Title: **Anti-proliferative agent and method of preparing the same**

Abstract

The present invention relates to a anti-proliferative agent which act as potent osteogenic inhibitor. More particularly, the present invention relates to the anti-proliferative agent which prepared from the extract of the marine gastropod mollusc having therapeutic value. Moreover this invention also relates to the process of preparing the above agent from the whole body extract (without shell) of *Terebra dislocata* (Say, 1822) as Terebra dislocata Bioactive Compound (TdBC).

I claim:

1) A anti-proliferative agent which comprises an effective amount of Inorganic phosphorus, Non Protein Nitrogen; Lithium; Iron; Urea; Magnesium; Triglycerides-; HDL; Total protein; Albumin; Globulin; Sodium; Potassium; Bicarbonate-; Chloride-; Glucose-; Insulin-; GGT-; CPK; TSH; SGPT; SGOT; LDH; Acid phosphatase; Alkaline phosphatase. Urea; Testosterone; Progesterone; Amylase-; Lipase; Vitamin.

2) The anti-proliferative agent as claimed in claim 1, wherein said composition comprises Inorganic phosphorus-18.2 mgm/dl; Non Protein Nitrogen- 5.2 mgm/dl; Lithium-7.0 mEq/L; Iron 319 μg/dl; Urea- 3,3 mgm/dl; Magnesium 0.97 mgm/dl; Triglycerides-60 mgm/dl; HOL-12 mgm/dl; Total protein-0.44 gm/dl; Albumin-0.39 gm/dl; Globulin-0.05 gm/dl; Sodium 260 mEq/L; Potassium-5.6 mEq/L; Bicarbonate-70 mEq/L; Chloride-141 mEq/L; Glucose-57.14 mgm/dl; Insulin-8.7 - IU/ml; GGT-16.0 U/L; CPK-16 U/L; TSH-0.12 IU/ml; SGPT-22 U/L; SGOT-190 U/L; LDH-24mgm/dl; Acid phosphatase-1.5 KA Units; Alkaline phosphatase- 7,27 KA. Units; Testosterone-1.6 ng/ml; Progesterone-0.372 ng/ml; Amylase-46 IU/L; Lipase-30 IU/L; Vitamin B-12-2150 pg/ml.

3) The anti-proliferative agent as claimed in claim 1which is produced from the whole body (without shell) extract isolated from the marine gastropod mollusc.

4) The anti-proliferative agent as claimed in claim 1which is capable of being used as potent osteogenic inhibitor medicament.

5) A process for preparing the anti-proliferative agent as claimed in claim 1 which comprises;

   a) removal of the inner body mass from the shell of the collected snails were washed with Phosphate buffer saline (PBS) solution (0.15M,pH7.4) and kept in a sterile beaker containing ice cold PBS;

   b) collected body masses were homogenized at 4°C and transferred in a sterilized beaker;

   c) homogenized material was then sonicated at 4°C; Sonication was performed for 40 seconds each time with a pause of 50 seconds at 6μ amplitude for a total period of 60 minutes and sonicated material was collected in a sterile beaker;

   d) Sonicated material was centrifuged at 20,000 rpm at 4°C, the supernatant was collected in a sterilized beaker, filtered through membrane filter and was collected in sterile beaker;

e) collected filtrate named as TdBC was aliquoted equally (2 ml) into the vials and were loaded in freeze drying machine setting the temperature of the machine 10°C below the eutectic point;

f) the primary drying was achieved by increasing the temperature gradually under vacuum and then final drying was done at 15-20°C and were then sealed with aluminum foils and kept in -20°C;

g) The Lyophilized TdBC in each vial was dissolved in 2 ml of double distilled water separately.

6) An anti-proliferative agent substantially as herein before described with particular reference.

7) The process for preparing the anti-proliferative agent substantially as herein before described with particular reference.

Dated this ....21st..... day of ...Feb..., 2014

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No. of Sheets: 06
Sheet No.: 01

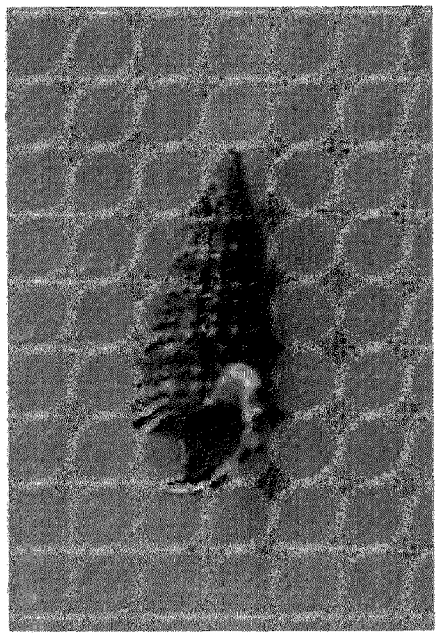

FIG.1: *Terebra dislocata.*

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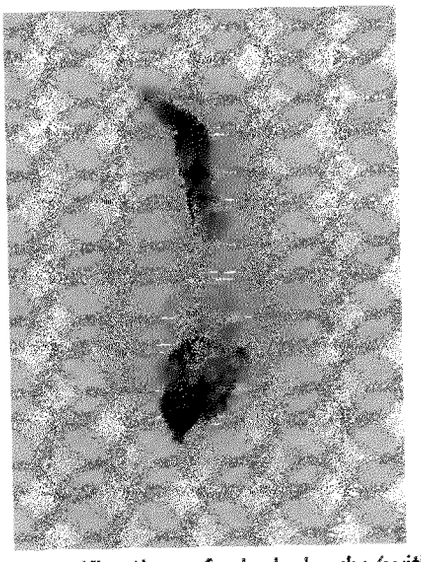

FIG. 2: Identification of whole body (without shell) of *Terebra dislocata*.

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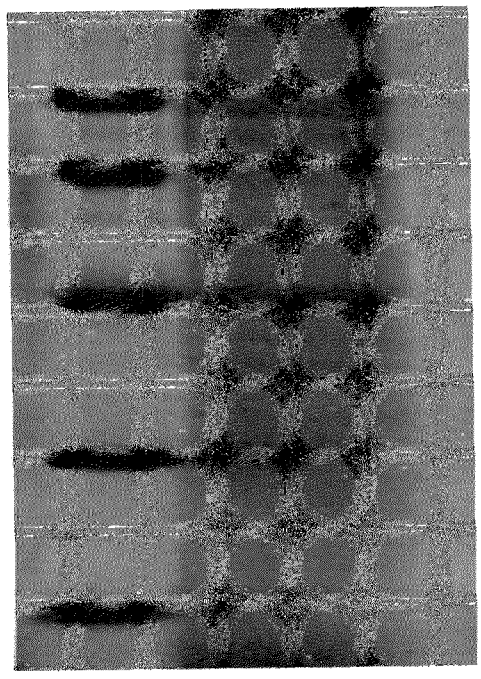

FIG. 3: SDS -PAGE analysis showed that the TDBC possess two (2) major protein bands.

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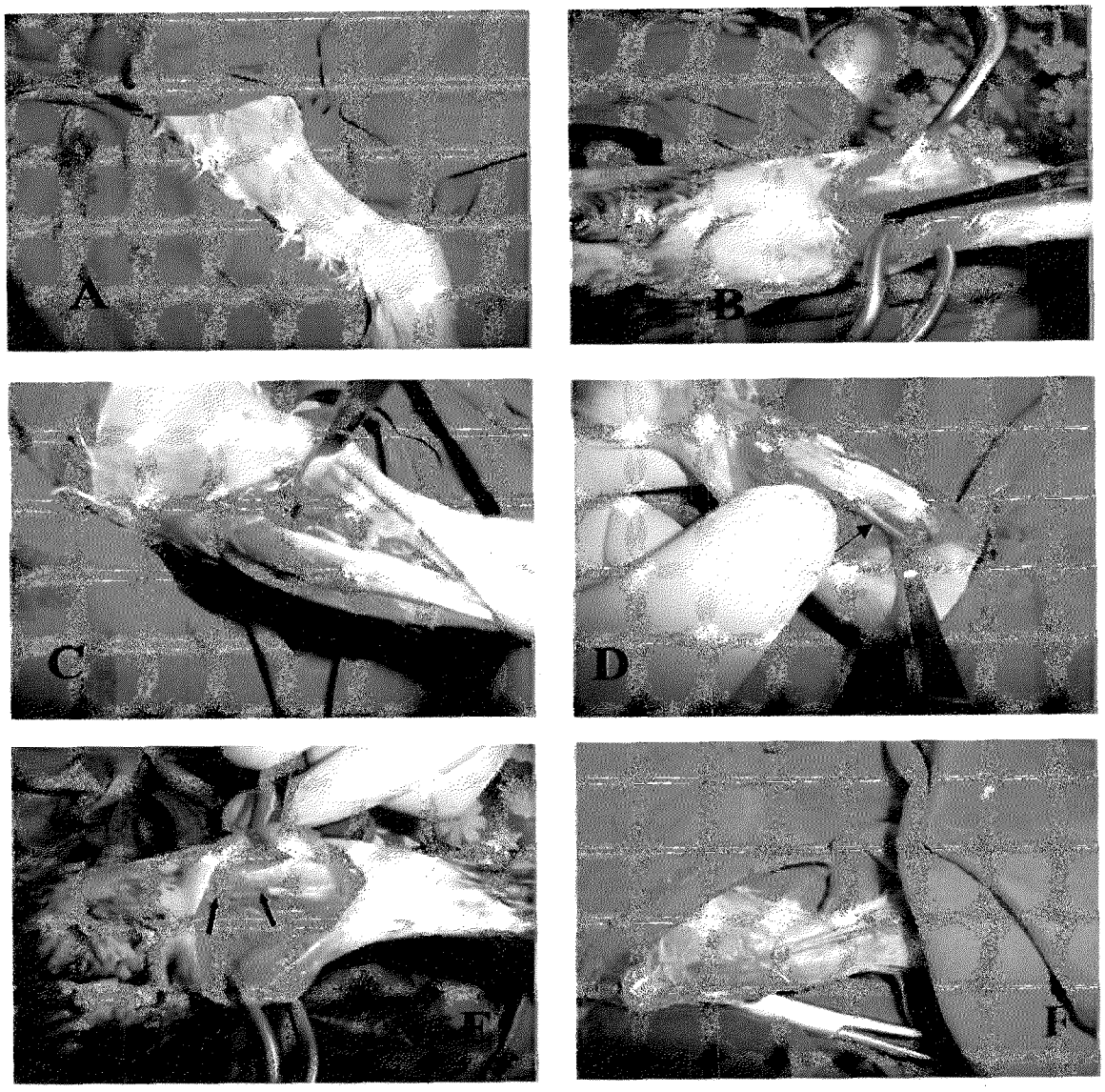

FIG. 4: Operative procedures performed showing the technique of fibular osteotomy. A. surgical preparation of the proposed site of incision. B. separation of subcuticular fascia and tissue. C. Division of muscles to expose the fibula. D. Identification of fibula (arrow marked). E. osteotomy performed (bold arrow). F. closure of wound.

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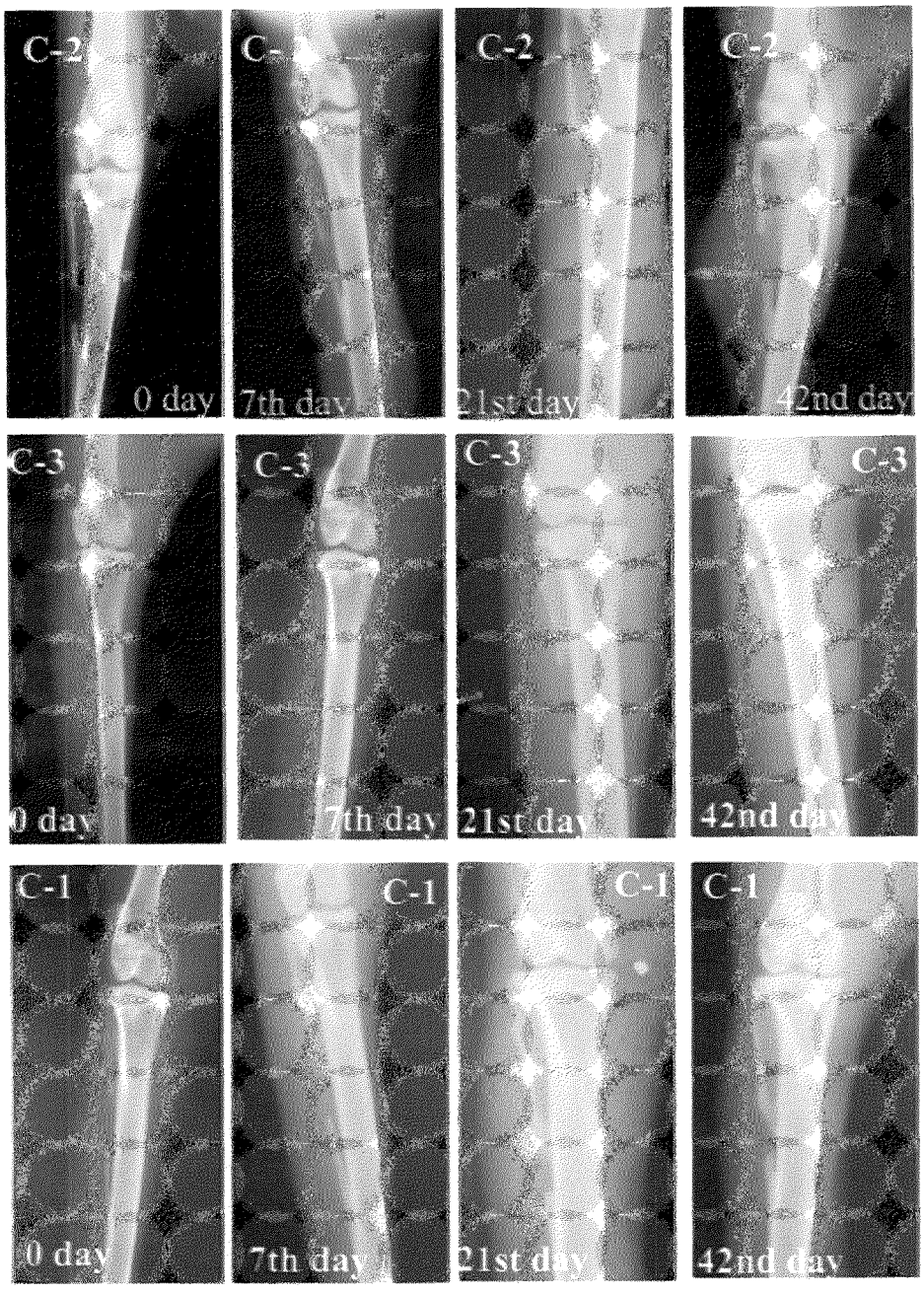

FIG. 5: Radiographs of different days interval of the control and treated groups showing comparative osteo-inhibition effect of treatment with no-treatment.

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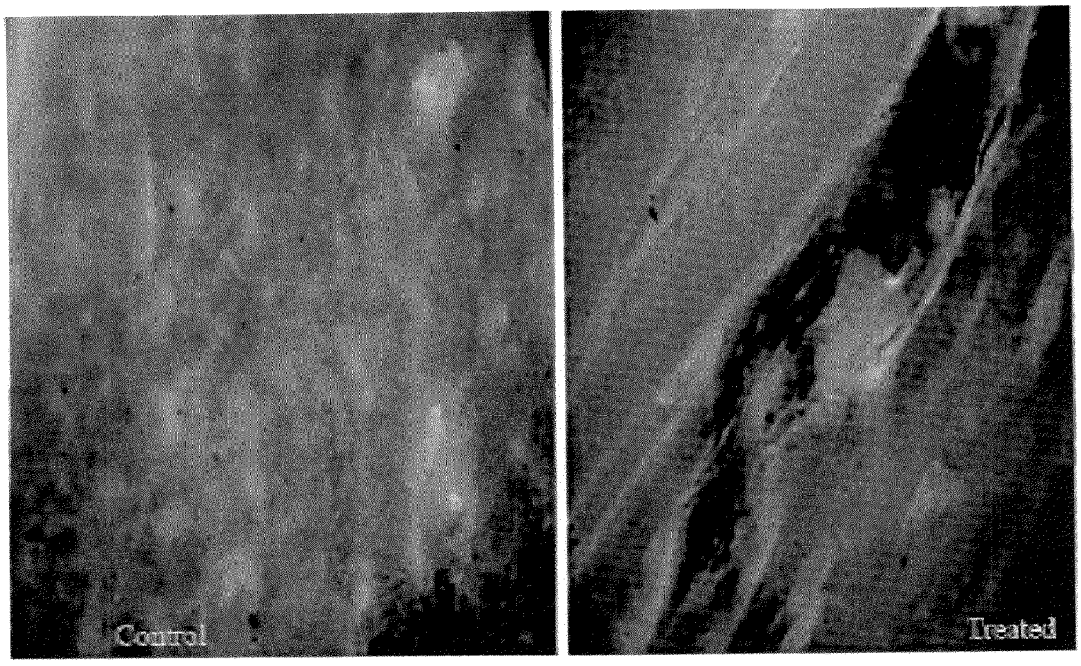

FIG. 6: Fluorochrome labeling study of treated and control groups.

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Field of the Invention

The present invention relates to an anti-proliferative agent which acts as potent osteogenic inhibitor. More particularly, the present invention relates to the anti-proliferative agent which prepared from the extract of the marine gastropod mollusc having therapeutic value. Moreover this invention also relates to the process of preparing the above agent from the whole body extract (without shell) of *Terebra dislocata* (Say, 1822) as *Terebra dislocata* Bioactive Compound (TdBC).

Background of the invention and related prior Art

It is well-understood that bone formation is indicated for treatment of a wide variety of disparate disorders in mammals including simple aging, bone degeneration and osteoporosis, fracture healing, fusion or arthrodesis, osteogenesis imperfecta, etc., as well as for successful installation of various medical orthopedic and periodontal implants such as screws, rods, titanium cage for spinal fusion, hip joints, knee joint, ankle joints, shoulder joints, dental plates and rods, etc.

Many children have abnormalities in bone growth that lead to a variety of conditions such as scoliosis or disproportional long bone growth, such as in the legs or arms. For children suffering from scoliosis, if the curvature of the spine is severe enough, the child will require surgery to correct the curvature of the

spine. Typically the surgery involves the fusion of one or more vertebrae of the spine together to correct the curvature. Spinal surgery however, is complicated, high risk, costly, and requires a long period of convalescence. Correcting disproportionate bone growth in an extremity is typically accomplished by performing an epiphysiodesis (Surdam *et al.*, 2003), which disrupts or destroys a selected growth plate for the purpose of delaying the longitudinal growth of the involved extremity. For example, a child may have a leg length discrepancy of 1 inch, which may be treated by performing an epiphysiodesis of a selected growth plate of the longer leg at a selected age. The epiphysiodesis of the selected growth plate inhibits residual longitudinal growth of the longer leg, and, as the child continues to grow, the continued growth of the shorter leg decreases the discrepancy. Timing of the current techniques is important since typically this ablation is permanent and its performance must be closely linked with the natural termination of growth. Inhibition of ossification is indicated in some clinical situations - that is, the initial early stage of heterotopic ossification, after an operative procedure for the treatment of craniosynostosis, and when there has been post-traumatic partial growth plate arrest. Local formation of bone may be inhibited physically by means of transplantation of autogenous free fat, bone wax, methylmethacrylate, on silicone rubber. However, implantation of non-resorbable foreign material may induce chronic

inflammation, predisposes to infection, and lead to a second operation for removal of the implant (Solheim *et al.*, 1992).

The document FR2922105 states that an active complex obtained from a mixture of a marine active ingredient (i) obtained from *Thermus thermophilus* culture, (ii) extracts of bivalve mollusc shells and (iii) marine sediments, is claimed. - Antimicrobial; Dermatological; Antibacterial; Virucide; Fungicide.

The invention stated in the document FR2880277 describes composition for equids, bovids and other domestic animals, comprises an active ingredient comprising soluble and insoluble biopolymers of marine origin, and mollusc aragonite biocrystals. Independent claims are also included for: (1) an orthopedic composition (I) for treating hair cracking and cracks, comprising calcium hydroxide and adragante gum; (2) a composition (II) for treating hair lesions and skin in horses, comprising excipients, vegetable fat content (obtained from shea tree, corn seed oil, onager oil or Rosa rubiginosa) and a aromatic extract mixture (obtained from *Helychrisum italicum*, *Inula helenium*, *Lavandula latifolia spica, Salvia officinalis* or *Citrus aurantium*); (3) a composition (III) comprising calcium hydroxide, allantoin, copper acetate and P-cresol; (4) a composition (IV) useful for the daily care of hoof and its appendices, comprising the excipients and the aromatic extract; (5) a composition (V) for the treatment of dysfunctions of the principal pad in the

growth of the horn of the hoof in horses, comprising vitamins and a mixture of aromatic extract; (6) a composition (VI) for the recovery and the cooling of the tendons after work in horses, comprising terpenic compounds; (7) a composition (VII) for the treatment of the dry scale and summer dermite comprising excipients and aromatic extracts; (8) a composition (VIII) for the treatment of breakdowns and damage of flexor muscles in horses, comprising components (acting on the muscular or articular pains) and essential oils; and (9) a composition (IX) for treating rotting of hoof plates in horses, comprising various excipients and healing substances. Dermatological; Anti-inflammatory; Muscular-Gen.; Analgesic; Endocrine-Gen.

The other document NZ525243 states the use of a kahalalide F formulation comprising a lyophilised mix of kahalalide F, a non-ionic surfactant, an organic acid and a bulking agent in the manufacture of a medicament for treatment to cancers such as prostate cancer, breast cancer, colon cancer, non-small cell lung cancer, ovarian cancer, for treating neuroblastoma, or against undifferentiated or mesenchymal chondrosarcomas or osteosarcomas.

The other document KR20120058956 states a composition containing HVEM inhibitors is provided to be used as a therapeutic agent for treating bone diseases. A composition for promoting bone differentiation or osteogenesis

contains HVEM (herpesvirus-entry mediator) gene expression inhibitor or HVEM protein activation inhibitor. The HVEM gene contains a base sequence of sequence number 1. The HVEM inhibitor is an antisense oligonucleotide, siRNA, or shRNA. A composition for preventing or treating bone diseases contains the composition as an active ingredient. A method for predicting or diagnosing risk of developing bone diseases comprises: a step of measuring HVEM gene expression level or HVEM protein level in a biological sample; and a step of comparing gene expression level or protein level between the biological sample and a control group.

The enormous biodiversity in the marine environment far exceed that of terrestrial environment and many potent biomedical compounds have been reported recently from marine animals like tunicates, sponges, soft corals, sea hares, nudibrances, bryozoans, sea slugs and from other organisms. It is reported that more than 10,000 important metabolites with novel pharmacodynamic properties have been isolated from marine organisms obtained from shallow water to 900 meters of the sea therefore, is an important and vast arena for getting plenty of unique pharmacologically potent metabolites and resources in living or in dead form. Major sources of biomedical compounds have been found from sponges (37%), Coelenterates

(21%), microorganisms (18%), algae (9%), echinoderms (6%), tunicates (6%), mollusc (2%), bryozoans (1%) etc. (Blunt *et al.*, 2003). The growth and healing of bone is highly regulated process and related to over expression of growth factors for neovascularization. Many of the marine compounds have been reported to have anti-angiogenic properties on different types of cancers (Amador *et al.*, 2003; Broggini *et al.*, 2003; Erba *et al.*, 2001; Pettit *et al.*, 1995, 1998; Lee *et al.*, 2003; Garcia-Rocha *et al.*, 1996 and Reese *et al.*, 1996). Thus, the present study was to investigate the role of whole body extract (without shell) of *Terebra dislocata* in reduction of bone growth in experimental osteotomy model in rabbits.

As stated in the document 36/KOL/2012, the invention provided an effective biomarker of cypermethrin toxicity by utilizing the isolated hemocytes of a characteristic molluscan species *Pila globosa* of India. After an in depth screening on diverse aquatic organisms, hemocytes of *Pila globosa* was invented as a perfect cell population for establishment of biomarker of the toxicity of cypermethrin present in the natural and artificial water bodies. The isolated hemocytes of *Pila globosa* exhibited all of the biological responsiveness and reactivities needed to be qualified for dye retention as suitable biomarker of cypermethrin toxicity in aquatic environment. In recent time, the biomarker approach for screening of environmental toxicity has been

proved as an effective and accurate method and gained a wide range of acceptability among aquaculturists, environmental technologists, environmental engineers and managers and scientists. *Pila globosa* being a common and widely distributed molluscan species of India strengthens and justifies our claim as a suitable source of hemocytes needed for biomarking of cypermethrin toxicity. Hemocytes of no other species excepting *Pila globosa* exhibited a relative dose or concentration dependent sensitivity against cypermethrin - which is an important considerable factor for a biomarker of aquatic pollution. Moreover, the prolonged longitivity of the hemocytes outside the body of *Pila globosa* is a practical advantage of biomarking the toxicity of cypermethrin both in the field and laboratory condition *in vitro*. Entire process of biomarking is relatively, inexpensive, easy, rapid and involves less instrumentational support. Report of biomarker of cypermethrin using hemocytes of *Pila globosa* is absent in current literature and this is a first time claim produced by us.

The document 5347/DELNP/2010 discloses the use of compounds for the control of gastropods. In particular, it relates to a method of controlling gastropods using strobilurin compounds.

The bioactive molecules isolated from the glandular extract of the spermatheca and/or ovotestis from the marine gastropod mollusk, *Telescopium telescopium*

that shows a promising and significant result as potent anticancer agent in experimental tumor bearing mice. The invention also relates to identification of glands of the marine gastropod mollusk, isolation of the bioactive compound(s) from the glandular extract of the spermatheca and/ or ovotestis of the marine gastropod mollusk and partial characterization of the bioactive compound(s) present in the extract herein referred as TBC which has been described in the document 858/KOL/2007.

The applicants still felt a need to improve upon the anti-proliferative agent to achieve enhanced efficacy with more synergic effect and better patient compliance and which is cost effective for the treatment of abnormal bone growth.

Summary of the invention

The present invention relates to an anti-proliferative agent which acts as potent osteogenic inhibitor. More particularly, the present invention relates to the anti-proliferative agent which prepared from the extract of the marine gastropod mollusc having therapeutic value. Moreover this invention also relates to the process of preparing the above agent from the whole body extract (without shell) of *Terebra dislocata* (Say, 1822) as *Terebra dislocata* Bioactive Compound (TdBC).

Detailed description of the invention

For the purpose of facilitating an understanding of the invention, there is illustrated in the preferred embodiment thereof, from an inspection of which, when considered in connection with the following description with accompanying drawings which are in three sheets, the invention, its embodiment, and many of its advantages should be readily understood and appreciated.

The principal object of the invention is to an anti-proliferative agent which acts as potent osteogenic inhibitor.

Another object of the present invention is to provide the anti-proliferative agent which prepared from the extract of the marine gastropod mollusc having therapeutic value.

Still other object of the present invention is to provide the process of preparing the above agent from the whole body extract (without shell) of *Terebra dislocata* (Say, 1822) as *Terebra dislocata* Bioactive Compound (TdBC).

Still other object of the present invention is to provide the anti-proliferative agent which consists of biochemical composition of the compounds in Lyophilized TdBC are: Inorganic phosphorus-18.2 mgm/dl; Non Protein Nitrogen- 5.2 mgm/dl; Lithium-7.0 mEq/L; Urea- 3.3 mgm/dl; Magnesium 0.97 mgm/dl; Triglycerides-60 mgm/dl; HDL-12 mgm/dl; Total protein-0.44

gm/dl; Albumin-0.39 gm/dl; Globulin-0.05 gm/dl; Sodium 260 mEq/L; Potassium-5.6 mEq/L; Bicarbonate-70 mEq/L; Chloride-141 mEq/L; Glucose-57.14 mgm/dl; Insulin-8.7 ~IU/ml; GGT-16.0 U/L; CPK-16 U/L; TSH-0.12 ~IU/ml; SGPT-22 U/L; SGOT-190 U/L; LOH-24 mgm/dl; Acid phosphatase-1.5 KA Units; Alkaline phosphatase- 7,27 KA. Units; Testosterone-1.6 ng/ml; Progesterone-0.372 ng/ml; Amylase-46 IU/L; Lipase-30 IU/L; Vitamin B-12-2150 pg/ml.

Another object of the present invention is to provide the potent antiproliferative/osteo-inhibitor(s) from the whole body (without shell) extract of the marine gastropod mollusc, *Terebra dislocata* to maintain the quality life without any pain and discomfort of different pathological conditions where aberrant osteosynthesis is predominant.

Another object of the present invention is the manufacturing the anti-proliferative agent from which the manufacturing cost is low. However, in altered disease states and severity can be greatly heightened, and dramatically affect one's quality of life.

The novel features that are considered characteristic of the present invention are set forth with particularity in the appended claims. The invention itself, however, both as to its organization and its method of operation, together with

additional objects and advantages thereof, will best be understood from the following description of certain specific embodiments, when read in connection with the accompanying drawings, in which:

FIG.1: *Terebra dislocate* with shell;

FIG. 2: Whole body of *Terebra dislocata.* (Without shell);

FIG. 3: SDS -PAGE analysis showed that TdBC possess 2 major protein bands and 6 minor bands;

FIG. 4: Operative procedures performed showing the technique of fibular osteotomy. A. surgical preparation of the proposed site of incision. B. separation of subcuticular fascia and tissue. C. Division of muscles to expose the fibula. D. Identification of fibula (arrow marked). E. osteotomy performed (bold arrow). F. closure of wound.

FIG. 5: Radiographs of different days interval of the control and treated groups showing comparative osteo-inhibition effect of treatment with no-treatment;

FIG. 6: Fluorochrome labelling study of treated and control groups;

All the ingredients of the anti-proliferative agent of the present invention are well standardized with acceptable impurity profiles. All the ingredients were reported to be the safety and clinical efficacy of the anti-proliferative agent is proved on human with effective in abnormal bone growth and related problems.

The gastropod molluscs are members of the large and diverse phylum Mollusca, which includes a variety of familiar animals well-known for their decorative shells or as seafood. The vast majority of molluscs live in marine environments. However, some of the bivalves and the gastropods are also found to inhabit in freshwater environment or live on land.

The shells of the sea snails in this family are typically shaped like slender augers or screws. In that respect they share certain shell characters with the family Turritellidae, the turret shells. One characteristic that distinguishes Terebridae from Turritellidae is the short anterior canal or notch in the aperture of the shell. Terebridae shells also tend to have characteristically flattened versus convex whorls, and they often have one or two plaits on the columella. Numerous species in this family are grouped under either the Terebra or the Hastula genus, and a minority of species are placed in four other genera.

These snails are sand-dwelling carnivores which live in warmer water. In most species, a venomous barb similar to that of the cone snails is used to stun and immobilize prey, which typically consists of various marine worms. *Terebra dislocata,* measures on average up to 2 1/4 inches in length, with a pointed spire. Colours vary with exterior bands of pale gray, pinkish brown or orange-brown. This species lives in sounds and offshore on shallow sand flats. The shell is commonly found washed up on sound and ocean beaches. The species

is found from Virginia to Brazil and also found plenty in Indian peninsula. The Atlantic auger is a carnivore, but it lacks the radula and poison gland found in most other augers. The snail comes under Kingdom: Animalia, Phylum: Mollusca, Class: Gastropoda, Subclass: Orthogastropoda, Superorder: Caenogastropoda, Order: Sorbeoconcha, Suborder: Hypsogastropoda, Infraorder: Neogastropoda, Superfamily: Conoidea, Family: Terebridae

The process of preparing the anti-proliferative agent which comprising whole inner body mass (Fig-2) of the snail was removed after breaking the shell, washed with phosphate buffer saline (PBS) solution (0.15M, pH 7.4) and kept in a sterile beaker containing ice cold PBS. The separated body mass (without shell) were homogenized together at 4°C in Universal Motor- RQ-127 A and transferred in a sterilized beaker. The homogenized material was then sonicated at 4°C in Ultrasonic Generator G04004-2 US-50. Sonication was performed for 40 seconds each time with a pause of 50 seconds at 6 IJ amplitude for a total period of 60 minutes. The sonicated material was collected in sterile test tube and centrifuged at 20, 000 rpm at 4^0C for 30 minutes. The supernatant was aspirated and kept in a sterilized beaker, 'filtered through membrane filter and collected in a sterile beaker. The collected filtrate named as **TdBC** was aliquoted equally (2 ml) into sterilized glass vials and lyophilized at 10' below the eutectic point. Primary drying was achieved by increasing the temperature

gradually under vacuum. Final drying was done at 15-20'C. The processed vials were then sealed with aluminium foils and kept in -20°C till further experimentation.

For biochemical analysis, lyophilized TdBC in each vial was dissolved in 2 ml of double distilled water separately and the results so far obtained have given in Table-1

Table 1: Biochemical composition of the anti-proliferative agent of the instant invention is as follows:

Sl No	Composition	Concentration
1	Inorganic phosphorous	18.2 mgm/dl
2	Iron	319µg/dl
3	magnesium	0.97 mgm/dl
4	Non protein nitrogen	5.2 mgm/dl
5	Lithium	7.0 mEq/L
6	Urea	3.33 mgm/dl
7	Lipase	30 IU/L
8	VitamineB12	2150 pg/ml
9	Triglycerides	60 mgm/dl
10	HDL	12 mgm/dl
11	Total Protein	0.44 gm/dl
12	Albumin	0.39 gm/dl
13	Globulin	0.05 gm/dl
14	Sodium	260 mEq/L
15	Potassium	5.6 mEq/L
16	Bicarbonate	70 mEq/L
17	Chloride	141 mEq/L

18	Glucose	57.14 mgm/dl
19	Insulin	8.7 μIU/ml
20	GGT	16 U/L
21	CPK	16U/L
22	TSH	0.12 μIU/ml
23	SGPT	22U/L
24	SGOT	190U/L
25	LDH	24 mgm/dl
26	Acid phosphate	1.5 KA. Unit
27	Alkaline Phosphate	7.27 KA. Unit
28	Testosterone	1.6 ng/ml
29	Progesterone	0.372 ng/ml
30	Amylase	46 IU/L

SDS -PAGE analysis as shown in Fig. 3 represents a way of biochemical characterization stating number of proteins contains in the compound and furthermore one or combination of major/minor proteins or altogether may contribute the major role in antiproliferative/osteo-inhibitor property.

Clinical trial in rabbit osteotomy model, following doses of TdBC was injected subcutaneously.

Group-A 1- control (without treatment)

Group- A 2-1 mg/kg for 2 alternative days.

Group- A3 -1mg/kg single dose.

Group-B 1- control (without treatment)

Group- B 2- 2 mg/kg for 2 alternative days.

Group- B 3- 2 mg/kg single dose.

Group-C 1- control (without treatment)

Group- C 2-3 mg/kg for 2 alternative days.

Group-C 3- 3 mg/kg single dose.

Group-D 1- control (without treatment)

Group-D 2- 5 mg/kg for 2 alternative days.

Group-D 3- 5 mg/ kg single dose.

In the animals of the respective control group only osteotomy was done but no treatment with the bioactive compound was considered. These control animals received respective amount of normal saline only. The extract was injected at the rate of 3mg/kg body weight single dose in two groups of rabbit that were already undergone osteotomy in fibula. In Group C-3, the compound was injected in single dose as per design and found that radiographically osteoblastic activity and formation of callus is evident only from 21st day of treatment which continues beyond 42no day and the healing of osteotomy wound is still under process but (Figure 5).

When the compound was injected in 3mg/kg body weight in twice dose as per schedule (group C-2), it showed remarkable changes on bone healing. Radiographically the signs and appearance of osteotomy wound between different days interval almost remain same and evinced with no healing. The evidence of bridging callus was completely absent from the proximal and distal

margin of the osteotomy wound. Loss of sharpness of both the ends of wounds as early signs of healing was also not found. The compound not only resulted non-healing of the osteotomy wound but also imposed a stop-signalling to healing mechanism (Figure 5). On the contrary, in control group (group C-1), healing process was as per normal schedule as evinced from radiographic callus, shortening of wound gap and above all radiographic union of the wound. The osteoblastic activity started on 7th day and by 42nd most the callus formation was complete so as to bridge the osteotomy wound (Figure5).

Oxytetracycline @ 50mg/kg body wt was injected intramuscularly on days 30, 31 and later after 6 days interval on days 37 and 38 (2-6-2) postoperatively for double toning of the new bone which was found Sufficient to label the newly formed osteoid tissue. Oxytetracycline labelled new bone emitted greenish yellow florescence when observed under UV light, whereas, matured old bone appeared dark sea green.

In treatment group, the process of new bone formation was less active in the healed area a~ evidenced from lesser golden yellow fluorescence. However, mostly the healed site appeared as homogenous non-fluorescent is as evidenced by presence of sea green appearance (Fig. 6).

On the other hand, in the control group the process of new bone formation was more active as evidenced by presence of golden yellow fluorescent. The golden

yellow fluorescent is present in the central area indicative of new osseous tissue formation and presence of dark sea green fluorescence at the periphery indicating host bone (Fig. 6). Toxic effect is one of the limitations of currently available synthetic drugs. So we investigated toxicity of TdBC, if any, in the experimental animals. Since 3-mg/Kg body weight multiple dose is the most effective dose of TdBC as observed, toxicological effect(s) was/were evaluated and the result shows no significant toxicological consequences. The compound inhibited the osteoblastic proliferation/ favoured the osteoclastic activity as evident from in vitro (cell culture) experiments.

In vivo (when drug was administered in rabbit with fibular osteotomy) studies revealed that the drug can also act indirectly by inhibiting the neovascularization /suppressing revascularization so as to prevent the proliferation of osteoblast and arrest the bone growth.

The following examples, which include preferred embodiments, will serve to illustrate the practice of this invention, it being understood that the particulars shown are by way of example and for purpose of illustrative discussion of preferred embodiments of the invention. Although the present invention has been described hereinabove by way of preferred embodiments thereof, it can be modified without departing from the spirit and nature of the subject invention as defined in the appended claims.