SAFETY AND EFFICACY OF RESVERATROL IN HEALING OF MAXILLOFACIAL FRACTURES: A RANDOMIZED CONTROLLED STUDY

THESIS

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AIIMS JODHPUR ***DR. SHIVKUMAR CHOPANE***

ALL INDIA INSTITUTE OF MEDICAL SCIENCES
JODHPUR

CERTIFICATE

This is to certify that thesis entitled "Safety and Efficacy of Resveratrol In Healing of Maxillofacial Fractures: A Randomized Controlled Study" is an original work of Dr. Shivkumar Suresh Chopane carried out under our direct supervision and guidance at Department of Dentistry, All India Institute of Medical Sciences, Jodhpur.

GUIDE

Dr. Ankita Chugh

Additional Professor
Oral and Maxillofacial Surgery
Department of Dentistry
AIIMS Jodhpur

CO-GUIDES

Dr. Kirti Chaudhry

Additional Professor
Oral and Maxillofacial Surgery
Department of Dentistry
All India Institute of Medical Sciences, Jodhpur

Dr. Mithu Banerjee

Additional Professor
Department of Biochemistry
All India Institute of Medical Sciences, Jodhpur

Dr. Pravin Kumar

Professor & HOD
Department of Dentistry
All India Institute of Medical Sciences, Jodhpur

ALL INDIA INSTITUTE OF MEDICAL SCIENCES

JODHPUR

DECLARATION

I, hereby declare that the work reported in the thesis entitled **"Safety and Efficacy of Resveratrol in Healing of Maxillofacial Fractures: A Randomized Controlled Study"** embodies the result of original research work carried out by me in the Department of Dentistry, All India Institute of Medical Sciences, Jodhpur.

I further state that no part of the thesis has been submitted either in part or in full for any other degree of All India Institute of Medical Sciences or any other institution/ University.

Dr. Shivkumar Chopane

Junior Resident
Department of Dentistry
Oral and Maxillofacial Surgery
All India Institute of Medical Sciences, Jodhpur.

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- ***Dr. Shivkumar Chopane***

CONTENTS

LIST OF ABBREVIATIONS

ALP	:	Serum Alkaline Phosphatase
BALP	:	Bone Alkaline Phosphatase
BF	:	Bite Force
BGP	:	Bone Gla Protein
BMC	:	Bone Mineral Content
BMD	:	Bone Mineral Density
Ca	:	Calcium
CS	:	Cigarette Smoking
CsA	:	Cyclosporin A
CTX	:	Carboxy-Terminal Collagen Crosslinks
DMSO	:	Dimethylsulfoxide
ELISA	:	Enzyme-Linked Immunoassay
MBF	:	Mean bite force
METS	:	Metabolic Syndrome
OCN	:	Osteocalcin
ORIF	:	Open Reduction And Internal Fixation
PBF	:	Posterior Bite Force
PDGF	:	Platelet-derived growth factor
PINP	:	Procollagen Type I N Propeptide
PO-MSCs	:	Periosteum-derived Mitochondrial stem cells
PTH	:	Parathyroid Hormone
RSV	:	Tablet. Resveratrol
SIRT1	:	Sirtuin (Silent Mating Type Information Regulation 2 Homolog)

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INTRODUCTION

Healing of fractured bone, following any insult, is an ever-evolving complex process(1). Variable biological, mechanical, systemic and local regulatory factors like hormones and cytokines modulate to restore previous anatomic structure and functional form(2). These processes range from osteo-conduction and induction involving cells of various hue as well as extra-cellular molecular signalling pathways(3). The post-operative sequelae of bone healing following maxillofacial procedures is also orchestrated by normal physiological processes with the eventual goal of restoring function and stability. However, any unanticipated events in these well-co-ordinated regenerative events may lead to sub-optimal healing and inadequate bone formation. In order to achieve optimum results and return to normal function, maxillofacial fractures require open reduction and miniplate fixation (ORIF)(4). However, as this fixation is semi-rigid, the need for faster bone formation cannot be understated. Also, the exponential increase in the medically compromised patients in the maxillofacial operatory makes the entire physiological healing process extremely unpredictable. Therefore, the surgeon should look for avenues to aid in bone healing with extreme alacrity to dispense patient centric quality healthcare.

Natural healing of fracture starts with extravascular blood clot or hematoma formation. This process begins in few hours after the incidence of the fracture (1). This collected blood contains many signalling molecules including interleukin (IL)-1, IL-6, transforming growth factor β (TGFβ), fibroblast growth factors (FGF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), tumor necrosis factor-α (TNF-α) and vascular endothelial growth factor (VEGF). These factors modulate subsequent recruitment of endothelial cells, platelets, macrophages, monocytes and multipotent mesenchymal stem cells at fracture site and induce a cascade of cellular events to recruit osteoblast and osteoclast (1, 2). Healing of bone is contingent on differentiation of osteoblast into osteogenic cells and their migration and proliferation. The process of fracture repair can be monitored with the help of some markers found in serum that are associated with healing of fracture. For the determination of osteoblasts activity, we can examine the levels of Osteocalcin (OCN) & Alkaline Phosphatase (ALP) in serum(5). Levels of both ALP & OCN after fracture insult consistently increase, duration to attain the first peak may although vary. It is found that the activity of IGFBP-3, IGF-I, ALP and osteocalcin fluctuates during several months after the

fracture, which might partially reflect activity of osteoblasts and can be used to monitor fracture healing. Clinical assessment of maxillofacial fracture healing can be done by evaluation of mechanical load and pain(6). As we already know, there will be decreased functional masticatory forces and chewing efficiency following maxillofacial fracture. This is one of the important parameter to evaluate considering efficacy of treatment modalities for fracture management in maxillofacial region. In this study, clinical evaluation of bone healing in terms of restoration of normal bite force was used as an indicator for bone healing.

In recent times, systemic enhancement of bone formation has gained attention. This has led to widespread interest in drugs like Resveratrol (RSV) that are proposed as bone enhancers(7). RSV belongs to polyphenols' stilbenoids group, possessing two phenol rings linked to each other by an ethylene bridge, detected in more than 70 plant species, especially in grapes' skin and seeds, and was found in discrete amounts in red wines and various human foods. Its bioactive effects, namely as anti-inflammatory, anticarcinogenic, cardioprotective, vasorelaxant, phytoestrogenic and neuroprotective have also been reported(8). The drug RSV has antioxidant property which helps to remove free radicals generated as a result of tissue necrosis associated with fracture site (7,8). RSV is believed to show cell proliferation, osteoblastic maturation, osteoblast differentiation, inhibition of osteoclastic activity, and thus protection against bone loss. The dose dependent effect of RSV have been studied considering that this drug enhances biogenesis of mitochondria or osteogenic differentiation of periosteum-derived MSCs (PO-MSCs) & increases both calcium deposits and ALP activities, which are important in maintenance of bone tissue and healing of fracture(9). Studies have proven that Resveratrol promotes human PO-MSCs mitochondrial biogenesis and osteogenesis and this further suggests a potential application of RSV as an adjunct for osteoporotic fractures and/or in osteoporosis(10). Although, RSV has been widely gaining attention for acceleration of post-operative bone formation, there still exists ambiguity over its efficacy, as the literature is replete with conflicting studies that too mainly in animals(11). Its use as a bone healing agent in healthy individual has not been studied.

Therefore, the objective of this study is to determine resveratrol role in early bone healing of maxillofacial fractures if any by correlating it with the change in the level of bone biomarkers like osteocalcin and serum alkaline phosphatase and restoration of normal bite forces.

REVIEW OF LITERATURE

FRACTURE HEALING

Giannoudis et al (2007)(12) stated that growth factors utilisation, mesenchymal stem cells, scaffolds (triangular concept), and mechanical environment are crucial elements in regeneration of bone. The traditional triangular concept should be accepted as the 'diamond concept'.

Marsell et al (2011)(13) found in his study that fractured bone heals by either indirect fracture healing or direct intramembranous, which consists of both endochondral or intramembranous bone formation. Indirect healing the most common pathway, whereas direct healing of bone demands anatomical reduction and stable fixation, which is obtained by ORIF leading to direct healing cascade. In non-stable conditions, an acute inflammatory response sets in.

Mukhopadhyay et al (2011)(5) studied 36 patients with fracture of long bone. The levels of urinary total & free hydroxyproline, serum alkaline phosphatase were measured & analysed and compared. Results showed favourable correlation between total serum alkaline phosphatase & urinary hydroxyproline excretion which was statistically significant and it indicates advancement towards satisfactory healing. Bone turnover biochemical markers serial monitoring can be utilized as a supplement to radiological & clinical evidence of healing of fracture.

Bigham et al (2014)(1) presented various treatment modalities of the bony fracture and explained normal healing of fractures and also factors interfering with fracture healing. In this study overview of the fracture healing processes and discussion of the latest therapeutic strategies were provided that might be effective in acceleration of fracture healing.

BITE FORCE

Gerlach et al (2002)(14) This study included 22 individuals associated with mandibular angle fracture. They were managed with ORIF with miniplate fixation using Champy's principles and maximal biting forces were evaluated. The load resistance was evaluated between incisors, canine and molars using electric test for 6 weeks following treatment and same procedure also done in controls. Study concluded that 1 week postoperatively

the maximal loading (vertical) found in 31% controls and at 6th week postoperatively value increased to 58%.

Kshirsagar et al (2011)(15) This study was done on 60 patients of age group between 18-60 year with mandibular parasymphysis fracture. The purpose of the study was to measure the amount of bite force generated by patients treated for mandibular parasymphysis fracture by open reduction and internal fixation at various time intervals. Bite force measurements were made on a bite force measurement device with upright relaxed position. Measurement of Bite forces were done at the incisor and right and left molar regions. The comparison of these bite forces were done with six isolated mandibular unilateral parasymphysis fracture patients. All patients were treated using open reduction and internal fixation using two miniplates at the fracture site. In the volunteer group, bite forces ranged from 22 to 50 kg in the molar region and 3 to 27 kg in the incisor region. Mean adult healthy values (male and female) in the molar region were 36 kg and in the incisor region, 15 kg. In mandibular parasymphyseal fractures, incisor bite forces were reduced significantly when compared with the control group in the first 2 postoperative weeks and regained significantly thereafter till 4 to 6 weeks. Bite forces in the molar region took 6 to 12 weeks to regain maximum bite forces when compared with the volunteer group. In mandibular parasymphysis fractures, functional forces are restored in 4 to 6 weeks and maximum bite forces in 8 weeks.

Gupta et al (2012)(16) conducted a study on 20 patients (10 patients in 2 group each) determined the efficacy & clinical stability of 1 miniplate combined with 1 microplate in mandibular fractures management of interforaminal region versus 2-miniplate standard treatment using measurements bite force. Results showed significant increase in bite force at incisor region compared with bite force measured preoperatively but no difference postoperatively. At molar region no significant difference was noted. Study concluded that fixation with microplate in mandibular fractures management is stable and sufficiently efficacious to bear the loads of mastication and force (torsional) acting in interforaminal region of mandible.

Sybil et al (2013)(17) The aim of this study was to assess the effect of mandibular fractures on the bite forces. These patients were surgically treated for isolated mandibular fractures. Measurements of bite force were done on the first, fourth, sixth, and ninth weeks postoperatively. These bite force values were compared with those of

age, sex, and weight-matched controls. A total of 60 patients were included in the study. It was found that maximum bite forces in patients were significantly less than in controls for several weeks after surgery. After the ninth postoperative week, the maximum bite force measured < 65% the normal in patients with isolated angle fractures and > 80% the normal in patients with isolated parasymphysis fractures. The same values reduced to < 60% in patients with fractures of angle and parasymphysis and < 70% in patients with fractures of parasymphysis and condylar complex. An inverse relationship was found between the bite force values and the number of fractures of the mandible. We also found lower bite forces and longer period for normalization in patients who had fractures in those regions of the mandible which are more significantly associated with the masticatory apparatus for example angle or condyle of the mandible.

Kumar et al (2014)(18) conducted a study to compare stability of fixation by internal locking miniplate and conventional miniplate on mandibular fractured fragments under functional load. Biting force was measured at incisor & molar regions. Studies showed that locking screw/plate system provides significant advantages.

Kinra et al (2017)(19) perfomed a comparative study including 40 patients sustained fracture of mandible for effectiveness of two different miniplates by evaluating the change in biting force. The assessment was performed on the basis of bite force at incisor, bilateral molar region. Study concluded the efficacy of 3D miniplates to bear loads of mastication during the fractures osteosynthesis.

Mustafa et al (2017)(20) Rigid internal fixation attained worldwide approval by utilizing compression and non-compression plating systems. This study was done to differentiate between the bite force recovery in patients with mandibular angle fractures treated by one monocortical miniplate and two monocortical miniplates. Out of 14 patients, 7 in each group with mandibular angle fractures were subjected to measure bite force (in kg) and comparatively analysed. Bite force was measured at incisor, premolar and molar. Patients had undergone fixation technique as mentioned above. Postoperatively bite force were noted weekly for 6 weeks and till 3 months. Bite force is raised gently in both categories in the anterior, premolar (right and left) molar region (right and left) during follow up period excluding a drop in category 1 during the 5^{th} week in right molar region. A statistically significant difference was established

between the change in bite force from the previous follow up visit in category 1 & 2. This was seen from week 1 to week 4 in incisor region, at week 1 in right premolar region, at week 3 in left premolar region, at week 5, 6 and 12 in right molar region and at week 6 and 12 in the left molar region. Thus use of either one or two miniplates osteosynthesis in fixation of angle fracture do not make much difference. So the suitable method for treating mandibular angle fracture is one miniplate osteosynthesis.

Abhinandan et al. (2021)(21) The sequalae of Maxillofacial fractures includes change in the skeletal architecture (anatomical) as well as in the masticatory apparatus (functional). Masticatory function refers to the ability to chew without any interference or pain. The major determinants of this is the range of mandibular motion, maximum occlusal forces, and the activity of the masticatory muscles. This function is affected in maxillofacial trauma and also pathological injuries to the jaws. Bite force measurements are an excellent criterion for the assessment of masticatory efficiency. The aim of this study was to assess the effect of maxillofacial fractures on the bite forces of patients treated for such fractures. 65 patients were divided into 7 groups based on type of maxillofacial fracture. All the cases underwent ORIF. Bite force measurement was done at immediate post-operative period, 1st, 4th and 12th post-operative week. The bite force instrument (transducer) was positioned between the cusps of Left and Right First Molar region. After 3rd post-operative week, all the groups showed a statistically significant increase in the bite force as compared to the immediate post-operative bite force recording. Thus the study provides a basis for similar studies with a longer follow up period and larger sample size in order to assess the different kinds of maxillofacial trauma and its effect on bite force.

RESVERATROL

Uysal et al (2011)(22) A randomised control animal study was conducted to evaluate the effects of local RSV administration on bone formation. Twenty 50-60-day-old male Wistar rats were divided into two equal groups. Both groups were subjected to expansion, and 30 cN of force was applied to the maxillary incisors with helical-spring. A day after appliance placement, single-dosage of 10 l-mol ⁄ kg local RSV in the Dimethylsulfoxide (DMSO) was administered to the inter-premaxillary suture in the experimental group. The same amount of DMSO was injected to the suture of rats in control group. Bone formation in the suture was evaluated

histomorphometrically. Statistical evaluation was done with Mann–Whitney U-test. Statistically significant difference was found in bone formation between the two groups. Areas of new bone formation were significantly larger in the experimental group.

Poulsen et al (2014)(23) Conducted a randomised, placebo-controlled, double-blinded and parallel-group study to identify the bone metabolic effects of resveratrol in human subjects. The study randomly assigned 24 obese non-diabetic men to 500 mg RSV or placebo treatment three times daily for four weeks. Biomarkers of bone metabolism, inflammatory parameters and circulating hormones were measured before and after the intervention period. Plasma levels of bone-specific alkaline phosphatase increased significantly in the RSV group as compared to placebo. This was paralleled by a tendency of total alkaline phosphatase to rise within the RSV group (P = 0.061), whereas no changes were detected in other biomarkers of bone and calcium metabolism, including PINP, osteocalcin, CTx, or PTH. Therefore, the study concluded that resveratrol does influence bone metabolism. However, more studies are required to evaluate it's clinical implications.

Ornstrup et al (2014)(9) This study was conducted to evaluate effects of RSV treatment on bone in men with Mets. The study was conducted at Aarhus University Hospital as a randomized, double-blinded, placebo-controlled trial assessing changes in bone turnover markers, bone mineral density (BMD), and geometry. The study population comprised 74 middle-aged obese men with MetS recruited from the general community, of which 66 completed all visits. Mean age of participants was 49.3 +/- 6.3 years and mean body mass index was 33.7 +/-3.6 kg/m2. Intervention: Oral treatment with 1gm RSV (RS high), 150mg RSV (RSV low), or placebo daily for 16 weeks. Prespecified primary endpoint was change in bone alkaline phosphatase (BALP). Results shows that high-dose RSV supplementation positively affects bone, primarily by stimulating formation or mineralization.

Ozge et al (2015)(24) conducted a study on rats for 4 weeks, rats were exposed to cigarette smoke at the equivalent of 6 cigarettes per day. After this period, monocortical defects were created in femurs by a trephine bur on day 28. Starting from the day of defect creation to the 28th postoperative day, rats were given 20 mg/kg body weight RSV. Histomorphometric examination of the number of osteoblasts and osteoclasts, as well as new bone area, was conducted on 33 rats. Differences between osteoblast

numbers in the control and Cigarette group (CS) were significant, and CS resulted in a reduction in the number of osteoblasts. Areas of new bone formation in the RSV and control groups were higher than in the smoking and smoking and RSV groups. Therefore, it was concluded that smoking had adverse effects upon bone healing and RSV administration helped to reduce these effects.

Simona et al (2018)(25) In this double-blind randomized placebo-controlled trial 192 T2DM outpatients were randomized to receive RSV 500 mg/day RSV, 40mg/day or placebo for 6 months. BMD, bone mineral content (BMC), serum calcium, phosphorus, alkaline phosphatase, and 25-hydroxy vitamin D were measured at baseline and after 6 months. Patients were found to have stastically significant lower bone density loss in T2DM patients who received RSV supplements(25). In subgroup analyses, in Resv500 treated-patients BMD values increased to higher levels in those with lower calcium and 25-hydroxy vitamin D values. They have found a significant increase in ALP values within-group in the RSV arms, though the differences were not significantly different from placebo.

Ayse et al (2018)(26) conducted a study to investigate the effects of RSV on alveolar socket healing after tooth extraction in normal and cyclosporin A (CsA)-treated rats. Seventy-two female Sprague Dawley rats were separated into four groups of 18. Group 1 was injected with a placebo solution intraperitoneally. Group 2 was injected with resveratrol (10 µmol/kg) intraperitoneally. Groups 3 and 4 were injected with CsA (10 mg/kg) subcutaneously for 8 days once daily before the tooth extraction. This was followed by the extraction of teeth and continuation of CsA injection until the animals were sacrificed. Eight days after commencing the CsA injections, Group 4 was injected with RSV while continuing with CsA injections. Nine rats from each group were sacrificed on days 14 and 28, and sections were examined to assess the degree of inflammation, the formation of connective tissue, and new bone formation. Immunohistochemical analysis was employed to evaluate the alveolar socket healing process using osteocalcin and osteopontin markers. There was more new bone formation in Group 2 patients who received RSV administration in comparison to the other three groups on day 14 after the tooth extraction.

Denise et al (2019)(7) A review was conducted to determine the biological effects of natural polyphenol RSV on health and as adjuvant for treatment of several chronic

diseases. They have shown that RSV has positive aptitude effects of both as promoter of osteoblasts proliferation and antagonist of osteoclasts' differentiation in bone formation. There could be interesting applications in the field of dentistry and maxillofacial surgery. This experimental finding comprises that the RSV has potential for bone regeneration.

Marzieh et al (2020)(27) In this systematic review and meta-analysis of randomized controlled trials (RCTs) on RSV and bone health biomarkers, they conducted Six RCTs (8 treatment arms with 264 subjects) which shows no significant reduction of serum Ca, osteocalcin, C-terminal telopeptide of type I collagen and procollagen I N-terminal propeptide (P1NP) values after RSV supplementation. The study indicate that the resveratrol supplementation increased some bone biomarkers, such as alkaline phosphatase (ALP) and bone alkaline phosphatase (BALP).

Qiangqiang et al (2021)(28) in their review determined the effects of RSV on bone mineral density and serum bone biomarkers, they have concluded that RSV has no significant effect in increasing BMD. There was no change in serum bone markers including serum ALP and BALP, serum Osteocalcin, PINP, CTX and PTH hormone, BMD.

SERUM BIOMARKERS IN BONE HEALING

Nyman et al (1991)(29) In this study the serum osteocalcin (BGP) concentration and alkaline phosphatase (ALP) activity were measured prospectively during the healing phases of crural fractures in 15 patients. They were divided into two groups, the time of union of the fracture being under (group 1) or over 16 weeks (group 2). The mean values of BGP and ALP were somewhat higher from the outset in the group 1 than in the group2, but the difference was not significant. A significant increase in BGP and AP (P lessthan 0.05) was evident in both groups at 6 weeks. In cases with undisturbed healing offractures (group 1) the values of serum BGP and AP then declined towards the values at the time of accident. Contrary to this, in group 2 both the values of the serum BGP and AP were still at a significantly higher level than those at the day of the fracture. However, no significant difference in the serum BGP or ALP was seen between the twogroups at 6 or 12 weeks. The results support some earlier ones: the changes in serum BGP and ALP may provide a prognostic indicator for consolidation of a fracture.

Bowles et al (1996)(30) conducted a study on 20 subject with tibial shaft fracture in which they noted changes in concentrations of osteocalcin and total and bone specific alkaline phosphatase activity occurring in the twenty week period. Up to 5 weeks Bone formation during the healing process is reflected by progressive increases in the concentration of osteocalcin and bone specific alkaline phosphatase after that it is correlated with the height and weight of the subject. In the early post injury period, total alkaline phosphatase activity increased whereas that of the bone isoenzyme initially fell, starting to rise again during the second week. After an immediate post injury rise, osteocalcin concentration also decreased, reaching by week 5. As only three of subjects demonstrated delayed union, study have not been able to demonstrate that biochemical monitoring of the healing process can provide an indication of prognosis in tibial shaft fracture.

Taniguchi et al (2003)(31) The purpose of this study was to determine the changes in serum markers relating to bone formation during fracture healing. 10 consecutive patients with fractures treated with or without surgery were included. Serum of all were collected in time intervals from patients for 80–280 (average 180) days after the fracture. The concentrations of intact osteocalcin, bone-specific alkaline phosphatase (ALP), insulin-like growth factor (IGF)-I and IGF-binding protein (IGFBP)-3 in the serum were measured by ELISA. All these serum markers increased or decreased after fracture and fluctuated during fracture healing, however, this pattern differed among the cases. It was concluded that the serum markers such as osteocalcin, ALP, IGF-I and IGFBP-3 reflected in part the osteoblastic activity during fracture healing.

F.H. et al (2006)(32) studied the efficacy of the bone markers carboxyterminal crosslinked telopeptide of type-I collagen (ICTP) OCN in new bone formation in dogs by using commercially available immunoassay kits. Significant differences in the amount of newly formed bone were found, although the finding was not reflected in levels of OCN and ICTP in the plasma. In conclusion, OCN and ICTP were not efficacious as markers of bone formation and resorption during osteogenesis in this canine model.

AIM AND OBJECTIVES

AIM:

To evaluate and compare the efficacy and safety of Resveratrol on maxillofacial fractures healing.

OBJECTIVES:

Primary

- To evaluate and compare the bite force measurements in both groups
- To compare levels of serum Alkaline phosphatase & Osteocalcin between Resveratrol & Placebo group in maxillofacial trauma cases and correlate with bite force.

Secondary

- To evaluate any adverse outcomes like infection, delayed union and non-union.
- To evaluate the incidence of drug related adverse events in experimental group.

RESEARCH QUESTION:

Is the procedure of bone healing accelerated on administration of RSV?

NULL HYPOTHESIS:

Fracture healing is same with or without the use of RSV.

MATERIALS AND METHODS

CLINICAL TRIAL

A single centre, randomized, parallel group, prospective, double blind clinical trial was conducted with 1:1 allocation ratio, in Placebo and Tab. Resveratrol group.

SETTING AND LOCATION

This study was conducted in accordance with ICH-GCP and ICMR guidelines. The clinical trial was submitted to and approved by the ethics committee of the institute (REF/2020/10/037660). The trial was strictly carried out in accordance with Consolidated Standards of Reporting trials (CONSORT) guidelines after prospective registration with the Clinical Trial Registry of India: CTRI/2021/10/037437.

A total of 40 patients, who reported to Department of Dentistry, AIIMS Jodhpur with maxillofacial fractures between the age of 20-60 years, fulfilling the inclusion criteria were selected for the study between 10 October 2020 to September 2021. Informed consent was obtained from all the selected patients. The study was started with administration of tab. resveratrol and placebo followed by evaluations and follow up visits up to 12 weeks.

INCLUSION CRITERIA

1. Patients between the age of 20-60 years with maxillofacial fractures
2. ASA I or II

EXCLUSION CRITERIA

1. Age <20 years and >60 years
2. Pregnant or lactating females
3. Hypercalcemia, Paget's disease or any other bone disorder
4. Malignant tumours
5. Patients earlier having radiation treatment.
6. Patients on Vitamin D therapy or any other bone medications

Based on the above mentioned screening criteria, all the patients were randomly divided into two groups:

a. **Group 1 (Placebo):** Patients with maxillofacial fractures who received Placebo tablet BD for 1 month following Open reduction and Internal fixation of fractured segments.
b. **Group 2 (Resveratrol):** Patients with maxillofacial fractures who received Tablet Resveratrol 500mg BD for 1 month following Open reduction and Internal fixation of fractured segments.

Patient were administrated Placebo or Tab. Resveratrol, on 1st day of admission. After discharge patients were motivated and instructed on every follow up, thus 100% compliance for medication was maintained in both the groups.

Image A: Tab. Resveratrol

RANDOMIZATION, ALLOCATION CONCEALMENT AND BLINDING

The randomization was done using randomization allocation software 2.0. The generated codes were sealed in sequentially numbered opaque envelopes for allocation concealment. Sealed envelopes were sequentially allotted to the patients requiring ORIF. Immediately following admission, an envelope was opened to allocate administration of either placebo medication or Tab. Resveratrol. The randomization, allocation concealment & assigning of participants was performed by an individual unrelated to trial.

BLINDING

This study was double blinded as the assessor and the patients both were blinded.

DATA COLLECTION:

BITE FORCE:

Maximum voluntary bite force was recorded in both side molar region and the force was calculated by Flexi force sensor which converted the generated force into numerical values. The values were taken preoperatively and at 1st, 4th, 8th and 12th week postoperatively in both the groups. Mean bite force of both side molar region was used for statistical analysis. To compare the change in bite force from preoperative values, the difference was calculated and designated as BF 1-0, BF 4-0, BF 8-0, BF 12-0 for 1st, 4th, 8th and 12th week respectively.

BLOOD BONE MARKERS:

Blood samples were taken from patients in both the groups and analysed for Serum Osteocalcin and Alkaline Phosphatase preoperatively and at 4th and 12th week postoperatively. The change between preoperative and 4th week and preoperative and 12th week was calculated and designated as 4-0,12-0.

INTERVENTION

After obtaining the written consent from the patient, both the groups were evaluated for predictor variables like bite force at the molar region, serum osteocalcin and serum alkaline phosphatase levels. ORIF was done as per the standard protocol. Outcome variables were post-operative bite force calculated at 1st, 4th, 8th and 12th week postoperatively using Flexi force sensor, serum osteocalcin and alkaline phosphatase levels calculated at 4th and 12th week postoperatively.

ELISA METHODOLOGY

1. The well, blank and sample were adjusted to the standard values. 100 µL of each blank and sample dilution of standard was added into the appropriate wells. The plate was covered with the sealer and was incubated for 90 min at 37ºC.
2. Decanted liquid was not washed. 100 µL of Biotinylated Detection AB solution was added to each well. Plate was Covered with a new sealer. Incubated for 1 hour at 37ºC.

3. Decanted the solution from each well and 350 µL of washed buffer was added to each well. Decanted solution from each well and was dried against clean absorbent paper. This step was repeated 5 times.
4. 100 µL of HRP Conjugate working solution was added to each well. The plate was covered with new sealer and incubated for 30 min at 37ºC.
5. 90 µL of Substrate reagent was added to each well and plate was covered with a new sealer. Solution was Incubated for about 15 min at 37ºC. Plate was protected from light.
6. 50 µL of Stop Solution was added to each well.
7. The optical density (OD value) of each well was determined once with micro-plate reader set to 450 nm.

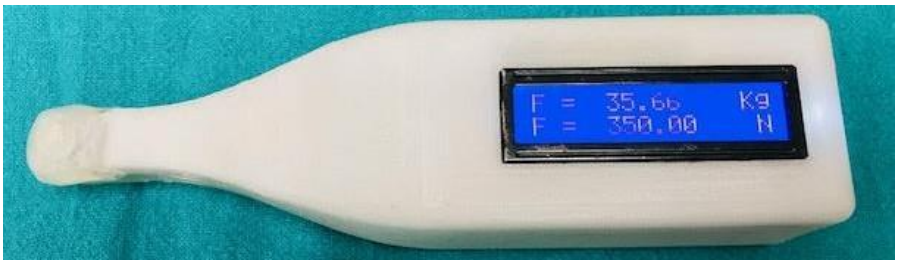


Image B: Flexiforce sensor device to record bite force

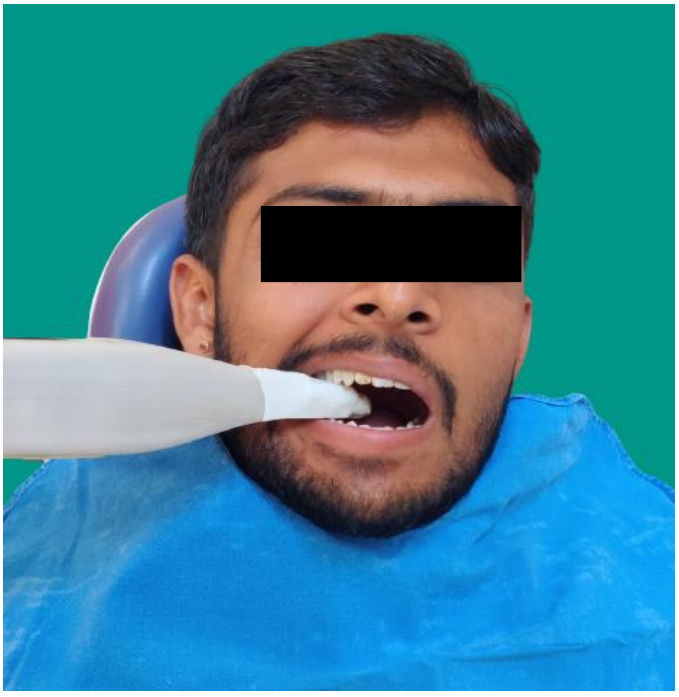


Image C: Measurement of bite force

Image D: ELISA Kit

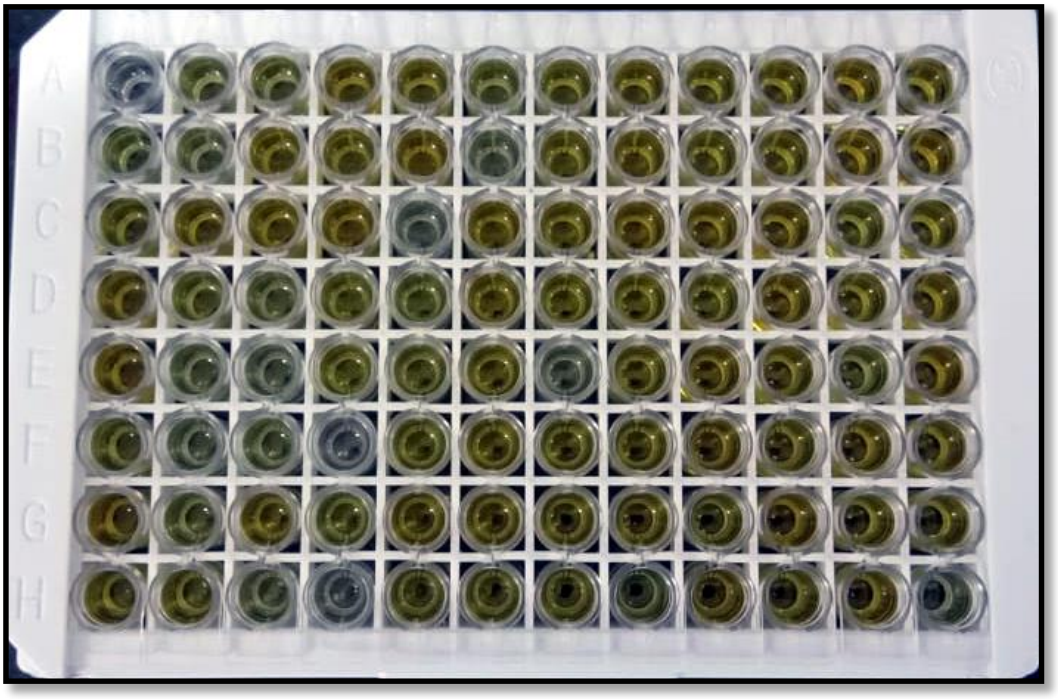


Image E: ELISA Wells

STATISTICAL ANALYSIS

SAMPLE SIZE CALCULATION

Sample size was calculated based on previously published study by Poulsen et al(23).

Sample Size was calculated using the following formula:

$$\mathbf{(n) = [Z\ (1-\alpha) + Z\ (1-\beta)]^2 \times 2\ Sp^2 / \mu d^2}$$

Z (1-α) = 1.96 as significance level of 95%

Z (1-β) = 0.842 as Power is 80%.

Sp^2= Pooled variance

μd = Mean Difference between two groups

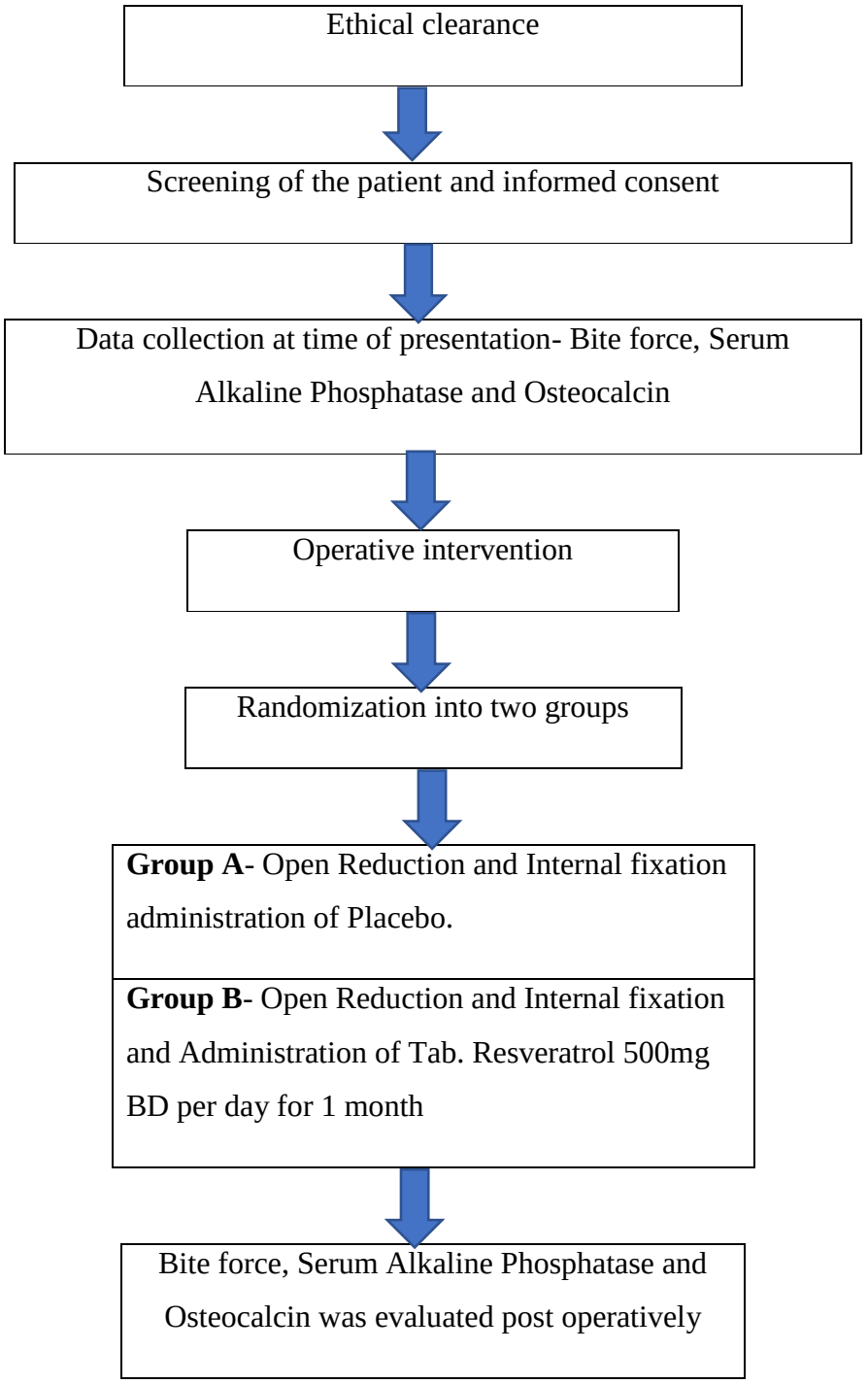
n = Sample size

The sample size was estimated to be 16 per treatment group. However, we assumed 20% dropouts during follow up, therefore 20 patients were recruited per treatment group.

STATISTICAL ANALYSIS

Data was expressed as mean ± standard error. Independent Student t-test was used for comparison of numerical variables between two groups. Chi-square or Fischer's Exact test was used to compare categorical variables. Intragroup comparison of mean changes in outcomes was evaluated by Paired t-test. Analysis was done using SPSS version 23 (IBM Corp. Ltd, Newark, USA). $P<0.05$ was considered as significant.

FLOW OF STUDY



RESULTS

40 patients with maxillofacial fractures were randomly divided into group 1 (Placebo) and group 2 (Tab. Resveratrol). In Group 1, 20 patients (19 male, and 1 female; mean age of 27.2±9.22 years) were administered placebo medication for 1 month and in group 2, 20 patients (19 male, and 1 female; mean age of 34.0±13.07 years) were administered Tab Resveratrol 500mg BD for 1 month. In both the groups we had started the medication after baseline investigation for one month on the day of admission depending on the randomization sequence.

Baseline demographic characteristics of two groups were compared and no significant difference was observed between the groups ($p>0.05$). Both the groups had similar and comparable demographics (Table 1).

Table 1: Baseline demographic characteristics of Placebo and Resveratrol groups

Variables		**Group 1 (Placebo)**	**Group 2 (Tab. Resveratrol)**	**p-value**
Age (Mean ±SD)(in years)		27.2±9.22	34.0±13.07	0.065
Gender	**Male**	19	19	NC
	Female	1	1	
Number of fractures	**Single**	30%	30%	NC
	Multiple	70%	70%	

p-Value $\leq$ 0.05 was considered statistically significant. Intergroup comparison was done by t-test.

NC- Not calculated

All the cases were evaluated for bite force, serum osteocalcin and serum alkaline phosphatase. Baseline mean value of each was noted preoperatively. Baseline bite force, serum ALP and serum OCN were similar in both the groups with non-significant p-values (Table 2).

Table 2: Preoperative Baseline measurements in the Placebo and Resveratrol groups

Parameters	Group 1 (Placebo)	Group 2 (Tab. Resveratrol)	p-value(t-test)
	Mean ± SD		
Mean Bite force	11.09 ± 31.07	9.17±21.42	0.821
Serum ALP	99.2±35.42	98.7±35.68	0.965
Serum OCN	75.38±20.85	74.37±19.93	0.877

p-Value $\leq$ 0.05 was considered statistically significant. Intergroup comparison was done by t-test.

Bite force readings preoperatively ranged from 0 to 139.22 in Placebo group and 0 to 82.98 in Resveratrol group. Serum alkaline phosphatase preoperatively ranged from 48 to169 IU/L in placebo and 52 to 199 IU/L in RSV group. Serum Osteocalcin preoperatively ranged from 2.55 to 84 in Placebo group and 11.16 to 84 in RSV group.

BITE FORCE (BF)

Bite force (BF) were measured at the right and left molar region and mean value was taken preoperatively and postoperatively at 1st week, 4th week, 8th week and 12th week. Preoperatively there was statistically no significant difference between the two groups (p=0.821) (Table 2). Mean of BF showed progressive increase in both the groups at all the time points. At 1st week on intergroup comparison group 1 showed more mean BF than group 2 though the difference was not statistically significant. At all consequent weeks group 2 showed greater mean BF than group 1 (Table 6) though difference was again non-significant statistically.

Table 3: Mean bite force in two groups at different time points

Mean Bite Force	Group 1 (Placebo) (Mean ± SD)	Group 2 (Tab. Resveratrol) (Mean ± SD)	p-value
Mean Preop	11.09 ± 31.07	9.17±21.42	0.821
Mean Postop 1st week	97.19 ± 91.47	89.54 ± 128.03	0.829
Mean Postop 4th week	270.72 ±170.62	338.02± 304.47	0.394
Mean Postop 8th week	428.27 ± 264.96	529.71± 281.62	0.248
Mean Postop 12th week	564.36±308.67	699.72± 259.54	0.142

p-Value ≤ 0.05 was considered statistically significant. Intergroup comparison was done by t-test.

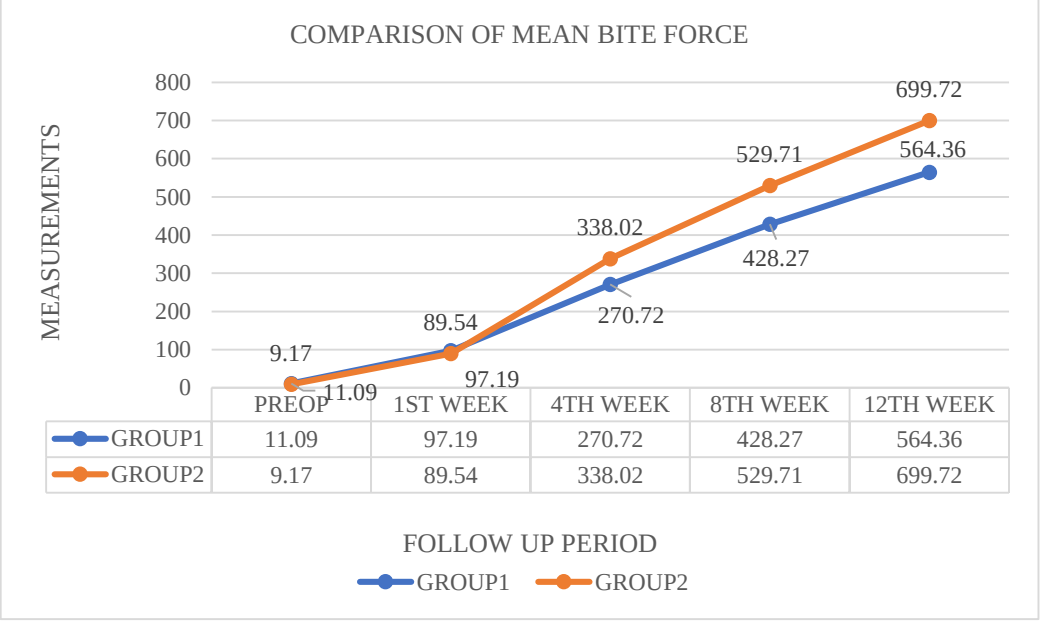


Figure 1: Comparison of mean bite force in both the groups

Table 4: Bite force in two groups at different time points on Right side

Bite Force Right side	Group 1 (Control) (Mean ± SD)	Group 2 (Tab. Resveratrol) (Mean ± SD)	p-value
Preop	7.84 ±18.13	5.26±9.04	0.574
Postop 1st week	88.58±94.49	103.67 ±181.73	0.744
Postop 4th week	251.30±175.63	382.45 ±362.47	0.154
Postop 8th week	414.52±275.76	544.73±299.06	0.160
Postop 12th week	554.95±307.08	720.41±270.05	0.078

p-Value ≤ 0.05 was considered statistically significant. Intergroup comparison was done by t-test.

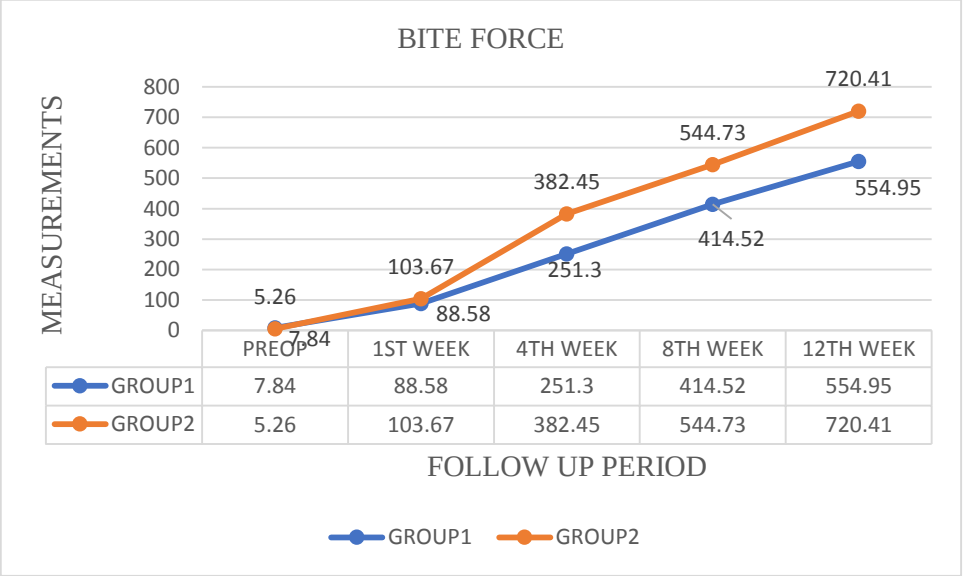


Figure 2: Comparison of bite force on right side in both the groups

Table 5: Bite force in two groups at different timepoints on Left side

Bite Force Left side	Group 1 (Placebo) (Mean ± SD)	Group 2 (Tab. Resveratrol) (Mean ± SD)	p-value
Preop	14.35±44.47	13.07±35.89	0.921
Postop 1st week	105.81±109.24	75.40 ±128.55	0.425
Postop 4th week	290.13±176.97	293.58±284.62	0.963
Postop 8th week	442.03±260.58	514.68±280.85	0.402
Postop 12th week	573.78±313.94	679.03±258.45	0.254

p-Value ≤ 0.05 was considered statistically significant. Intergroup comparison was done by t-test.

At 12th week range of bite force in group 1 was 119 N to 1000 N while in RSV group it was 76.44 N to 1000 N.

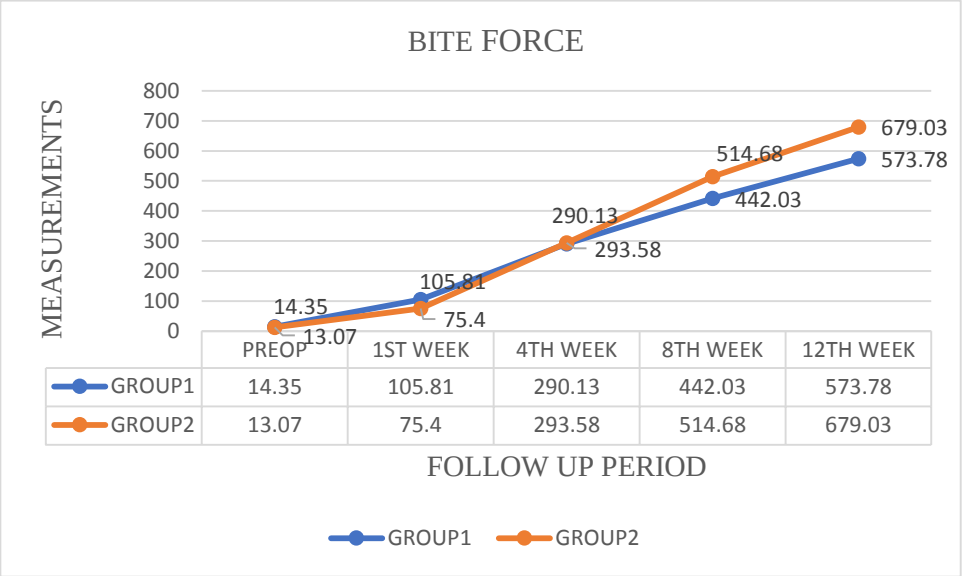


Figure 3: Comparison of bite force on left side in both the groups

In placebo group left side mean BF is more as compared to right at all time points. In RSV group except the preoperative reading mean BF at right side was higher than the left side at all time points. Except preoperative value, at all time points on both sides mean bite force in RSV group was higher than control.

In both the groups on intra group comparison the mean change of bite force was statistically significant at all the time points (Table 6).

On evaluation of change in mean bite force when compared to preoperative readings over these time periods it was observed that mean change was higher in RSV group than control at all time points (4-0, 8-0, 12-0) except at 1st week (Table 6), though the difference was statistically non-significant.

Table 6: Comparison of Change bite force in the Placebo & RSV groups

Group	Group 1		Group 2		p-value with Levene's Test
	Mean ± SD	p-value	**Mean** ± SD	p-value	
Mean BF 1-0	86.10±82.10	0.000*	80.37±129.58	0.012*	0.868**
Mean BF 4-0	259.62±167.99	0.000*	328.85±302.44	0.000*	0.376**
Mean BF 8-0	417.18±250.64	0.000*	520.54±281.01	0.000*	0.227**
Mean BF 12-0	553.27±300.08	0.000*	690.55±262.00	0.000*	0.132**

*Intragroup comparison was done with paired t-test. **Intergroup comparison was done by t-test. ($p - Value \leq 0.05$ was considered statistically significant.)

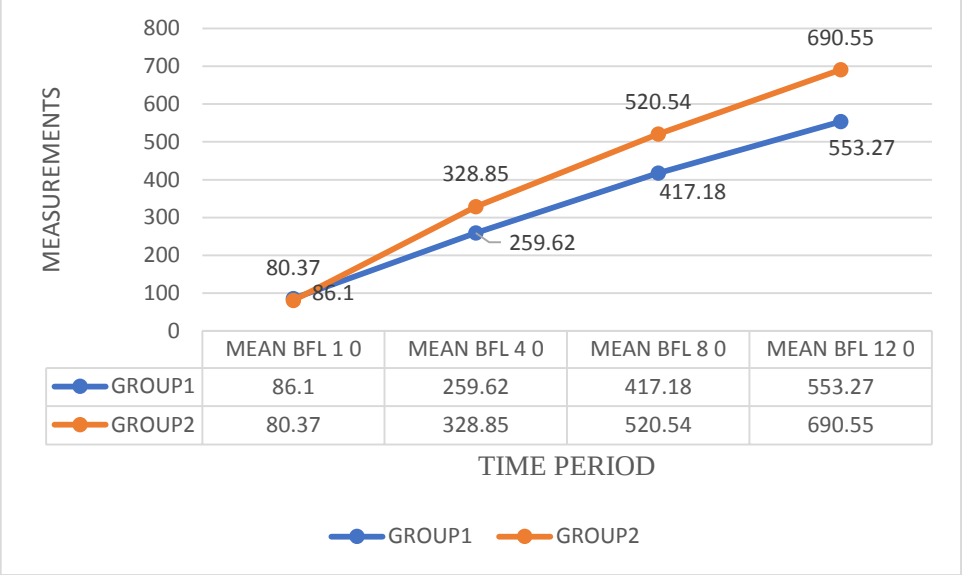


Figure 4: Mean change in bite force at various time points

SERUM OSTEOGENIC MARKERS

Serum OCN and ALP were evaluated preoperatively and at 4th and 12th week postoperatively. Mean value in each group was calculated and used for analysis. Paired t-test was used for the statistical analysis to compare the preoperative and postoperative values.

Table 7: Intragroup comparison of Mean Change of Serum markers

	Group 1 (Placebo)		**Group 2 (Tab.Resveratrol)**	
	(Mean±SD)	**p-value**	**(Mean±SD)**	**p-value**
Mean difference ALP 4-0	-28.80±46.88	*0.013	-29.00±43.13	*0.007
Mean difference OCN 4-0	13.75±21.34	*0.010	5.8±21.96	*0.247
Mean difference ALP 12-0	8.7±28.7	*0.018	18.1±66.35	*0.006
Mean difference OCN 12-0	-13.4±27.24	*0.011	0.24±23.41	*0.269

*p-value on intragroup comparison.

Significant difference were found in mean change of ALP and OCN levels on intragroup comparison in placebo group, though the difference in mean change was present only in ALP levels but not in OCN levels in RSV group. There correlation could not be justified on intergroup comparison because of lesser number of samples.

SERUM ALKALINE PHOSPHATASE(ALP)

No statistically significant difference was found in the preoperative serum ALP level among the two groups (p=0.96) (Table 2). At postoperative 4th week the mean serum ALP level was increased in both the groups similarly and intergroup there was no significant difference (p=0.98). At 12th week postoperatively mean serum ALP levels showed reduction as compared to 4th week on intragroup comparison but the reduction in group 2 (RSV) is less pronounced than group 1 (placebo) though the results showed that there was no significant difference on intergroup comparison despite having higher readings in RSV group (p=0.57) (Table 8).

Table 8: Mean Preoperative and Postoperative serum ALP level

Group	Group 1 (Placebo)	Group 2 (Tab.Resveratrol)	p-Value
	(Mean ± SD)		
Preop	99.20±34.52	98.70±35.68	0.965
Postop 4th Week	128.00±63.92	127.70±43.08	0.986
Postop 12th Week	107.90±42.99	116.80±55.25	0.573

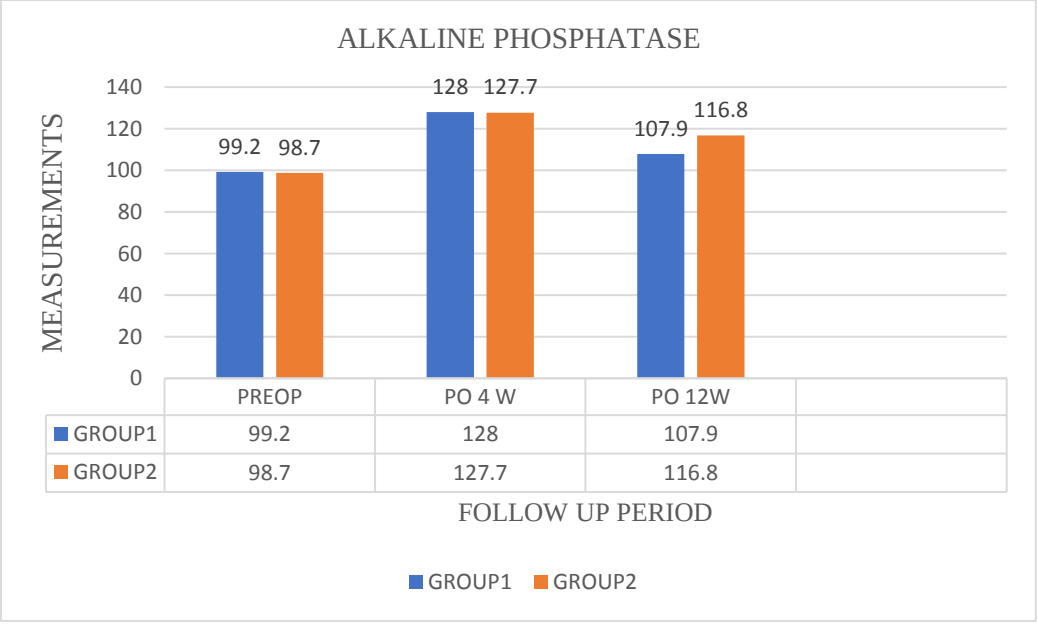


Figure 5: Comparison of serum ALP in two groups

SERUM OSTEOCALCIN (OCN)

No statistically significant difference was found in preoperative serum OCN level among the two groups (p=0.87) (Table 2). At 4th week postoperatively both the groups showed a decrease in the mean serum OCN level however it had reduced more in group 1 as compared to group 2 (Tab. Resveratrol), but the difference was not statistically significant (p=0.35). On 12th week postoperatively mean serum OCN level in group II (Resveratrol) is increased as compared to 4th week and is back to preoperative values while in group I (Placebo) the values are same as 4th week and thus reduced as compared to preoperative levels. OCN levels were higher in RSV group at both postoperative time points but on intergroup comparison it did not yield pure significant difference (p=0.06) (Table 9).

Table 9: Mean Serum Osteocalcin level

Group	**Group 1 (Placebo)**	**Group 2 (Tab.Resveratrol)**	**p-Value**
	(Mean±SD)		
Preop	75.38±20.85	74.37±19.93	0.877
Postop 4th Week	61.62±26.32	68.50±19.84	0.357
Postop 12th Week	61.88±25.85	74.61±15.21	0.065

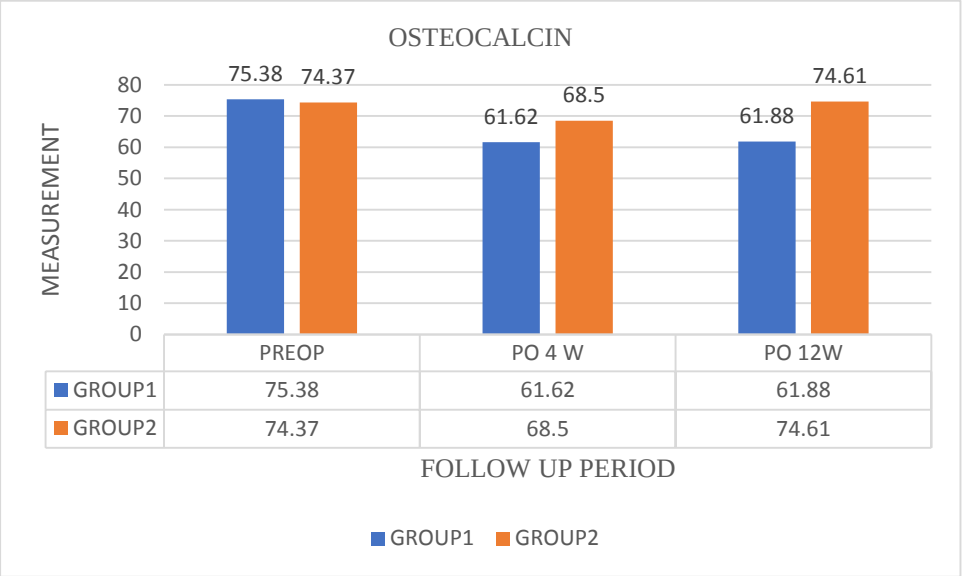


Figure 6: Comparison of serum OCN in two groups

DISCUSSION

Maxillofacial fractures are one of the most common injuries resulting from road traffic accidents, assaults, fall etc(33). These fractures warrant special attention and early intervention for optimization of aesthetics as well as masticatory function. Optimal correction of the resulting disfigurement and dysfunction is essential for early return to a healthy lifestyle. The bone healing is a natural biological process and has different stages which is governed by various biochemical and mechanical factors. The process can be direct/primary healing or indirect/secondary healing and associated factors are age, local, chemical, vascular, systemic and treatment factors. Usually from day one, patients treated with open reduction and internal fixation (ORIF) for maxillofacial fractures are allowed for functional use of the jaws for mastication. To ensure faster bone healing and bone formation irrespective of all unfavourable factors, supplements that improve bone healing process with minimal complications can be a promising option. The quintessential presence of maxillofacial fractures in an Oral and Maxillofacial office has, thus, made surgeons of various hues to evaluate the efficacy of innumerable drugs in initiating bone formation and early return to function following ORIF. Resveratrol (RSV) is a polyphenol group obtained from the plant and was primarily considered as an antioxidant agent. **Yu T et al** studied the anti-osteoporotic effects of resveratrol through molecular mechanisms, which was associated with the positive effect on osteogenesis and bone formation(34). Although, RSV, a SIRT1 agonist, has been widely touted as a possible agent for initiating faster bony healing, there still exists ambiguity over its efficacy, as the literature is replete with conflicting studies(35).

There are studies by **Feng J et al, Ornstrup et al, Uysal et al** that have shown promising effects of resveratrol as a bone anabolic agent. **Feng J et al** evaluated the effects of RSV on bone density, serum alkaline phosphatase and osteocalcin levels in osteoporotic rats. RSV was given in three different doses of 5 mg/kg, 25 mg/kg and 45 mg/kg. The trial exhibited improved bone density as well as higher alkaline phosphatase and osteocalcin levels in rats that were given RSV in doses of 25 and 45 mg/kg(35). **Ornstrup et al** evaluated the effect of RSV in men with metabolic syndrome (MetS). This study concluded that bone alkaline phosphatase (BALP) increased dose dependently in the RSV high (1gm daily for 16 weeks) group compared with placebo

at all the time-points(9)**. Uysal et al** studied local application of RSV during the early stages to orthopedically expanded inter-premaxillary suture area in male Wistar rats. RSV was injected into the inter-premaxillary suture in the experimental group and compared with control group. Evaluation of bone formation in the suture showed that RSV stimulates faster bone formation and shortens the retention period(22). **Ayse et al** studied effects of resveratrol (10 µmol/kg) on healing of extraction socket in cyclosporin A (CsA) treated rats. They found that RSV has a significant effect on healing of extraction sockets as compared to other groups, but it was statistically non-significant. It was found that osteocalcin and osteopontin marker levels and new bone formation was higher in RSV group compared to the other groups on day 14 after the tooth extraction. This was found to be statistically significant(26).

However, in contrast to this, a systematic review and meta-analysis conducted by **Qiangqiang et al** in 2021 suggested that resveratrol supplementation ranging from 16 weeks to 12 months did not have any statistically significant effect on the bone mineral density (BMD). Supplementation of resveratrol did not result in significant change in bone serum markers, including serum alkaline phosphatase (ALP), bone alkaline phosphatase (BALP) and even OCN(28).

In our study we had included 40 patients with maxillofacial fracture of age group ranging between 20-60 years. Maxillofacial trauma affects the masticatory functions due to loss of biting force as a result of changes in architecture and loss of balance between the structures. Therefore, to assess the jaw functions and bone healing in patient with maxillofacial fractures after surgery bite force measurement is a reliable parameter. Restoration of mean bite force in these patients indicates restoration of skeletal architecture and satisfactory healing of the masticatory system. In our study, we had used flexi force sensor for recording bite force which was customized at Indian Institute of Technology (IIT) Jodhpur. A pilot study was conducted on 20 healthy individuals to standardize and calibrate the instrument. Bite force was measured by placing a flexi force sensor between the molar cusp tips. The mean posterior bite force (PBF) based on this pilot study was 350 N. We have used these bite forces as the normal mean bite force for further comparison. There are several factors that influence the bite force like age, sex, muscle strength etc. In flexi force transducer that we have used for bite force recording, the sensor was covered with soft foam to reduce pain on biting and to reduce patient’s fear to fracture of tooth during biting on hard object and thus should

yield true values.

ALP is the most widely recognized biochemical marker for osteoblast activity in early bone healing and essential for skeletal mineralization. OCN is the most abundant small non-collagenous protein, synthesized by mature osteoblast in bone. Serum osteocalcin is found to be a marker for bone remodelling, as it couples and reflect both bone resorption and bone formation. It is mostly used to assess osteoporosis and to predict fracture risk in elderly. For these reasons we chose ALP and OCN as markers to correlate with bone healing(30).

In our study we have used ORIF as a standard treatment protocol for all fracture patients and Tab. Resveratrol was given for study group and placebo was given to other group for one month as a adjunctive treatment modality. Serum bone markers and bone healing after fracture were assessed.

RSV was used because of its promising role in bone healing as shown in the existing literature and also as it is affordable and has minimal side effects. Cost for one month of dose of RSV was Rupees 1500 which is less in comparison to other bone anabolic agents like Teriparatide (Rs4500/month).

There was an ambiguity about the dose of RSV. Various doses have been experimented yet a conclusive ideal recommendation for dose is not there. **Marzieh et al** conducted a systematic review and metanalysis that included all human RCTs that were conducted to evaluate the dose dependent effects of RSV on bone biomarkers. The duration of intervention varied from 6 to 24 weeks. Their study revealed that 1000 mg/day and more resveratrol supplementation had significant effect on ALP increase but supplementation of 500 mg/day and less did not show any significant change. In accordance with this and other literature, we have used 500mg BD of resveratrol which was shown to be an effective dose with minimal complications(27).

In our study the bite force was the prime clinical indicator of the fracture healing. So magnitude of bite force at various time points both in Placebo and RSV group were noted by placing the transducer in the right and left molar region. As expected lower values of preoperative bite force is noted in the molar region as compared to normal mean bite force (350 N) due to the decrease of action of masticatory muscles immediately after fracture. We have seen that the mean bite force (BF) preoperatively

was in the range 11.09 ± 31.07 N and 9.17±21.42 N in placebo and RSV group respectively. Bite force increased gradually from 1 week postoperatively. At 1st week 2 out of 20 patients in RSV group had achieved normal mean bite force (350N) while none in the placebo group had achieved it. At 4th week placebo group showed a better catch up and more number of patients, 7 out of 20 patients had achieved normal mean bite force in contrast to 6 out of 20 patients of RSV group. Though the mean BF in RSV group was higher than placebo. At 8th and 12th week, RSV group had more number of patients with bite force greater than normal mean bite force (15 out of 20 patients in RSV group and 12 out of 20 patients in placebo at 8th week, 19 out of 20 patients in RSV group and 16 out of 20 patient in placebo group at 12th week). **Pepato et al** conducted a study to assess the bite force in patient with mandibular fracture after surgical treatment and he found that, about 50% to 60% of molar bite force were obtained within the control group at 6 weeks postoperatively(36). Similar to this **Gerlach et al** reported restoration of only 58% of the bite forces by 6th week(14).

In our study mean bite force at 4th week was less than the normal mean bite force value in both the groups but in RSV it was 338 N which is nearly close to the normal mean bite force (350 N). **Sybil et al** in their study showed that the maximum bite force in the patients with maxillofacial fracture was achieved between 6th to 12th postoperative weeks(17). **Kshirsagar et al** in their study showed restoration of functional bite forces was evident by 6 to 8 weeks. However, the restoration of maximum bite forces may require up to 12 weeks in parasymphyseal fracture(15). **Kumar et al** conducted a study to detect the stability of fractured mandibular fragments under functional load following open reduction and internal fixation. They found that bite force function was returned to 92% of normal bite force within 3 months(18).

In our study by 8th week mean BF values in both the groups had crossed the normal bite force of 350 N and it was greater in RSV group when compared to the placebo group though not statistically significant. **Abhinandan P et al** had done a study to evaluate the bite force on right and left side in patient with maxillofacial fractures. They have shown that there was steady increase in bite force from 1st week and maximum bite force was achieved on 12th post-operative weeks. There was no significant difference in bite force on right and left side of jaw(21). **Gaurav et al** did a study to compare the bite force on right and left side in patient with mandibular fracture, they have shown that the normal bite force on both right and left side was achieved between 3rd to 6th

month postoperatively(37). **Elham et al** carried a study to determine the maximum bite force in student (age between 20-23 years) with premature occlusal contacts, their study results has shown that the average MBF ranged between 290 and 965 N. The average MBF on right side was 575.15 ± 146.71, while on left side was 571.69 ± 148.86 N(38). An interesting finding reported in study by **Pepato et al** had revealed that the mean bite force was more on the left side compared to the right molar region, during the 2-month period for both the groups, with a statistically significant difference ($P < 0.05$)(36). These findings were also supported in studies by **Elham et al, Agarwal et al**(38,39). In our study in placebo group left side mean BF is more as compared to right. This is in accordance to the above studies. But in Group 2 in which resveratrol was used as intervention at all postoperative time points readings of mean BF were higher on right side as compared to the left. In our study, in RSV group, normal mean bite force on right side was achieved by 4^{th} week itself while on left side in both the groups and on left side in RSV it was achieved by 8^{th} week. According to **Sybil et al** after the ninth postoperative week, the maximum bite force measured < 65% the normal in patients with isolated angle fractures and > 80% the normal in patients with isolated parasymphysis fractures. The same values reduced to < 60% in patients with fractures of angle and parasymphysis and < 70% in patients with fractures of parasymphysis and condylar complex(17). In our study though both the groups had mean bite force values greater than the maximum biting force (350 N) at 8^{th} week and RSV had greater mean but force at 4^{th}, 8^{th} and 12^{th} week than placebo but due to small sample size subgroup analysis cannot be done.

The difference in bite force was calculated in both the groups, the RSV group patients achieved higher bite force at all the time points than placebo group though the difference was not statistically significant. There is a subtle change in pattern of bite force reflected with higher readings on right side in treatment group however the mean bite force in two groups still could not show a statistically significant difference. A larger sample size may be able to reflect if it is actually the effect of drug intervention.

SERUM ALKALINE PHOSPHATASE AND OSTEOCALCIN

The process of normal fracture healing involves increased osteoblastic activity. In this osteoblasts have major role and responsible for both new bone formation and its mineralization and it involves large quantities of ALP, which is crucial in this process.

Normal serum alkaline phosphatase level is 30–120 IU/L.

Most of ALPs are produced in liver and some of ALPs are generated in the bones, intestines and kidneys. **Sarac et al** revealed bone-specific alkaline phosphatase (BALP) is synthesized by the osteoblasts and is presumed to be involved in the calcification of bone matrix. Conditions like bone growth, healing of fracture shows increased levels of BALP. The total ALP is examined by the measurement of the total amount of alkaline phosphatase enzyme in the bloodstream. Many factors may cause an increase of ALP activity in serum, the most common being obstructive liver disease and metabolic bone disease. Ideally measurement of bone ALP rather than serum ALP may be a true indicator of effects of RSV on bone(40). However, **Taniguchi et al** has stated that with normal liver function in adults, about 50% of total ALP is produced from the bone in the serum. BALP being an expensive test was thus avoided and serum ALP was chosen in our study as our patients had normal liver function(31).

Preoperative serum ALP levels in our study were not significantly different among the RSV and Placebo groups. At 4^{th} week the ALP levels in both the groups showed similar increased values on comparison to preoperative values. At 12^{th} week postoperatively in both the groups ALP levels showed a decline. In RSV group ALP was seen to be dropped from 127.70±43.08 to 116.80±55.25 and in Placebo group, ALP levels dropped way more from 128.00±63.92 to 107.90±42.99. On intragroup comparison ALP levels had changed significantly both at 4^{th} and 12^{th} week in both the groups, however lower p-value in group 2 (0.006, 0.007) in contrast to group 1 (p-value=0.01) indicates a stricter significance and a possible effect of drug resveratrol on maintenance of ALP (Table 7). However on Intergroup comparison the difference between the two groups both at 4^{th} and 12^{th} week was not statistically significant (Table 8).

There are reported cases of elevated level of serum ALP after RSV medication**.** **Marzieh et al** in their systematic review and meta-analysis of randomized controlled trials (RCTs) had depicted the dose dependent effect of resveratrol on bone markers(27). In our study also serum ALP levels at 12^{th} week was higher in RSV group than placebo group. The studies like **Poulsen et al, Ornstrup et al** which had shown significant increase in ALP levels after RSV had used it in higher doses or for duration greater than our study(23,9). A shorter duration in our study could have been the reason for non-significant results. From the data of our study we can see the better maintenance

of ALP seen in RSV group but to check for statistically significant difference in ALP values may be a larger sample size is needed.

Osteocalcin (OCN) is a protein derived from osteoblast during bone remodelling. During bone formation osteoblast produce large amount of OCN. Once transcribed, this protein undergoes posttranslational modifications within osteoblastic cells before its secretion, including the carboxylation of three glutamic residues in glutamic acid, which is essential for hydroxyapatite binding and deposition in the extracellular matrix of bone. For serum OCN blood sample collection was done at various time points as mentioned above and centrifuged. The serum sample was then stored at -80ºC. Test result of serum OCN were obtained with the help of ELISA OCN kit (Elabscience) using standard method. Normal OCN level is 1.1-11ng/ml. In this study based on the used ELISA kit we have considered the maximum OCN value as 84 and the minimum as zero. In a study done by **Sudhir et al** in, they had evaluated the bone mineral density and serum osteocalcin levels in women with osteoporosis. They had proven that serum OCN is a bone turnover marker in osteoporotic patients. He stated that increased levels of OCN indicates bone remodelling(41). In study by **Bowles et al** they have studied a change in serum Osteocalcin and BALP after tibia fracture. They have observed decreased bone specific ALP till fourth day after injury and further rise throughout the study period, and achieved maximum concentration by 10th week and OCN concentrations increased significantly immediately after tibia fracture but then fall again reaching day 1 concentrations by week 5(30). **Stoffel et al** conducted a pilot study to check the change in levels of serum biomarkers in 20 patients with lower limb fracture with the study duration of 24 weeks. Changes were comparable but more pronounced in the tibia group, and marker concentrations (BALP, OC, ICTP) remained increased than malleolar group at the end of study at day 84 after osteosynthesis(42). **Seibel et al** showed in their study that OCN is involved in the process of osteoid mineralization, during initial phase of bone formation. Serum levels of immunoreactive OCN have direct correlation with rate of bone formation. Rapid degradation of OCN occurs in serum, so that both intact peptides and OCN fragments of various sizes coexist in the circulation. Some investigators have suggested that OCN fragments may be released even during bone resorption. According to this study however these fragments gets dissoluted and absorbed quickly making their assessment difficult(43).

Literature reports varied responses of OCN levels drug bone healing or after trauma. OCN was either increased or decreased in the studies. OCN being a marker of osteoblastic and osteoclastic activity both, should ideally increase as also seen in most literature. But in our study, OCN levels in placebo group reduced during postoperative phase with a rapid decline from preoperative values to 4th week (75.38 to 61.62). It remained at these reduced values of 4th week even at 12th week. This seems to be in accordance with study done by Bowles et al where OCN concentration fell during initial weeks(30). OCN decreased in RSV group also at 4th week though the change was less pronounced than Placebo group. At 12th week however in RSV group the OCN levels increased from 4th week levels and attained the preoperative levels**.** On intragroup comparison there was significant decrease in OCN levels in placebo group at both 4th and 12th week (p value= 0.010, 0.011), while the decrease in RSV was not significant (p value =0.247,0.269) (Table 7). In RSV group though change of ALP on intragroup was significant but OCN change both at 4-0 and 12-0 was non-significant. This may indicate that OCN levels were maintained partially in RSV group unlike the significant reduction seen in placebo group. Despite high levels and increase noted within the group at 12th week in RSV, on intergroup comparison the difference was not statistically significant.

Our result is in contrast to the studies that have shown elevated OCN levels during bone healing. It could be a possibility that the readings recorded preoperatively reflect the initial rapid rise of OCN that occurs immediately after the injury. After that OCN continued to decrease and this reduction was more evident in placebo group. This is reflected in our readings of 4th week where OCN in RSV group is higher than placebo. Another reason for reduced OCN levels could be its difficult assessment as reported by Siebel et al. At 12th week gain of OCN and return to preoperative values only in RSV group may be a reflection of the effect of drug intervention. In support of this there are some studies in the literature. **Bowles et al** have reported an initial rise in levels of OCN and then return to pre values by 5th week(30). **Poulsen et al** in a RCT on 24 obese non diabetic individual reported statistically significant rise in ALP after 4 weeks of 500 mg TDS RSV administration while OCN levels did not differ when compared to controls. **Lars F H et al** also in their study on concentration model had shown that despite significant difference in amount of new bone formation levels of osteocalcin in plasma were not a reflection of the same(32).

Literature has studies that support both increase and decrease in OCN levels post trauma. Our study reports a reduction in OCN levels in initial weeks even in placebo group. To resolve this confusion more precisely planned and executed studies to evaluate OCN changes physiologically at different time intervals post trauma should be undertaken.

The effect of RSV on OCN and ALP values shows better elevation in RSV group but yet a larger sample size with sequential evaluation at more frequent time points is required to draw a conclusion.

All patients completed the follow up period in our study of 3 months. None of our patients had presented with complications like infection, non-union or malunion. No adverse effects were reported with the use of resveratrol drug.

In future to investigate the effectiveness of resveratrol on human, more precise time period and study sample size should be advocated to achieve significant outcomes.

STRENGTHS OF THE STUDY

1. Most of the studies have evaluated effect of resveratrol in animal studies or patient with osteoporotic bone or in post-menopausal or immunocompromised status like metabolic syndrome etc. This study might be a first study to evaluate the efficacy of Resveratrol in maxillofacial fracture in healthy patients.
2. All the patients have completed the follow up period of 12 weeks and there was no sample attrition.
3. This was a randomized controlled study in which all participants got equal chance of distribution. We have used computer base generated codes for patient selection so no bias in our study. Our study was double blinded so there was less interpretation bias in our study.
4. We had used all objective parameters like bite force and serum markers.
5. We have strictly adhered to the methodology as committed in the protocol.

LIMITATIONS OF STUDY

1. Our study sample size was small, more sample size may be required to give conclusive results.
2. Confounders of bone healing like Vitamin D and Calcium were not evaluated and correlated in the study. Though any patient on vit D/calcium on any such supplement was excluded from the study.
3. Because of small sample size subgroup analysis based on age and type of fractures could not be done.

CONCLUSION

1. Resveratrol molecules is reported to have a potential to activate particular signalling pathways for osteoblastic growth and differentiation especially in compromised bone or immune status situation cases.

2. Bite force significantly increased in both our groups at all time points but on intergroup comparison mean bite force at 4^{th}, 8^{th} and 12^{th} week was more in RSV group than Placebo group though not statistically significant. In placebo group left mean bite force were more than right. In RSV group mean bite force at right side were more than left at all time points.

3. Alkaline phosphatase levels were increased at 4^{th} week and then showed decrease at 12^{th} week in both the groups but at 12^{th} week values of ALP were higher in RSV group though not statistically significant on intergroup comparison. On intragroup evaluation both the groups showed statistically significant increase in ALP with smaller p values in RSV group.

4. Osteocalcin was higher in RSV group than placebo both at 4^{th} and 12^{th} week though not statistically significant. OCN showed reduction in placebo group at both time points while in RSV after a decrease at 4^{th} week it increased to return towards preoperative values. On Intragroup comparison OCN in placebo group showed a statistically significant decrease at 12^{th} week while it was unaltered in RSV group.

5. In our study on maxillofacial trauma in healthy individuals a pattern of better bite force and higher levels of bone markers was noted in the experimental group of resveratrol but the results were non-significant on comparison to the placebo group. Studies with larger sample sizes are required to have conclusive assertion regarding utility of Resveratrol in bone regeneration in healthy patients.

SUMMARY

Background: In the past number of surgical and non-surgical methodologies have been used in facilitating and enhancing fracture healing. There is insufficient data in literature regarding the use of osteogenic drugs in maxillofacial fracture healing. A prospective, randomized controlled trial was planned aiming to assess the safety and efficacy of Tablet Resveratrol in maxillofacial fracture healing. The primary objective was to compare the bite forces and levels of serum alkaline phosphatase and osteocalcin in Resveratrol and Placebo group in patients of maxillofacial fracture.

Methods: 40 patients of maxillofacial fracture patients were recruited from Dental OPD of All India Institute of Medical Sciences, Jodhpur and were randomly divided into two equal groups. Group 1 (Placebo) in which ORIF intervention was followed by Placebo tablets for 1 month. In Group 2(RSV) tablet Resveratrol 500 mg were administered in BD doses for 1 month after ORIF. Patients were assessed preoperatively and postoperatively at regular intervals till 12 weeks. Posterior bite force using bite force machine and serum markers like serum alkaline phosphatase (ALP) and serum Osteocalcin were assessed.

Results: The result of our study showed that there was no statistically significant difference in Tab Resveratrol group and Placebo group in terms of bite force and level of serum markers of fracture healing. However more patients in RSV group (19 out of 20 patients) had achieved maximum mean BF within 12^{th} weeks in comparison to Placebo group (16 out of 20 patients). BF in RSV group was higher than placebo at 4^{th},8^{th} and 12^{th} post operative week. RSV group had showed better long-term maintenance of Serum ALP level as compared to Placebo group though the results were not statistically significant. On intragroup comparison ALP levels showed a statistically significant increase in both the groups but in RSV group it indicated better correlation because of smaller p values. Both the group had shown decrease in serum osteocalcin at 4^{th} week postoperatively. At 12^{th} week in placebo group OCN values continued to remain at reduced levels. In RSV group OCN values increased and reached preoperative values. Though the results were again not statistically significant on intergroup comparison.

Conclusion: Both the groups showed satisfactory fracture healing and improving functional outcome (bite force restoration). However, there was no statistical difference in bite force, serum ALP level and serum Osteocalcin levels between the two groups. Though not statistically significant but early increased level of serum osteogenic markers, better restoration of bite force in group 2(tab. Resveratrol) indicates towards it's possible optimistic role in maxillofacial fracture healing. More studies with larger sample sizes are needed in order to confirm the efficacy of this drug in maxillofacial fracture.

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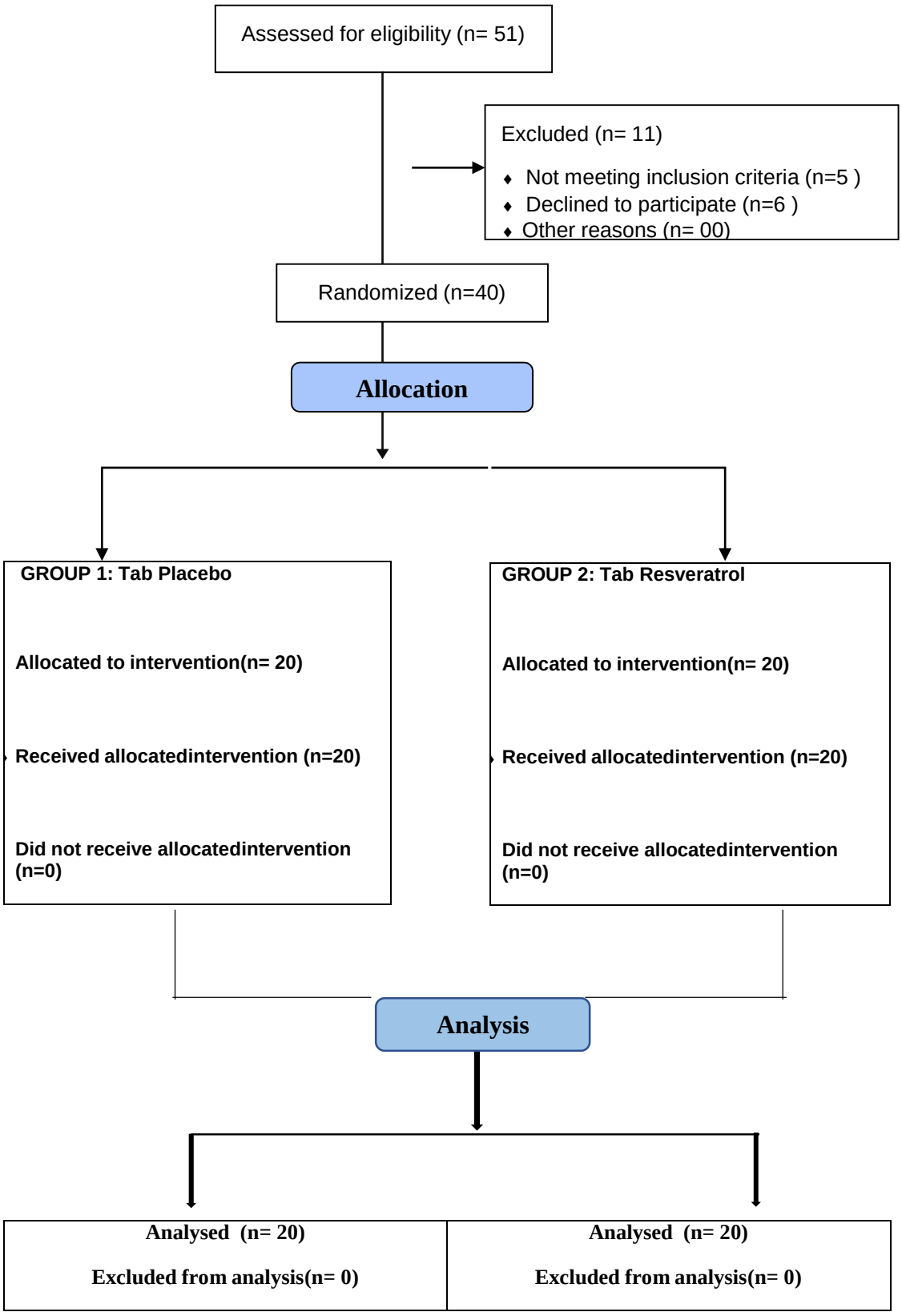
Annexure 1- CONSORT flow diagram



Annexure 2a: Patient Information Sheet (English)

All India Institute of Medical Sciences, Jodhpur

Department of Dentistry

PATIENT INFORMATION SHEET

TITLE: **"SAFETY AND EFFICACY OF RESVERATROL IN HEALING OF MAXILLOFACIAL FRACTURES: A RANDOMIZED CONTROLLED STUDY"**

You have been requested to volunteer for a research study which involves administration of oral tablet resveratrol after the usual reduction and fixation of fracture. Complications that occur after administration of resveratrol include nausea, head ache, leg cramps, liver dysfunction, diarrhoea, gastric problems and dryness of mouth. Bite force and blood markers will be evaluated preoperatively and postoperatively at regular intervals and co-related with fracture healing.

Confidentiality

Your medical records and identity will be treated as confidential documents. They will only be revealed to other doctors/scientists/monitors/auditors of the study if required. The results of the study may be published in a scientific journal but you will not be identified by name.

Ethics committee approval has been obtained for the study.

Your participation and rights

Your participation in the study is fully voluntary and you may withdraw from the study anytime without having to give reasons for the same. In any case, you will receive the appropriate treatment for your condition. You will not be paid any amount for the participation in the study. You will have to pay for the routine investigations that will be done.

For further queries, contact:

Dr Shivkumar Chopane
Post Graduate student
Department of Oral and maxillofacial surgery
AIIMS, Jodhpur
Mobile No: - 9765931438 Email ID: shivkumarchopane@gmail.com

Annexure 2b: Patient Information Sheet (Hindi)

अखिल भारतीय आयखविज्ञानु संस्थान,जोधपुर

दंत चिकित्सा विभाग

रोगी सूचना पत्र

शीर्षक: ''मैक्सिमोफैक्चियल फ्रैक्चर्स के स्वास्थ्य में रेस्वेराट्रोल के परिणाम और सुरक्षा: एक रैंडमाइज्ड नियंत्रण अध्ययन''

आपसे एक शोध अध्ययन के लिए स्वयंसेवक से अनुरोध करने का अनुरोध कीया गया है जिसमे सामान्य कमी और फ्रैक्चर के निर्धारण के बाद टैबलेट रेस्वेराट्रोल के उपकरणीय का प्रशासन शामिल है। रेस्वेराट्रोल के बाद निम्नलिखित समस्ट्याएं हो सकती हैं- सिरदर्द, पैर ऐंठन, यकृत की शिथिलता, दस्त, सिरदर्द, गैक्स्ट्रक समस्ट्याओं, मुंह का सूखापन। चबाने की शक्ति, खून की जाँच के आकलन के लिए नियमित अंतराल पर पूर्ववर्ती और बाद में मूल्यांकन कीया जाएगा।

गोपनीयता

आपके मेडिकल रिकॉर्ड और पहचान को गोपनीय दस्तावेज माना जाएगा। यदि आवश्यक हो तो केवल अध्ययन के अन्य डॉक्टरों / वैज्ञानिको / मॉनीटर / लेखा परीक्षकों को ही प्रकट किये जाएंगे। अध्ययन के परिणाम वैज्ञानिक पत्रिका में प्रकासशत कीए जा सकते हैं लेकिन आपको नाम से पहचाना नहीं जाएगा। अध्ययन के लिए नैतिकता समिति की मंजूरी प्राप्त की गई है।

आपकी भागीदारी और अधिकार

अध्ययन में आपकी भागीदारी पूरी तरह से स्वैच्छिक है और आप इसके कारणों के बीना कीसी भी समय अध्ययन में भाग लेने से मना कर सकते हैं। कीसी भी मामले में, आपको अपनी स्थिति के सलए उचित उपचार प्राप्त होगा। अध्ययन में भागीदारी के लिए आपको कोई राशि नहीं दि जाएगी। आपको नियमित जांच के लिए भुगतान करना होगा।

संपर्क व्यक्त: आगे के प्रश्नों के लिए -

डॉ शिवकुमार चोपने

पोस्ट्ट ग्रजुएट छात्र

ओरल एंड मैक्सिमोफैक्शियल शल्य चिकित्सा विभाग

एम्स, जोधपुर

मोबाइल नंबर: - 9765931438

ईमेल आईडी:- shivkumarchopane@gmail.com

Annexure 3a: Informed Consent Form (English)

Serial no._____

All India Institute of Medical Sciences, Jodhpur
Department of Dentistry
INFORMED CONSENT FORM

Title: "SAFETY AND EFFICACY OF RESVERATROL IN HEALING OF MAXILLOFACIAL FRACTURES: A RANDOMIZED CONTROLLED STUDY"

Participant's registration number: _____.

I declare that on date..... All the details of this information sheet given to me have been explained to me in the language that I comprehend the best. I have been informed that tablet resveratrol will be administered to me I have been told that complications might occurs after administration includes nausea, head ache, leg cramps, liver dysfunction, insomnia, diarrhoea, gastric problems and dryness of mouth. Bite force and blood markers will be evaluated preoperatively and postoperatively at regular intervals.

I understand that all information related to me in this research will be kept safe by the responsible staff of AIIMS Jodhpur. I, hereby, allow them to see all the information related to me. I have been told that all the information related to me will be kept confidential. I have also been told that the results of this research can be published in any book or journal and can be displayed in any conference. I have also been told that my name or any other identity will not be used without my consent. I am participating in this research with my consent and I am aware that I can refuse to participate in this research at any time without any reason.

I agree to participate in this research.

(Signature)

Place: Date:
Name of the Participant: _____
Son/Daughter/Spouse of: _____
Complete postal address: _____

This is to certify that the above consent has been obtained in my presence.

1) Witness – 1	2) Witness – 2
Name:	Name:
Address:	Address:

Signatures of the principal investigator:

Place: Date:

Annexure 3b: Informed Consent Form (Hindi)

सीरीयल नम्बर।_____

अखिल भारतीय आयखविज्ञानु संस्थान,जोधपुर

दंत चिकित्सा विभाग

सूचित सहमति प्रपत्र

शीर्षक: "मैक्सिमोफैक्चियल फ्रैक्चर्स के स्वास्थ्य में रेस्वेराट्रोल के परिणाम और सुरक्षा: एक रैंडमाइज्ड नियंत्रण अध्ययन"

रजिस्ट्रेशन नंबर : _____

मैं घोषणा करता हूं की ..... तारीख में मुझे दिया गया यह सूचना पत्र मेरी भाषा में समझाया गया है। मुझे सूचित किया गया है की टैबलेट रेस्वेराट्रोल को मुझ पर प्रशाससत किया जाएगा। रेस्वेराट्रोल के बाद निम्नलिखित समस्ट्याएं हो सकती हैं– सिरदर्द, पैर ऐंठन, यकृत की शिथिलता, दस्त, सिरदर्द, गैक्स्ट्रक समस्ट्याओं, मुंह का सूखापन। काटने की शक्ति और खून की जाँच का मूल्यांकन किया जाएगा। यह शोध अध्ययन और इलाज के लिए कीया जा रहा है। मैं समझता हूं की इस शोध में मुझसे सम्बंधित सभी जानकारी एम्स जोधपुर के जिम्मेदार व्यक्ति द्वारा रखी जाएगी। मैं उन्हें मुझसे सम्बंधित सभी जानकारी देखने की अनुमनत देता हूं। मुझे बताया गया है की मुझसे सम्बंधित सभी जानकारी गोपनीय रखी जाएगी। मुझे यह भी बताया गया है की इस शोध के लिए कीसी पुस्तक या पत्रिका में प्रकासशत कीए जा सकते हैं और कीसी भी सम्मेलन में प्रिसशित कीए जा सकते हैं। मुझे बताया गया है की मेरी मरजी के बिना मेरा नाम या कोई अन्य पहचान का उपयोग नहीं कीया जाएगा। मुझे पता है की मैं इस शोध मैं अपनी मरजी से भाग ले रहा हूं और मैं बिना कीसी कारण के कीसी भी समय इस शोध में भाग लेने से इंकार कर सकता हूं। मैं इस शोध में भाग लेने के लिए सहमत हूं।

(हस्ताक्षर)

जगह : तारीख :

प्रनतभागी का नाम : _____

पुत्र / पुत्री/ पनत / पत्नी : _____

पूरा डाक पता: _____

उपर्युक्त सहमती मेरी मौजूदगी में प्राप्त की गई है।

1) साक्षी - 1 2) साक्षी – 2

नाम : नाम :

Annexure IV: Case Record Form

Case Record Form

Sl. No.:

Name: CR No:

Age/Sex: Date:

Address: Occupation:

Contact number

Randomization Code:

Group Allocated:

Inclusion criteria

1.	Patients with between 20-60 years with maxillofacial fractures	**Y** / **N**
2.	ASA I or ll	**Y** / **N**

Exclusion criteria

1.	Age <20 years and >60 years	**Y** / **N**
2.	Pregnant or lactating females	**Y** / **N**
3.	Hypercalcemia, Paget's disease or any other bone disorder	**Y** / **N**
4.	Malignant tumours	**Y** / **N**
5.	Patients earlier having radiation treatment.	**Y** / **N**
6.	Patients on Vitamin D therapy or any other bone medications.	**Y** / **N**

SITE OF MANDIBULAR FRACTURE

PARASYMPHYSIS	
BODY	
ANGLE/RAMUS	
CONDYLE	
CORONOID	

MIDFACE FRACTURE

LEFORT I	
LEFORT II	
LEFORT III	
ZMC	
ORBITAL	
FNOE	

A. Biteforce

TIME PERIOD	RIGHT MOLAR	LEFT MOLAR	MEAN
Preoperative			
1st week Postoperatively			
4th Week Postoperatively			
8th Week Postoperatively			
12th Week Postoperatively			

B. Blood investigations

TIME PERIOD	OSTEOCALCIN	ALKALINE PHOSPHATASE
Preoperative		
4th week Postoperatively		
12th week Postoperatively		

Annexure 5 : Ethical clearance certificate

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2020/3328 **Date: 14/10/2020**

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2019-20/987**

Project title: **"Safety and efficacy of Resveratrol in healing of maxillofacial fractures: a randomized control study"**

Nature of Project:	**Research Project**
Submitted as:	**M.D.S. Dissertation**
Student Name:	**Dr. Shivkumar Suresh Chopane**
Guide:	**Dr.Ankita Chugh**
Co-Guide:	**Dr. Mithu Banerjee, Dr. Kirti Chaudhary Dutt & Dr. Pravin Kumar**

Institutional Ethics Committee after thorough consideration accorded its approval on above project.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

Please Note that this approval will be rectified whenever it is possible to hold a meeting in person of the Institutional Ethics Committee. It is possible that the PI may be asked to give more clarifications or the Institutional Ethics Committee may withhold the project. The Institutional Ethics Committee is adopting this procedure due to COVID-19 (Corona Virus) situation.

If the Institutional Ethics Committee does not get back to you, this means your project has been cleared by the IEC.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member Secretary

Member secretary
Institutional Ethics Committee
AIIMS Jodhpur

Annexure 6: Plagiarism Certificate

ORIGINALITY REPORT

18%
SIMILARITY INDEX

PRIMARY SOURCES

1	www.ncbi.nlm.nih.gov Internet	176 words — 2%
2	repository-tnmgrmu.ac.in Internet	121 words — 1%
3	www.science.gov Internet	116 words — 1%
4	worldwidescience.org Internet	77 words — 1%
5	ihcoedu.uobaghdad.edu.iq Internet	76 words — 1%
6	www.thieme-connect.com Internet	72 words — 1%
7	jpbsonline.org Internet	63 words — 1%
8	juniperpublishers.com Internet	62 words — 1%
9	link.springer.com Internet	62 words — 1%

Annexure 7: CONSORT Checklist

SECTION	ITEM #	CONSORT-SPI 2010	CONSORT-SPI 2018	REPORTED ON PAGE#
TITLE AND ABSTRACT				
	1a	*Identification as a randomised trial inthe title§*		*Cover page*
	1b	*Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for Abstracts)§*	*Refer to CONSORT extension for social and psychological intervention trial abstracts*	*39*
INTRODUCTION				
Background and Objectives	*2a*	*Scientific background and explanationof rationale §*		*1-2*
	2b	*Specific objectives or hypotheses §*	*If pre-specified, how the intervention was hypothesied to work*	*11*
METHODS				
Trial Design	*3a*	*Describe of trial design (such as parallel, factorial), including allocationratio §*	*If the unit of random assignment is not the individual, please refer to CONSORT for Cluster Randomized Trials*	*12*
	3b	*Important changes to methods after trial commencement (such as eligibility criteria), with reasons*		*NA*
Participants	*4a*	*Eligibility criteria for participants§*	*When applicable, eligibility criteria for settings and those delivering the interventions*	*12*
	4b	*Settings and locations where the data were collected*		*12*
	5	*The interventions for each group with sufficient details to allow replication, including how and when they are actually administered §*		*14-16*

Interventions	*5a*		*Extent to which interventions were actually delivered by providers and taken up by participants as planned*	*14*
	5b		*Where other informational materials about delivering the intervention can be accessed*	*13,14*
	5c		*When applicable, how intervention providers were assigned to each group*	*14*
Outcomes	*6a*	*Completely defined pre-specified outcomes, including how and when they were assessed§*		*14,15*
	6b	*Any changes to trial outcomes after thetrial commenced, with reasons*		*NA*
Sample Size	*7a*	*How sample size was determined§*		*17*
	7b	*When applicable, explanation of any interim analyses and stopping guidelines*		*NA*
RANDOMISATION				
Sequence generation	*8a*	*Method used to generate the random allocation sequence*		*13*
	8b	*Type of randomisation; detail of any restriction (such as blocking and block size)§*		*13*
Allocation concealment mechanism	*9*	*Mechanism used to implement the random allocation sequence, describingany steps taken to conceal the sequence until interventions were assigned§*		13

Implementation	*10*	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions§		*13*
Awareness of assignment	*11a*	Who was aware of intervention assignment after allocation (for example, participants, providers, thoseassessing outcomes), and how any masking was done		*13*
	11b	*If relevant, description of the similarityof interventions*		*NA*
Analytical methods	*12a*	*Statistical methods used to compare group outcomes§*	*How missing data were handled, with details of any imputation method*	*17*
	12b	*Methods for additional analyses, such as subgroup analyses, adjusted analyses, and process evaluations*		*17*
RESULTS				
Participant flow (a diagram is strongly recommended)	*13a*	*For each group, the numbers randomly assigned, receiving the intended intervention, and analysed for the outcomes§*	*Where possible, the number approached, screened, and eligible prior to random assignment, with reasons for non-enrolment*	*18*
	13b	*For each group, losses and exclusions after randomisation, together with reasons§*		*18*
Recruitment	*14a*	*Dates defining the periods of recruitment and follow-up*		*12*
	14b	*Why the trial ended or was stopped*		*12*
Baseline data	*15*	*A table showing baseline characteristics for each group§*	*Include socioeconomic variables where applicable*	*19*

Numbers analysed	*16*	*For each group, number included in each analysis and whether the analysis was by original assigned groups§*		*19*
Outcomes and estimation	*17a*	*For each outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)§*	*Indicate availability of trial data*	19-27
	17b	*For binary outcomes, the presentationof both absolute and relative effect sizes is recommended*		NA
Ancillary analyses	*18*	*Results of any other analyses performed, including subgroup analyses, adjusted analyses, and process evaluations, distinguishing pre-specified from exploratory*		28-36
Harms	*19*	*All important harms or unintended effects in each group (for specific guidance see CONSORT for Harms)*		36
DISCUSSION				
Limitations	*20*	*Summarize the main results (includingan overview of concepts, themes, and types of evidence available), link to thereview questions and objectives, and consider the relevance to key groups.*	*Trial limitations, addressing sourcesof potential bias, imprecision, and, if relevant, multiplicity of analyses*	*38*
Generalisability	*21*	*Discuss the limitations of the scoping review process.*	*Generalisability (external validity, applicability) of the trial findings§*	*39*
Interpretation	*22*	*Provide a general interpretation of the results with respect to the review questions and objectives, as well as potential implications and/or next steps.*	*Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence*	*28-39*
IMPORTANT INFORMATION				
Registration	*23*	*Registration number and name of trial registry*		*12*

Protocol	*24*	*Where the full trial protocol can be accessed, if available*		*CTRI*
Declaration of Interests	*25*	*Sources of funding and other support; role of funders*	*Declaration of any other potential interests*	*AIIMS,JODHPUR*
Stakeholder investments	*26a*		*Any involvement of the intervention developer in the design, conduct, analysis, or reporting of the trial*	*NA*
	26b		*Other stakeholder involvement in trial design, conduct, or analyses*	*NA*
	26c		*Incentives offered as part of the trial*	*NA*

This table lists items from the CONSORT 2010 checklist (with some modifications for social and psychological intervention trials) and additional items in the CONSORT-SPI 2018 extension. Empty rows in the 'CONSORT-SPI 2018' column indicate that there is no extension to the CONSORT 2010 item

*We strongly recommended that the CONSORT-SPI 2018 Explanation and Elaboration (E&E) document be reviewed when using the CONSORT-SPI 2018 checklist for important clarifications on each item

§An extension item for cluster trials exists for this CONSORT 2010 item

Citations

Montgomery, P., Grant, S., Mayo-Wilson, E., Macdonald, G., Michie, S., Hopewell, S., & Moher, D. (2018). Reporting randomised trials of social and psychological interventions: the CONSORT-SPI 2018 Extension. *Trials*, *19*(1), 407.

Grant, S., Mayo-Wilson, E., Montgomery, P., Macdonald, G., Michie, S., Hopewell, S., & Moher, D. (2018). CONSORT-SPI 2018 Explanation and Elaboration: guidance for reporting social and psychological intervention trials. *Trials*, *19*(1), 406.