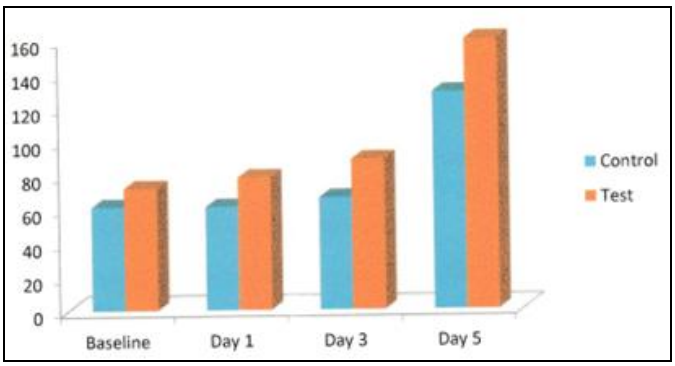

FIG. 1: MEAN PLATELET COUNT (IN THOUSANDS) OF GANJHUVIR^R TREATED VERSUS WITHOUT TREATED PATIENTS

Fig. 2 exhibits the comparative analysis of body temperature (^{o}F) in GanjhuVirR treated group compared to control group. On the baseline, the mean value of body temperature found in the treated group (101.54) in comparison with control group (101.6) while in day 1 the data was comparable (101.2 and 101.03) but day 3 and day 5 significantly (P<0.001) decreased (99.84 and 98.52) in treated group when compared to untreated (control) group (100.65 and 100.14).

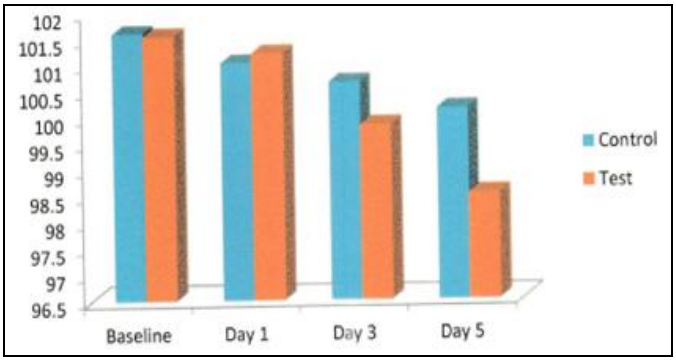

FIG. 2: MEAN BODY TEMPERATURE (IN OF) OF GANJHUVIRR TREATED VERSUS WITHOUT TREATED PATIENTS

Fig. 3 depicts the comparative analysis of number of days of hospitalization (days) in GanjhuVirR

treated group compared to control group. The mean hospitalization period was significantly (P<0.01) reduced in treated group (3.65 ± 0.97) when compared to control group (6.20 ± 0.98).

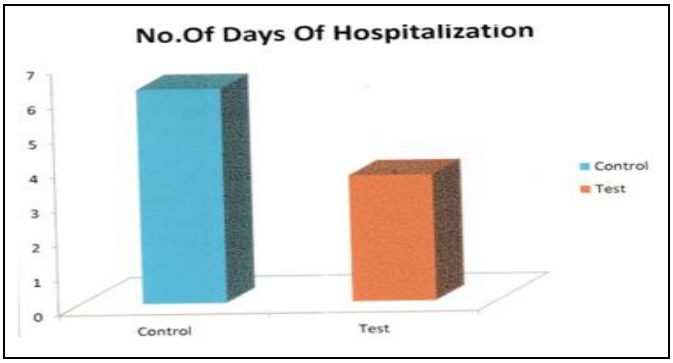

FIG. 3: MEAN DURATION OF HOSPITALIZATION (IN DAYS) OF GANJHUVIR^R TREATED VERSUS WITHOUT TREATED PATIENTS

Fig. 3 evaluates the comparative analysis of mean viral load reduction (VLR) in GanjhuVirR treated group compared to control group. The mean VLR was increased in treated group (-1.72 ± 1.07) when compared to control group (-1.48 ± 0.42).

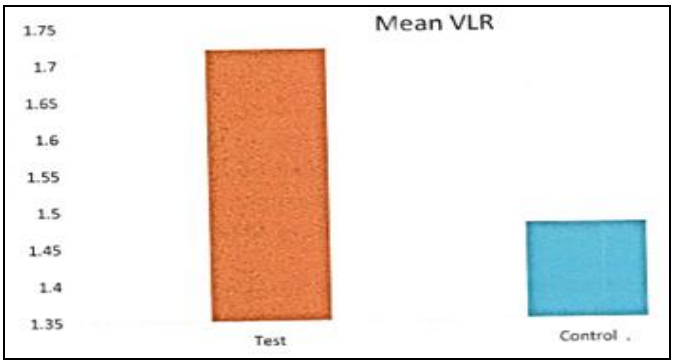

FIG. 4: MEAN VIRAL LOAD REDUCTION (IN DAYS) OF GANJHUVIRR TREATED VERSUS WITHOUT TREATED PATIENTS

Several phytochemicals have capacity to prevent various diseases. Among several medicinal plants, *Desmodium gangeticum* (L.) DC. is well known medicinal plant to prevent major health disorders [16, 17]. According to Rastogi *et al.* [16], this plant has potential antiviral activity when used the extract. Interesting research by Lelešius *et al.* [18] indicated the extract of *Desmodium canadense* prevent avian infectious bronchitis viral activity. From earlier study by Ganjhu *et al.* [19] explained the potential of this plant as an antibacterial and antiviral activities, but they mentioned that further research is required to know that mechanism of combating microbial and viral infections.

In the present study, it was established that the GanjhuVir^R extracted from *Desmodium gangeticum* is a suitable non-toxic antiviral drug potential for the therapeutic agent to cure dengue fever along with thrombocytopenia among patients. This is a first-time endeavour to develop GanjhuVir^R Patented (Patent no. 302868) as an antiviral phytomedicine.

CONCLUSION: It is concluded that GanjhuVir^R is a phytomedicine extracted from *Desmodium gangeticum* (L.) DC. and capable to treat viral activity especially for Dengue virus. Moreover, this phytomedicine observed non-toxic when tested in rat at acute and subacute oral exposure without mortality and morbidity as well as normal behavioural features. The clinical trial revealed that this phytomedicine is safe without any adverse events among patients. Also, this increased the platelet counts and decreased body temperature, hospital stay and viral load among treated patients in comparison with untreated (control) patients. It is suggested in future research to know the efficacy of antiviral activity of Mild to Moderate Covid-19 Patients.

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CONFLICTS OF INTEREST: Authors declare none.

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