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Introduction

Journal of Pharma Innovative Research (JPIR) is intended primarily to promote prompt publication (original research articles and review articles) in all fields of pharmaceutical science. Specific scientific topics of interest to the journal include: Pharmaceutics, Pharmaceutical Chemistry, Industrial Pharmacy, Pharmacology, Pharmacognosy, Phytochemistry, Pharmacodynamics, Pharmacokinetics, Pharmacogenomics, Biopharmaceutics, Physical Pharmacy, Drug Design, Pharmaceutical Analysis, Drug Stability, Quality Control and Assurance, Pharmaceutical Engineering, Hospital and Clinical Pharmacy.

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INVESTIGATION OF HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *AMARANTHUS DUBIUS* LEAVES, BY *IN VIVO* AND *IN SILICO* METHODS

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Abstract

Background: Hepatoprotective activities of medicinal plants is important for researchers and clinicians. The herbal treatment provides the advantages of wide spread distribution, cost effectiveness, and improvement in patient compliance through lesser adverse effects, and drug-interactions. Amaranthus species is distributed all over the world, growing under variety of climatic conditions, and traditionally used for inflammation, ulcer, diabetes, asthma, and hyperlipidemia. *Amaranthus spinosus* and *tricolor* had been previously studied for their hepatoprotective effects. With this background, the current study is designed to evaluate the hepatoprotective potential of *Amaranthus dubius* leaf extract. **Methods:** In CCl4 induced hepatotoxicity in mice the in vivo study was designed, pretreatment with the ethanolic extracts of *A.dubius*, for seven days followed by hepatic injury. The protective effects were evaluated through changes in body weight, feed and water consumption, and histopathological changes in liver. The *in silico* studies, the molecular mechanism of phytoconstituents were studied with the macromolecule 1ILH, by AutodockVina. **Results:** The *in vivo* study, treatment with the A.dubius improved the body weight, feed and water consumption, and liver damage was found to be reduced, which proves the hepatoprotective activities of the ethanolic extract. In the *in silico* docking studies, the Stigmastane derivatives phytoconstituents, was found to have best docking scores. **Conclusion:** The hepatoprotective potential of the Amaranthus dubius was proved in the mice model of hepatic injury and the Phytoconstituent, Stigmastane derivative bound to the Pregnane X Receptor (PXR), which could be responsible for this therapeutic activity.

Keywords: Amaranthus, Hepatoprotective, CCl4, Mice, Docking, Pregnane

Introduction

Liver is an important organ where blood coagulation factors, fibrinogen, and heparin are formed and stores sugar as glycogen. Liver diseases are common, caused by intoxication with alcohol, viral hepatitis, aflatoxins, and numerous drugs cause hepatotoxicity. Liver diseases are critical and extend the length of hospital stay. Majority of the healthcare professionals rely on indigenous herbal medications for treatment of liver disorders. In different countries various medicinal plants are explored for addressing the liver toxicity [1]. Studying the potential use of valuable herbals, the respective phytoconstituents and assessment of their safety plays an important role in traditional herbal research. With the advent of modern tools, such as computer aided drug discovery, the role of phytoconstituents could be studied at the molecular level,

which adds value to preclinical research. With this background, the current study was designed to carry out pharmacological research to study the effectiveness of *Amaranthus dubius*, and investigate the phytochemical *in silico* research to explore its mechanism of action [2, 3].

Amaranthus genus has different plants such as *Amaranthus hybr*, *Amaranthus hypochondriacus*, *Amaranthus spinosus*, and *Amaranthus dubius*, having geographical distribution in India, Kenya, Indonesia, Thailand, and Laos. In traditional system of medicine, it is used as antidote, astringent, diaphoretic, emmenagogue, emollient, and febrifuge [4,5]. In the current study, we investigated the hepatoprotective potential of *Amaranthus dubius* in carbon tetrachloride (CCl4) induced hepatotoxicity in mice, and the phytoconstitutents were taken for the *in-silico* docking study.

Materials and methods

Plant material collection and identification

The leaves of *Amaranthus dubius* was collected in and around Gerugambakkam, Chennai, and the leaf material was taxonomically identified and authenticated by Prof.Jayaraman, Head of Botany, Mudichur, Tambaram, Chennai.

Extraction method

The leaves of *Amaranthus dubius* were shade dried at room temperature, pulverized, and 100g of coarse powder was defatted with petroleum ether (40-60°C) in a Soxhlet extractor. The above defatted powdered leaf material was successfully extracted with petroleum ether, chloroform, ethyl acetate, and ethanol in increasing order of polarity. The extracts were concentrated under reduced pressure using rotary flash evaporator and the residues were dried in desiccator over sodium sulfite.

Preparation of extract for administration

0.1g of ethanolic extract of the plant was suspended in 0.006g Carboxy methyl cellulose (CMC) dissolved in 10ml warm water to get uniform suspension.

Experimental animals and Ethical considerations

A total of 18 Swiss albino mice, of both sexes, weighing 20-25g were obtained from the Animal house, K. K. College of Pharmacy. The mice were maintained under standard condition (12:12 hours light/dark cycle and at ambient temperature of 25 ± 2°C, with 65 ± 5% humidity) for 7 days as adaptation period. They were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), in the Department of Pharmacology, K.K. College of Pharmacy, Chennai (IAEC approval number – KKCP/2019/05).

Experimental design and protocol : Animals were randomly divided into 3 groups each containing six mice.

Group I was served as control in and received saline daily (3 served as negative control after induction of liver toxicity)

Group II received 100mg/kg of Silymarin

Group III received 200mg/kg of ethanolic extract of *Amaranthus dubius*.

All the treatments were given for seven days.

Induction of hepatotoxicity

A single dose of CCl4-olive oil solution (1:1 v/v) at 2ml/kg was intraperitoneally injected into each of the mice to induce liver injury, when the mice in the control group received dose of olive oil. After 24 hours all the mice were euthanized.

Biological evaluation

The body weight, feed consumption, water consumption was measured on all the days and also after induction of hepatotoxicity. The liver was dissected out after 24 hours of induction of hepatotoxicity and subjected to histopathological analysis.

Molecular docking

Ligands and Protein preparation

The ligands of Amaranthus were collected from the Collective Molecular Activities of Useful Plants (CMAUP). The ligands were downloaded in PDB formats, then converted to PDBQT format to be identified by AutoDockVina using Open babel [6].

The crystallographic structure Human Pregnane X receptor bound to SR12813 was retrieved from protein data bank as a PDB three-dimensional structure file (PDB ID: IILH) with a resolution [Å]: 2.76, with a R-value Free: 0.282, R-value work: 0.222 deposited by Watkins et al, on 2001-05-2008, and released on 2001-06-27.

Docking protocol

The complexed ligands were extracted from the initial X-ray crystal structure followed by removal of water molecules. Polar hydrogens and Gastieger charges were added using the Autodock tools. The compounds were drawn using Chem Draw Ultra 8.0 software and were optimized for energy and geometry using MMFF94 force field. Grid boxes were established to cover the active sites of the macromolecule under study, with a spacing of 1.0 Å between the grid points. The exhaustiveness and the number of poses were set to 12 and 10 respectively. The docking results were visualized utilizing Discovery Studio 2020 Client (Biovia, San Diego, USA)

Statistical analysis method

The data were exhibited as mean ± S.E.M for six mice. ANOVA test was followed by individual comparison by Posthoc-Bornferroni test using Graph pad prism (version 5.0) (GraphPad software, Inc., USA) for the estimation of level of significance. The values of $p<0.05$ was considered statistically significant.

Results

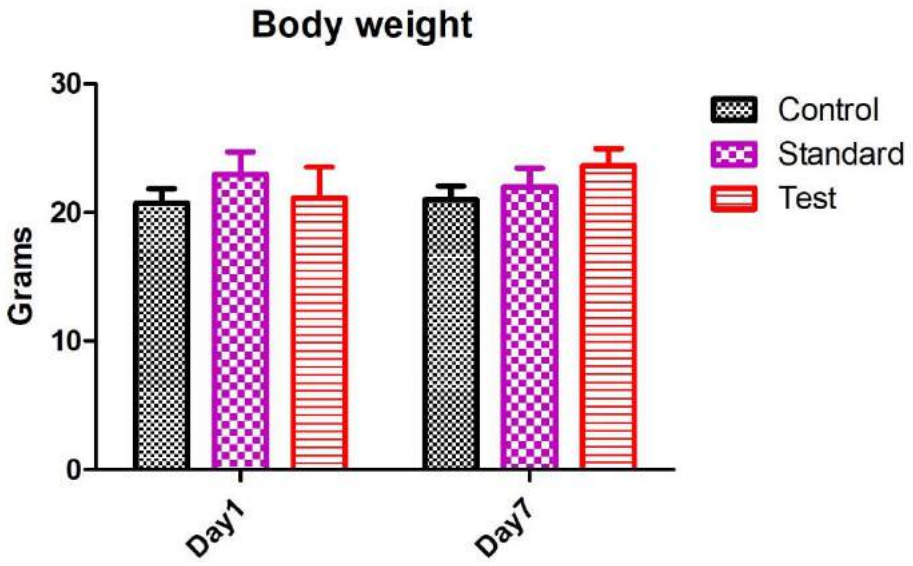


Figure 1: Body weight changes at baseline and after induction of liver toxicity – Control (Vehicle), Standard (Silymarin 100mg/kg), Test (Ethanolic extract of *Amaranthus dubius* 200mg/kg)

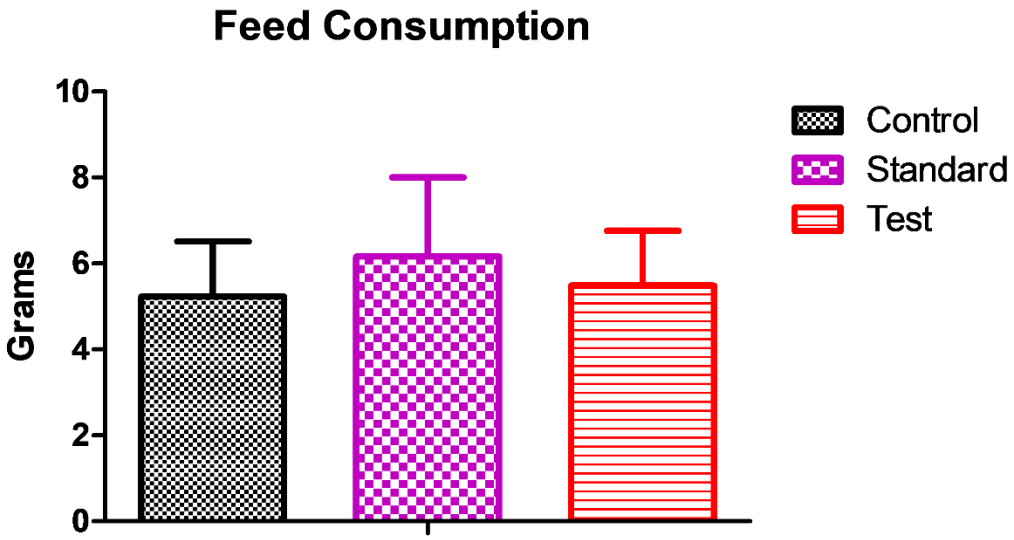


Figure 2: Changes in Feed consumption at baseline and after induction of liver toxicity – Control (Vehicle), Standard (Silymarin 100mg/kg), Test (Ethanolic extract of *Amaranthus dubius* 200mg/kg)

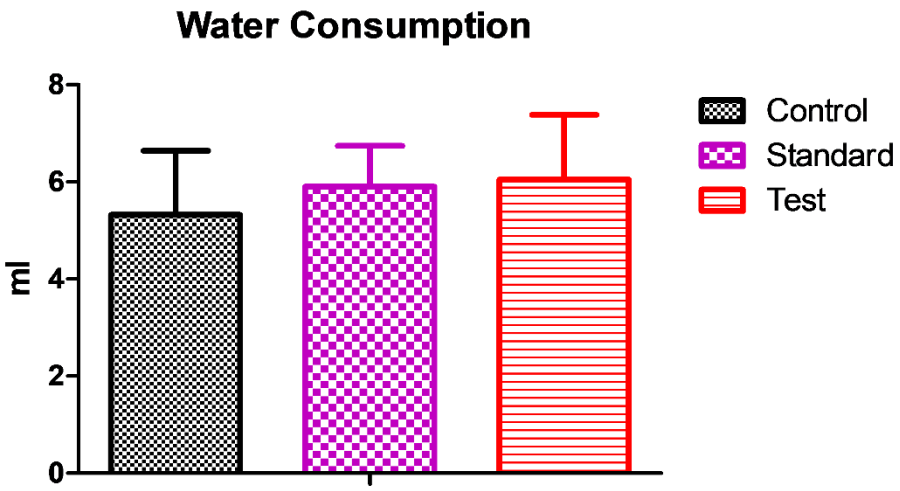


Figure 3: Changes in water consumption at baseline and after induction of liver toxicity – Control (Vehicle), Standard (Silymarin 100mg/kg), Test (Ethanolic extract of *Amaranthus dubius* 200mg/kg)

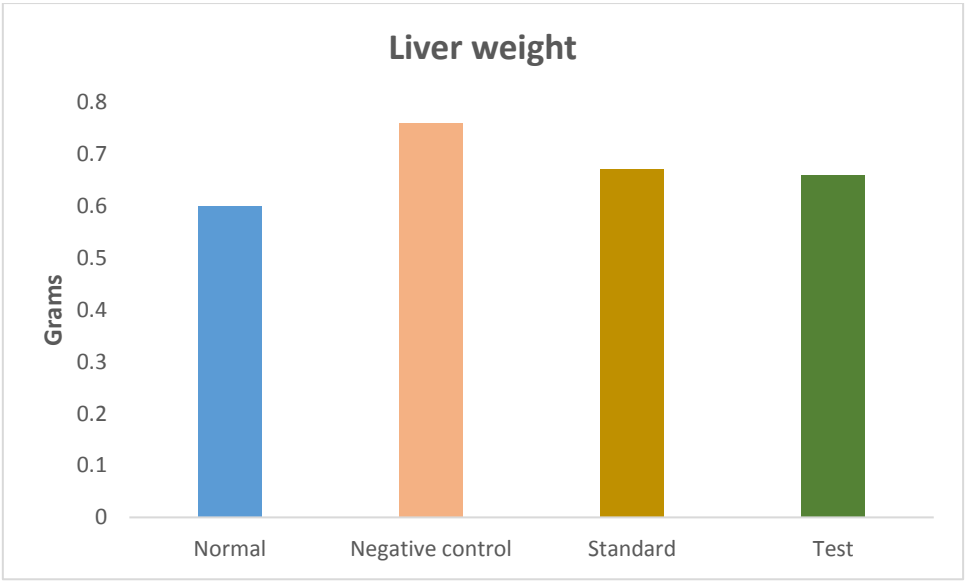
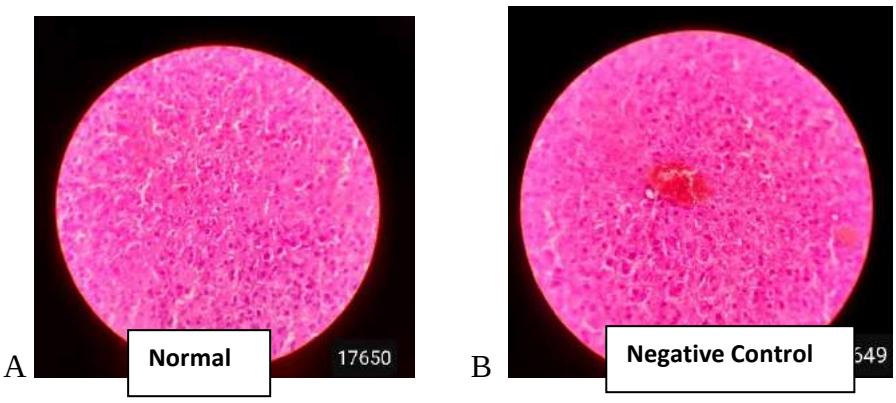


Figure 4: Liver weight in the treatment groups – Control (Vehicle), Negative Control (No treatment), Standard (Silymarin 100mg/kg), Test (Ethanolic extract of *Amaranthus dubius* 200mg/kg)



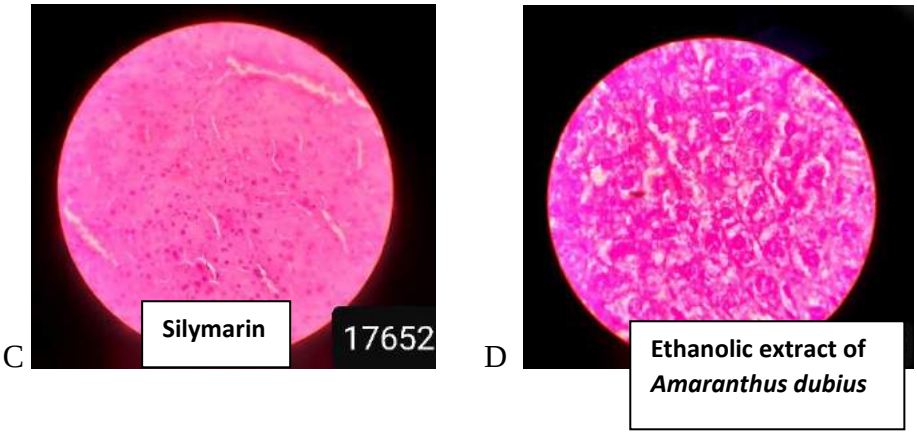


Figure 5: Histopathological changes – Control (Vehicle), Negative Control (No treatment), Standard (Silymarin 100mg/kg), Test (Ethanolic extract of *Amaranthus dubius* 200mg/kg)

A. Normal liver with no congestion of blood vessels. B. Marked disrupt or liver architecture, Severe and diffuse areas of necrosis predominantly in the centrilobular areas with compressed sinusoidal spaces and congestion C. Compressed sinusoidal spaces with congestion D. Liver architecture normal with slight congestion of blood vessels.

Molecular docking

In current study phytochemical constituents of Amaranthus species were taken, which are, S-2,6- diaminohexanoic acid, hentriacontane, Stigmastane derivative, amaranthin betacyanin, betaine, and isoamaranthin were analyzed.

Stigmastane derivative have shown stronger binding at the receptor's binding site (-9.8 kcal/mol) when analyzed using AutoDockVina

Table 1: The Docking and binding energy of selected phytoconstituents (ranked by kcal/mol)

Ligand	Ligand ID	Binding energy (kcal/mol)
Stigmastane derivative	CID_521229	-9.8
Isoamaranthin	CID_102154177	-6.7
Hextriacontane	CID_12410	-5.9
S-2,6- diaminohexanoic acid	CID_5460926	-4.6
Betaine	CID_247	-3.5
Amaranthin betacyanin	CID_112500418	-3.2

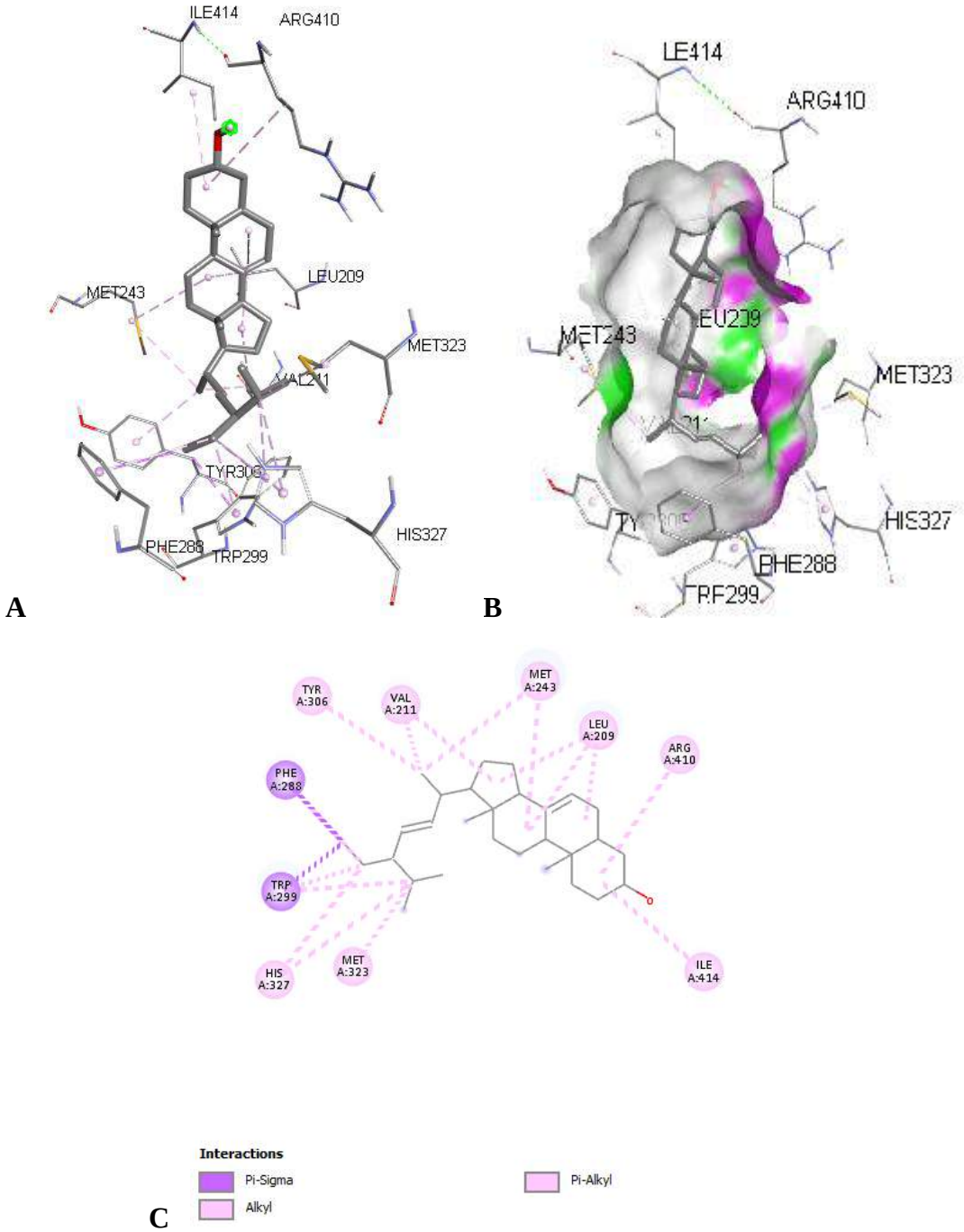


Figure 6: Binding modes and the corresponding interactions for the phytoconstituents of *Amaranthus dubius*

A. Overlay of best-docked 3D poses of Stigmastane derivative with the human pregnane X receptor (PDB-code 1ILH). B. Stigmastane derivative 3D pose with its binding in the receptor pocket of the macromolecule. C. 2D interactions of the binding pocket with the corresponding Amino acid interactions within the binding pocket of the macromolecule

Discussion

The hepatotoxic agents disturb the normal physiology of liver and lead to deleterious effects, and chronically it leads irreversible liver damage. The ability of herbal medications in this

context, had been proven in different studies [7, 8, 9]. In previous studies the hepatoprotective potential of *Amaranthus spinosus* whole plant and *Amaranthus tricolor* roots extract was evaluated in CCl4 induced hepatotoxicity in rats. In the current study, the ethanolic extract of *A.dubius*, was evaluated in the same model, in mice. To our knowledge, this is the first study in this plant, and we tried to correlate its mechanism through in silico studies. It is suggested that the hepatic injury created by this model is similar to acute viral hepatitis. The hepatotoxicity is induced by the hepatotoxins released by the inducing agent, that alkylates different proteins in the liver tissue, causing impairment of liver functions. It causes hepatocellular necrosis and release of stored enzyme, which act as biomarkers to measure its toxicity.

Previous study reports in *Amaranthus spinosus* implicated the dose dependent hepatoprotective activity through reduction of oxidative stress, but the role of phytoconstituents was not studied. In the present study, the body weight, water consumption and feed consumption were slightly increased in the treatment groups when compared to the normal control. In the liver weight was increased in the negative control mice, which proves the liver damage. The reduction in the liver weight when compared to the negative control, proves the hepatoprotective potential of the herbal treatment. From the results, it is clear that the treatment with *A.dubius*, alleviates the liver injury. The histopathological changes revealed the normal architecture was disrupted in the negative control group, whereas the it was improved to a greater extent in the *A.dubius* treatment, which is a proof of evidence, for its hepatoprotective activity.

The Pregnane X Receptor (PXR) is a member of nuclear receptor, involved in herb-drug interactions, through gene expression [10, 11, 12]. The molecule formed Pi sigma interactions with TRP299, and Pi alkyl interactions with MET243, PHE288, and VAL211. From the Ligplot, it was found that all the interaction aminoacids were found in the receptor pocket. Other interactions such as with LEU209, TRP306, MET323, ARG410, and ILE414 were out of the binding pocket.

Conclusion

The hepatotoxic model in mice was found to be alleviated by pretreatment with ethanolic extract of A.dubius leaves. Molecular docking study provides virtual screening approach to filter large molecular databases and predict the binding of strongest ligands. This study has provided the information that Stigmastane derivatives in A.dubius possess the hepatoprotective potential, by binding to PXR receptor pocket.

Limitations

The dose-dependent effect the *A.dubius* was not studied and the measurement of liver enzymes was not done. The antioxidant profile the liver homogenate was not performed, which could be studied further for justifying its hepatoprotective potential.

Acknowledgment

We would like to thank Dr. A.Meena, Principal, Dr. A.Shanthi, Vice-Principal, Dr. K.Senthilkumaran, Dean and Dr. V.Vaidhyalingam, Director, K.K.College of Pharmacy for motivating us to perform this research work.

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EVALUATION OF SIDDHA POLYHERBAL FORMULATION FOR THE MANAGEMENT OF WOUND HEALING IN MICE WITH DOCKING STUDIES TO EXPLORE THE MECHANISM

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2, 3, 4 – B.Pharm Final year, K.K.College of Pharmacy, Chennai, Tamil Nadu
Corresponding Author E-mail: cologyprem@gmail.com

Abstract

Background: Traditional medicine in wound healing is important due to the numerous phytoconstituents contributing to cell proliferation, angiogenesis, epithelization, cell adhesion and reduce the inflammation of wound healing. The polyherbal formulation in excision wound model with in silico studies to explore the mechanism, is less studied. **Methods:** This study investigates the role of polyherbal formulation in excision wound model in mice. Animals were divided into vehicle, povidone-iodine group, and polyherbal formulation treatment. Wound contraction, epithelization, and the recovery duration were noted. Docking studies were performed with GSK-3β and the phytoconstitutents of *Azadirachta indica*, *Andrographis paniculata*, *Curcuma longa*, and *Ricinus communis*, used in the polyherbal formulation. .**Results:** The wound healing was found to be faster in the polyherbal formulation treatment when compared to the negative control group, but standard treatment was found to be more effective. The docking studies revealed that dehydroandrographolide possess strong binding affinity to the GSK-3β, which promotes wound healing. **Conclusion:** Polyherbal formulation could be useful in promoting wound healing through inhibition of the key enzymes participating in wound pathology.

Keywords: Polyherbal, Azadirachta, Andrographis, Curcuma, Ricinus, GSK-3β

Introduction

Physical trauma of skin, torn, burn, or punctured is called a wound. In clinical practice, wound often causes discomfort. Wound healing occurs in three stages: inflammation, proliferation, and remodeling. Formation of extracellular tissue by collagen, strengthens the wound. Wound healing is a complex biological process influenced by numerous factors. Depending on the rapidity, the wound takes few days to several weeks to cure. A number of agents are investigated for the wound healing properties. Traditional medicines are used for comprehensive medical care, from the time immemorial [1]. In India, herbal medicines are widely used for the management of wound healing. *Azadirachta indica* is a member of Meliaceae family, a well-known neem tree, closer to human civilization from the vedic period and is used for variety of ailments like analgesic, curative of fever, twig in cough, asthma, piles, tumor, intestinal worms, urinary disorder, leaf in leprosy, epistaxis, intestinal worms, cancer, eye disorders, ulcers, scabies, and skin diseases. It has various chemical constituents such as flavonoids, terpenoids, glycosides, limonoids, fatty acids, and steroids [2, 3, 4, 5]. *Andrographis paniculata* is a member of acanthaceae family, is an important medicine, widely used in

Southeast Asian countries. Andrographolide is the key diterpene lactone, has multiple pharmacological properties, such as anti-inflammatory, anti-allergic, anti-platelet aggregator, anti-cancer, anti-HIV, and hepatoprotective [6]. Turmeric, called as *Curcuma longa*, contains curcumin, is a popular Indian spice has been used for variety of ailments such as diabetic ulcers, anorexia, rheumatism, cough, anorexia, and sinusitis. Curcumin has anti-inflammatory, anti-oxidant, anticarcinogenic, anti-infective, and has wound healing properties [7]. *Ricinus communis* is an important medicinal plant belonging to Euphorbiaceae, has flavonoids, phenolic compounds, fatty acids, phytosterol, and terpenoids. It has been reported to possess analgesic, antidiabetic, antifertility, anti-inflammatory, antimicrobial, hepatoprotective, antioxidant, and wound-healing properties [8]. It has been suggested that Wnt/b-catenin pathway enhances wound healing by inhibiting glycogen synthase kinase-3β (GSK-3β) protein, an important regulatory protein. Wnts constitute a family of glycoproteins which are secreted with distinct mechanism which controls cell differentiation, cell growth, and cell death. The genes coding the Wnts are expressed during regeneration of the skin [9]. In in silico studies, many phytocontstituents had been studied in this enzyme. In this paper we have made an attempt to screen the wound healing property of the polyherbal formulation of *Azadirachta indica*, *Andrographis paniculata*, *Curcuma longa*, and *Ricinus communis* in vivo on Swiss albino mice employing excision, and the mode of action was hypothesized in silico by docking the phytoconstituents to GSK3β protein, an important regulatory enzyme whose inhibition promotes wound healing through β-catenin dependent Wnt signaling pathway.

Materials and methods

Plant material collection and identification

The Plants *Andrographis paniculata* leaves, *Ricinus communis* seeds, *Curcuma longa* rhizomes and *Azadirachta indica* leaves were collected in and around Chennai, and was taxonomically identified and authenticated by Prof.Jayaraman, Head of Botany, Mudichur, Tambaram, Chennai.

Extraction of the plant material: The leaves of *Azadirachta indica*, the aerial parts of *Andrographis paniculata*, Rhizomes of *Curcuma longa*, and Seeds of *Ricinus communis* were shade dried and grounded to fine powder. Accurately weighted 500g of the plant material was macerated separately with water, ethanol, and ethanol-water (1:1 v/v) for 72 hours. All the extracts were filtered, concentrated in vacuo.

Preparation of ointment: The test samples were incorporated in to an ointment base (vehicle) composed of white wax and white petrolatum (5%), with a drug concentration of 5%, and homogenized

Experimental animals and Ethical considerations

A total of 18 Swiss albino mice, of both sexes, weighing 20-25g were obtained from the Animal house, K. K. College of Pharmacy. The mice were maintained under standard condition (12:12 hours light/dark cycle and at ambient temperature of 25 ± 2°C, with 65 ± 5% humidity) for 7 days as adaptation period. They were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), in the Department of Pharmacology, K.K. College of Pharmacy, Chennai (IAEC approval number – KKCP/2019/01).

Experimental design and protocol

Animals were randomly divided into 3 groups each containing six mice.

Group I was served as control

Group II received Povidone-Iodine ointment 10% cream

Group III received Polyherbal Siddha formulation ointment

All the treatments were given for seven days.

Excision wound model

The mice were anesthetized by administering pentobarbitone (90mg/kg b.w i.p). A full thickness of the excision wound of circular area (approx. 300mm^2) and 2mm depth was made on the shaved back of the mice, 10 min later the anesthesia. The wounding day was considered as day zero. The wounds were treated with the corresponding topical applications and the measurements were at day 5, 10 and 15. The period of epithelization was calculated as the number of days required for the formation of it.

Biological evaluation

The wound diameters were measured in millimeter at day0, day5, day10 and day15. The measurements were taken for analysis of wound healing. The corresponding photographs were taken for visual epithelization.

Molecular docking

Ligands and Protein preparation

The ligands of Amaranthus were collected from the Collective Molecular Activities of Useful Plants (CMAUP). The ligands were downloaded in PDB formats, then converted to PDBQT format to be identified by AutoDockVina using Open babel [10].

The Glycogen synthase 3 complexed with a inhibitor was retrieved from protein data bank as a PDB three-dimensional structure file (PDB ID: IQ5K) with a resolution [Å]: 1.94, with a R-value Free: 0.242, R-value work: 0.222 deposited by Bhat et al, on 2003-08-2008, and released on 2004-08-10.

Docking protocol

The complexed ligands were extracted from the initial X-ray crystal structure followed by removal of water molecules. Polar hydrogens and Gastieger charges were added using the Autodock tools. The compounds were drawn using Chem Draw Ultra 8.0 software and were optimized for energy and geometry using MMFF94 force field. Grid boxes were established to cover the active sites of the macromolecule under study, with a spacing of 1.0 Å between the grid points. The exhaustiveness and the number of poses were set to 12 and 10 respectively. The docking results were visualized utilizing Discovery Studio 2020 Client (Biovia, San Diego, USA)

Statistical analysis method

The data were exhibited as mean ± S.E.M for six mice. ANOVA test was followed by individual comparison by Posthoc-Bornferroni test using Graph pad prism (version 5.0) (GraphPad software, Inc., USA) for the estimation of level of significance. The values of $p<0.05$ was considered statistically significant.

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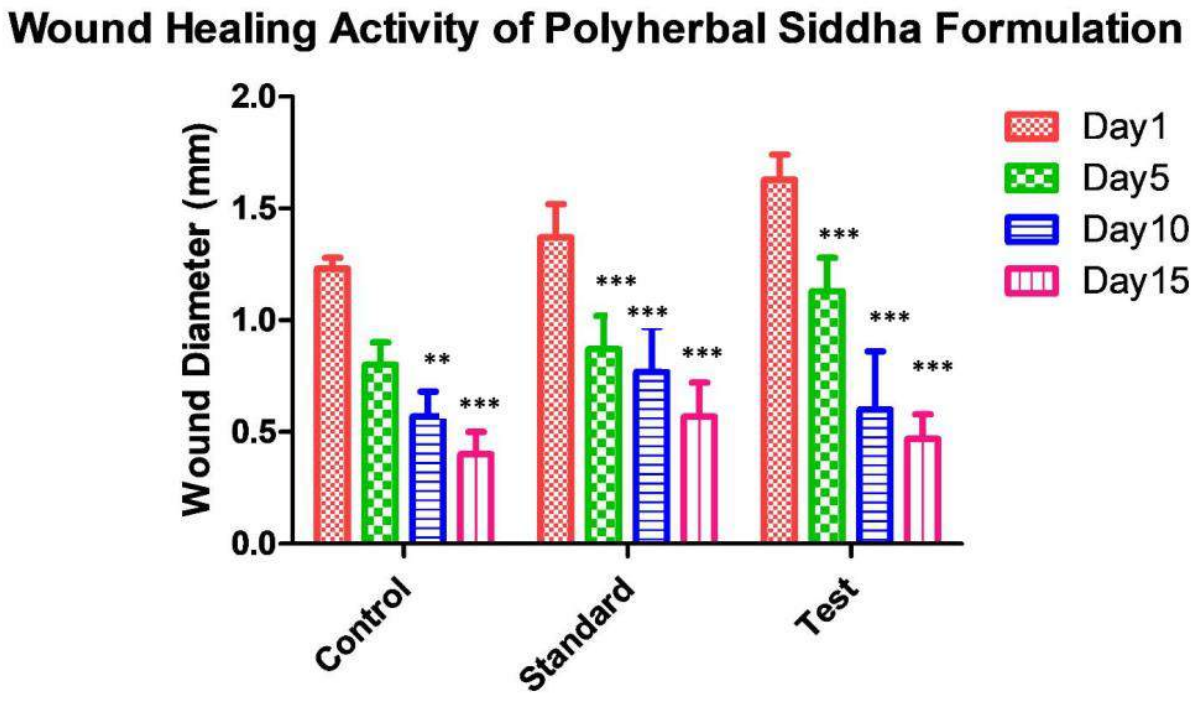


Figure 1: Wound healing at Day5, Day10 and Day15, compared to Day1 – Control (Vehicle), Standard (Povidone-Iodine ointment), Test (Polyherbal Siddha Formulation) – *p<0.05, **p<0.01, ***p<0.001

A

B

C 

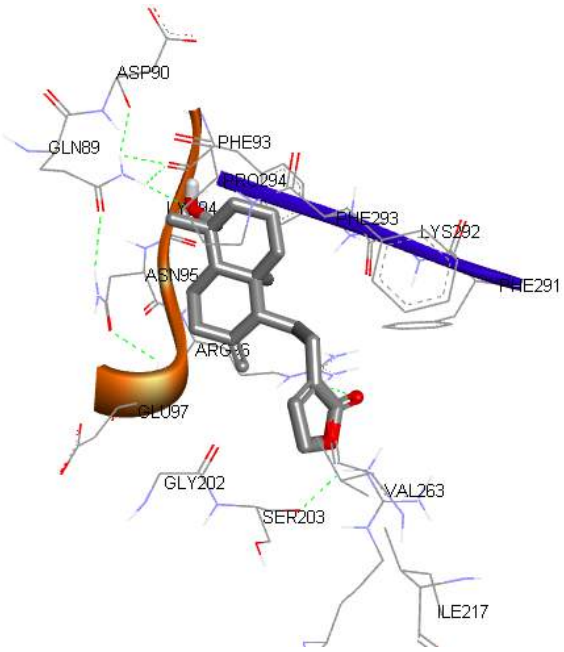
Figure 2: Histopathological changes – Wound healing

A. Control at Day1 and Day15 B. Povidone-Iodine ointment Day1 and Day15 C. Polyherbal formulation Day1 and Day15

Molecular docking: In current study 25 phytoconstitutents of the herbs under investigation were taken for docking studies. Dehydroandrographoline shown stronger binding at the receptor's binding site (-9.8 kcal/mol) when analyzed using AutoDockVina

Table 1: The Docking and binding energy of selected phytoconstituents (ranked by kcal/mol)

Ligand	Ligand ID	Binding energy (kcal/mol)
Dehydroandrographoline	CID_15411809	-9.3
Andrograpanin	CID_11666871	-9
Nimbidiol	CID_11334829	-8.6
Gedunin	CID_114923	-8.5
Azadirachtol	CID_23256847	-8.5
Azadiradione	CID_12308714	-8.4
Margosinolide	CASID_105404-75-9	-8.2
Nimbidinin	CID_101306757	-8
Vilasinin	CID_102090424	-8
Salannin	CID_118701505	-8
Azadirachtin	CID_102146586	-7.9
Azadirone	CID_10906239	-7.8
Andrographidine B	CASID_113963-38-5	-7.7
Andrographin	CID_5318506	-7.7
Andrographidine A	CID_13963762	-7.7
Andrographidine C	CID_5318484	-7.6
Agmatine	CID_199	-7.6
Apigenin	CID_5280443	-7.5
Casbene	CID_5280437	-7.2
ar-Turmerone	CID_160512	-7.2
Tetrahydrobisdemethoxycurcumin	CID_9796792	-7.2
Myrcene	CID_31253	-7.1
Piperine	CID_638024	-7
CHEMBL149285	CID_11078630	-7
Tetrahydrocurcumin	CID_11980944	-5.5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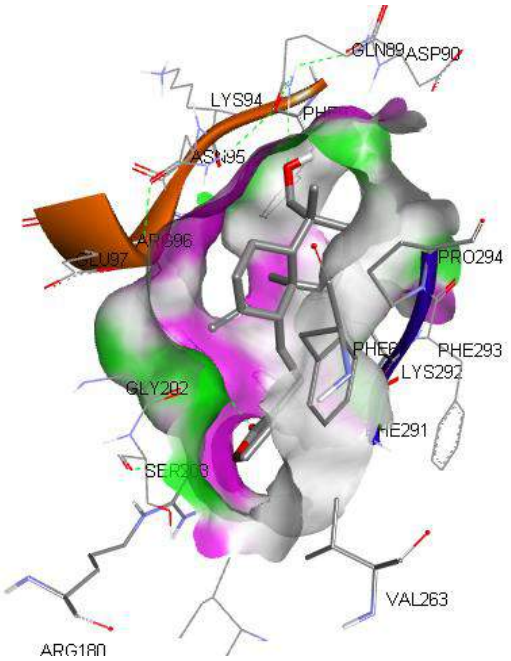
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VAL263

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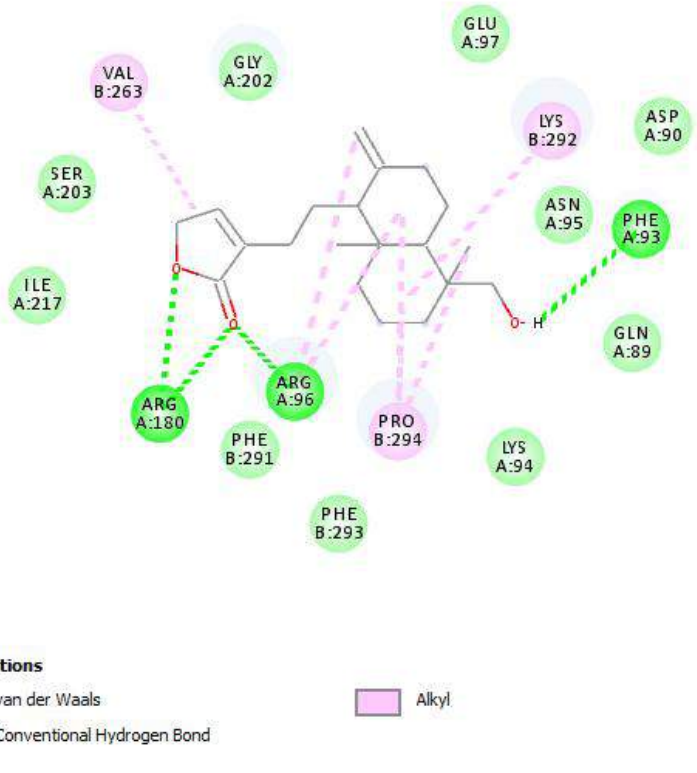

C

Figure 3: Binding modes and the corresponding interactions for the phytoconstituents of the Polyherbal formulation

A. Overlay of best-docked 3D poses of Dehydroandrographoline with the GSK1-3β (PDB-code 1Q5K). B. Dehydroandrographoline 3D pose with its binding in the receptor pocket of the macromolecule. C. 2D interactions of the binding pocket with the corresponding Amino acid interactions within the binding pocket of the macromolecule

Discussion

In the present study polyherbal formulation of *Azadirachta indica*, *Andrographis paniculata*, *Curcuma longa*, and *Ricinus communis* was used for evaluation of wound healing potential in the excision wound model in mice. Wound contraction is the process of formation of full thickness wound healed centripetally, to close the defect. In this study we have observed that the polyherbal formulation has faster rate of healing when compared to all the groups, while the standard povidone-iodine being the most effective.

Azadirachta indica significantly increases the protein and hydroxyproline content, causes migration of neutrophils, epithelial cells, causes synthesis of extracellular matrix, formation of collagen is enhanced, and also increases the DNA content of the wound. Thus, it promotes cell proliferation and hastens the wound healing [11]. Angiogenesis is the granulation tissues provides nutrients, and leads to re-epithelization. *Andrographis paniculata* improves angiogenesis, promotes fibroblast proliferation, improves collagen synthesis, causes neovascularization, increases the tensile wound strength, and thus accelerates wound healing [12]. In wound healing curcumin has antioxidant, radical scavenger, anti-inflammatory and antimicrobial properties, that play a vital role in wound healing. It stimulates the production of growth factors, acceleration of wound restoration [13]. It has been postulated that the *Ricinus*

communis Fraction1 (RCA_1) plays a important role in formation of epithelial cells during wound healing, by enhancing cell proliferation, and promotion of intercellular linkages [14].

In the docking studies it was found that dehydroandrographolide shows binding activity which regulates GSK-3β, has van der Waals interaction, hydrogen bonding, and some alkyl bonds. Developing the inhibitors of GSK-3β is challenging due to potential toxicity reports such as, hypoglycemia, tumorigenesis, and neuron deregulation [15]. So, inhibition without adverse effects is a challenging task.

Conclusion

We evaluated the wound healing activity of the polyherbal formulation in excision wound model in mice, which has shown promising results. Furthermore, the phytoconstituents of the herbs were found to be a potent competitor of inhibiting GSK-3β, which demonstrates the pharmacological basis of the formulation in promoting wound healing.

Acknowledgment

We would like to thank Dr. A.Meena, Principal, Dr. A.Shanthi, Vice-Principal, Dr. K.Senthilkumaran, Dean and Dr. V.Vaidhyalingam, Director, K.K.College of Pharmacy for motivating us to perform this research work.

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FORMULATION AND CHARACTERIZATION OF RESVERATROL LOADED SELF EMULSIFYING DRUG DELIVERY SYSTEM

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ABSTRACT

The aim of the study is to formulate and evaluate BCS class II drug resveratrol loaded SNEDDS. Solubility studies with various oils, surfactants and co-surfactant was screened. At the end of the solubility study kolliphor RH 40, propylene glycol and Eucalyptus oil was the combination of surfactant, co-surfactant and oil respectively were resveratrol was more soluble. By using different ratio 9 formulations of SNEDDS was formulated and evaluated for different characteristics such as self-emulsification time, dilution studies, globule size and *in vitro* drug release, self-emulsification time of F2 formulation was found to be faster, dilution studies showed that F1, F2 and F3 formulations gave clear solution in 100,150 and 200 times of formulation which other formulations showed unclear solution, globule size of F2 formulation was found to better than other formulation. *In vitro* release of different formulation was performed in phosphate saline buffer pH 7.4 and compared with drug in solution and F2 formulation maximum drug release of (95%). The result revealed the comparative *in vitro* study.

Key Words: Poorly soluble drugs, Solubility enhancement, Nano-emulsion, Dissolution rate.

INTRODUCTION

Nearly 70 % of the new chemical entities identified by pharmaceutical industry screening programs have failed to be developed because of poor water-solubility, which makes their formulation difficult or even impossible. According to BCS classification there are 4 classes of drugs. Class I, class II, class III and class IV. A Class II drug will typically exhibit low dissolution rate limited absorption and a Class IV drug will typically exhibit low permeation rate limited absorption. Some of the problems associated include low aqueous solubility, poor permeability, erratic and poor absorption, inter and intra subject variability and significant positive food effect which leads to low and variable bioavailability. [1]. Lipid-based drug delivery (LBDD) systems have gained much importance in the recent years due to their ability to improve the solubility and bioavailability of drugs with poor water solubility. The absorption of drug from lipid-based formulation depends on numerous factors, including particle size, degree of emulsification, and rate of dispersion and precipitation of drug upon dispersion. Lipid based formulations may include oil solution or suspensions, emulsions, self micro or self nano emulsifying drug delivery systems (SMEDDS/SNEDDS)[2]

Nano Emulsifying Drug Delivery System (SNEDDS)

Self emulsifying drug delivery system is one among the lipid based drug delivery systems that has been currently investigated for its advantages, providing a large interfacial area for partitioning the drug between oil and GI fluid. This technique improves the oral bioavailability of poorly soluble drugs by enhancing the solubility and maintaining the drug in a dissolved state, in small droplets of oil, all over its transit through the gastrointestinal tract.[3] Self nano emulsifying drug delivery system (SNEDDS) is an isotropic mixture of oil, surfactants and co-surfactants that form fine oil-in-water nano emulsion, upon mild agitation, followed by administration into aqueous media, such as GI fluids. Upon dilution, SNEDDS typically produces droplet sizes between 20 and 200 nm. These nano sized droplets may offer an improvement in dissolution rates as well as bioavailability which results in more reproducible blood time profiles.[5] SNEDDS is a physically more stable formulation when compared to emulsions, and easier to manufacture in a large scale. The rationale to use SNEDDS for the delivery of poorly soluble drugs is that, they are presented in the form of pre concentrated solution. Hence, the dissolution step required forsolid crystalline compounds shall be avoided. In addition, the formation of a variety of colloidal species on dispersion and subsequent digestion of SNEDDS facilitates drug absorption.[6]

Need of SNEDDS: SNEDDS are promising approach for oral delivery of poorly water-soluble drugs. It can be achieved by pre-dissolving the compound in a suitable solvent and fill the formulation into capsules. The oral drug delivery of hydrophobic drugs can be made possible by SNEDDS. The main benefit of this approach is that pre-dissolving the compound may overcome the initial rate limiting step of particulate dissolution in the aqueous environment within the GI tract.[7] However, a potential problem is that the drug may precipitate out of solution when the formulation disperses in the GI tract, particularly if a hydrophilic solvent is used (e.g. polyethylene glycol). If the drug can be dissolved in a lipid vehicle there is less potential for precipitation on dilution in GI tract, as partitioning kinetics will favor the drug to remain in the lipid droplets. [8]

Advantages of SNEDDS [9, 10]

- Improvement in oral bioavailability.
- Ease of manufacture and scale-up.
- Reduction in inter-subject and intra-subject variability and food effects.
- Ability to deliver peptides that are prone to enzymatic hydrolysis in GIT.
- No influence of lipid digestion process.
- Increased drug loading capacity.
- Sterilizable.
- More consistent temporal profile of drug absorption.
- Selective targeting of drug(s) towards specific absorption window in GIT.
- Protection of drug(s) from hostile environment in gut.
- Control of delivery profiles.

Disadvantages [11]

- No accurate predictive in-vitro models for the assessment of the formulations.
- Low Stability.
- Large quantity of surfactants in the formulations may induce G.I. irritation.

Criteria for Self –Nanoemulsifying Drug Delivery System [12, 13,14]

- The size of the oil globule should be 200nm or less upon dilution with water under gentle stirring.
- Upon dispersion it should be optically clear.
- The HLB value of surfactant should be greater than 12.
- It should be kinetically stable and spontaneously undergo nanoemulsification.

MATERIALS:

Resveratrol (Sami labs), Castor oil, Eucalyptus oil, Cod liver oil, Seasame oil (Local market), Olive oil, Linseed oil, Propylene glycol (Bestcareformulations), Kolliphor EL, Kolliphor HS, Kolliphor RH 40 (BASF chemicals), CAPMUL 2GPL, CAPTEX 300, CAPROL PGE860, CAPMUL MCM (ABITEC)

METHODOLOGY

1. ANALYTICAL METHOD FOR RESVERATROL [15,16]

Preparation of phosphate buffered saline (pH 7.4)

8g of Nacl was added to 800 ml of water. Then 200 mg of Kcl, 1.44 g of disodium hydrogen phosphate, 240 mg of potassium dihydrogen phosphate were added and made up to 1000 ml with distilled water pH 7.4.

Preparation of standard stock solution.

Standard stock solution of resveratrol was prepared by dissolving 10 mg of drug in 10 ml of Phosphate buffered saline.

Preparation of working stock solution.

From the stock solution 1ml was taken 10ml standard volumetric flask and was made up with phosphate buffered saline. Further it was diluted with phosphate buffered saline pH 7.4. To reach concentration range of 5 -100µg.

Determination of absorption maximum.

From the above working standard solution, 1 ml was transferred into a 10 ml volumetric flask and the volume was made up to the mark with phosphate buffered saline pH7.4 to prepare a concentration of 100 µg/ml. The sample was scanned in UV-VIS Spectrophotometer in the range 200-400nm using blank and the wavelength corresponding to maximum absorbance (λ max) was noted.

2. Solubility study [17,18]

The solubility of Resveratrol was determined in various essential oils, surfactants and co-surfactants by pouring an excess of drug into1 ml of each vehicles. The obtained mixtures were mixed continuously for 2 min using cyclo mixer. The mixtures were shaken (100 rpm) for 24 h at 25◦C in a thermostatically controlled shaking water bath followed by equilibrium for 12 h. The equilibrated samples were removed and centrifuged at 10,000 rpm (10,621 × g) for 5 min. The supernatant solution was taken and filtered through a Millipore membrane filter

(0.45 _m) and then suitably diluted with methanol. The concentration of Resveratrol was determined using UV Spectrophotometer (Shimadzu 1700, Japan) at 244 nm. The experiment was repeated in triplicates.

3. Formulation of SNEDDS:

The desired component ratios of SNEDDS were selected for drug incorporation. Ten milligram of drug and mixed surfactant and co-surfactant were incorporated in their determined ratios into oil phase containing drug. Finally homogeneous mixture was obtained by vortex mixing. The prepared SNEDDS was kept in a tightly closed bottle at 25◦C and from these the stable formulations were subjected to further study i.e. dilution studies, droplet size analysis, self-emulsification time, particle size analysis and zeta potential analysis.

Table 1: Ratio of oil , surfactant and co-surfactant content

Formulation	Drug(mg)	Oil (ml)	Surfactant (ml)	Co-Surfactant (ml)
F1	25	0.1	0.7	0.2
F2	25	0.1	0.6	0.3
F3	25	0.2	0.7	0.1
F4	25	0.3	0.6	0.1
F5	25	0.3	0.4	0.3
F6	25	0.3	0.5	0.2
F7	25	0.6	0.3	0.1
F8	25	0.7	0.2	0.1
F9	25	0.7	0.3	_

4. CHARACTERIZATION OF FORMULATIONS

1. Self-emulsification time [19, 20]

Self-emulsification time is the time required by the pre concentrate to form a homogeneous mixture upon dilution, when disappearance of SNEDDS is observed visually. The efficiency of self-emulsification of SNEDDS was assessed by using a standard USP XXII dissolution apparatus. One ml of each formulation was added dropwise to the medium (900 ml of water with a paddle speed of 100 rpm at 37.0 ± 0.5 ◦C) by a dropping pipette and the time required for the disappearance of the SNEDDS was recorded. The efficiency of self-emulsification was visually assessed.

2. Dilution test. [21]

It was studied by diluting 100, 150 and 200 times with water. The diluted samples were stored for 24 h and observed for any signs of phase separation or precipitation.

3. Globule Size Analysis.[22,23]

In this study, the effect of various dispersion medium and volume on globule size was investigated. The selected SNEDDS formulations (1 ml) were diluted with water and compared. The mean globule size of the formulations was determined using Microscope.

4. *In vitro* drug release study[24,25]

The release profile study of Resveratrol SNEDDS was performed using the dialysis bag method according to dissolution apparatus II in USP 24. Resveratrol SNEDDS was instilled in to the dialysis bag (Dialysis Membrane-110 (Mol. Weight12,000–14,000)). This was firmly sealed with dialysis clamp and was placed in 250 ml, pH 7.4 of phosphate saline buffer as the dissolution medium at 37^{o}C. The revolution speed of the paddle was maintained at 100 rpm. The samples (5 ml) were drawn at predetermined time intervals, and replenished with the same volume of fresh dissolution medium. The release of Resveratrol from SNEDDS formulation was noted and percentage release was calculated. The drug content in the samples was assayed using UV-160 a double beam spectrophotometer (Schimadzu, Japan).

RESULTS AND DISCUSSION

1. SOLUBILITY STUDIES

Solubility of Resveratrol was performed in various surfactants, co-surfactants and oils.10 mg of drug was taken and mixed with surfactants, co surfactants and oils taken in effendrof tube. It was mixed until continuously by adding 10 mg until supernatant was formed. The above solution was centrifuged and supernatant was analyzed using uv-visible spectroscopy. Solubility studies on various oils, surfactants, co-surfactants were performed and found that Kolliphor RH 40 was more soluble in Resveratrol in surfactant, propylene glycol was more soluble in co-surfactants and eucalyptus oil was more soluble in oils.

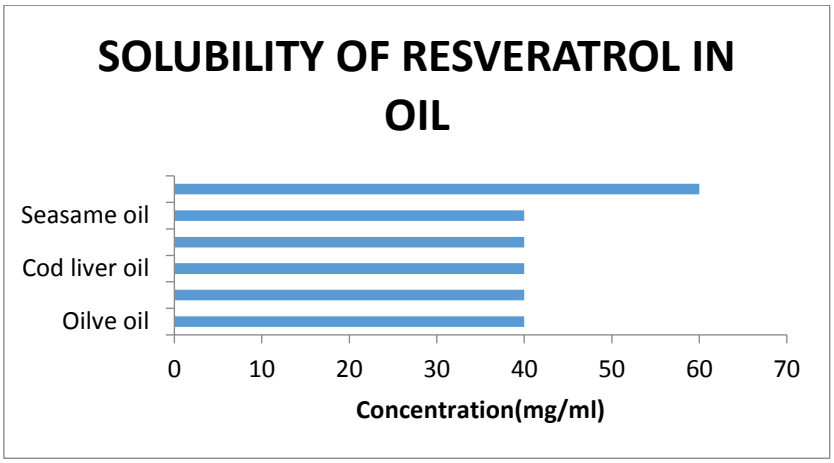

Fig 1: Solubility of Resveratrol in oil

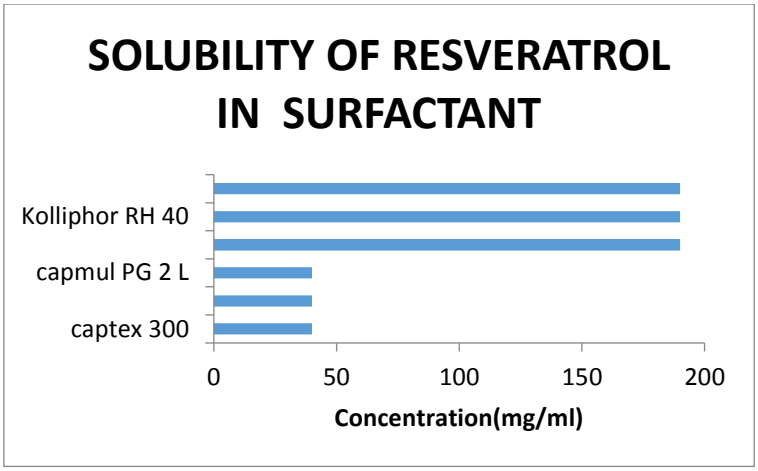

Fig. 2: Solubility of Resveratrol in Surfactant

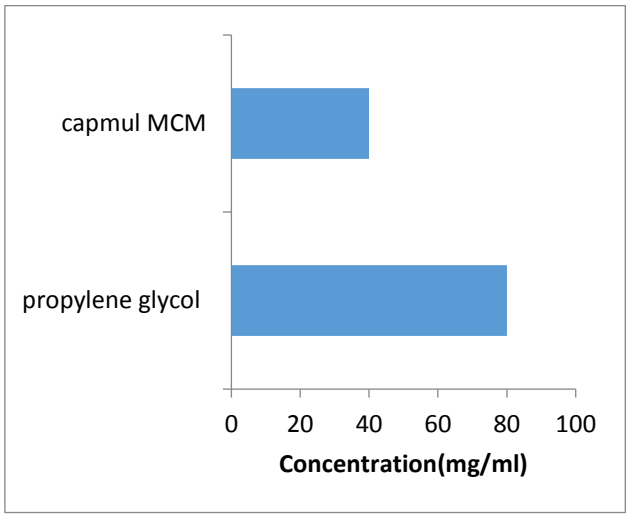

Fig 3: Solubility of Resveratrol in Co-Surfactant

2. ANALYTICAL METHOD FOR RESVERATROL

Determination of absorption maximum

The sample of resveratrol at a concentration of 500µg/ml was scanned in UV-VIS Spectrophotometer in the range 200-400 nm using blank and the wavelength corresponding to maximum absorbance (λ max) was recorded. The sample showed maximum absorbance at wave lengths 306 nm.

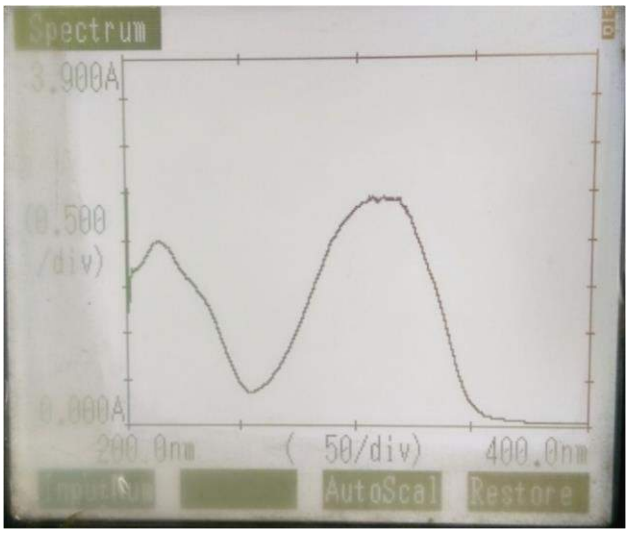

Fig 4: Absorbtion maxima of Resveratrol

From the above figure it was found that Resveratrol is having maximum absorption wavelength of 306 nm.

3. Preparation of Calibration Curve.

Aliquots of standard drug solution of Resveratrol containing 0.2-1.0 ml (100 µg/ml) are taken and transferred into series of 10ml volumetric flask. To a series of 10ml volumetric flasks aliquots standard solutions were taken and volume was made up using Phosphate saline buffer pH 7.4. The absorbance of these solutions measured at maximum absorbance of 306 nm as given in Table Absorbance values thus obtained were plotted against respective concentration to obtain standard calibration curve as shown in Figure 12. The standard concentrations of Resveratrol (5-100 µg/ml) showed good linearity with R^2 value of 0.996, were shown which suggests that it obey the Beer- Lamberts law.

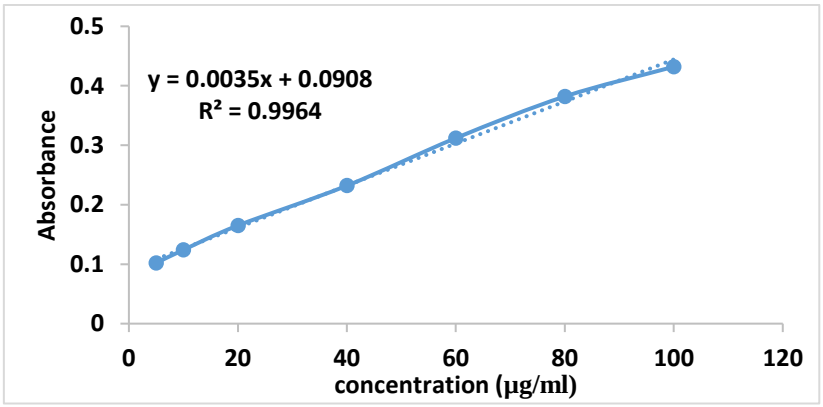

Fig 5: Calibration curve of Resveratrol

Table 2: Standard Calibration curve

Standard Calibration curve		
S.No.	**Concentration (µg/ml)**	**Absorbance**
1	5	0.102
2	10	0.124
3	20	0.165
4	40	0.232
5	60	0.312
6	80	0.382
7	100	0.432

4. Characterization of formulation.

1. Self-emulsification time.

The rate of emulsification was a major index for assessment of the efficiency of self-emulsification. The SNEDDS should disperse completely and quickly when subjected to dilution under mild agitation. FormulationF1,F2,F3,F4,F5 and F8 showed less emulsification time (less than 1 min) when compared to others.

Table 3: Self-emulsification time of different formulation.

S.NO	FORMULATION	SELF EMULSIFICATION TIME.(SECONDS)
1	F1	30
2	F2	10
3	F3	30
4	F4	60
5	F5	30
6	F6	80
7	F7	250
8	F8	50
9	F9	1070

2. Dilution Test.

Uniform emulsion formulation from SNEDDS is very important at different dilutions because drugs may precipitate at higher dilution *in vivo* which affects the drug absorption significantly. each formulation was subjected to 100, 150,200 times the dilution in distilled water. Even after

24 hrs formulations F1, F2 and F3 showed no signs of precipitation, cloudiness or separation which ensured the stability of the reconstituted emulsion. Rest of the formulations showed phase separation and turbidity.

3. Globule Size Analysis.

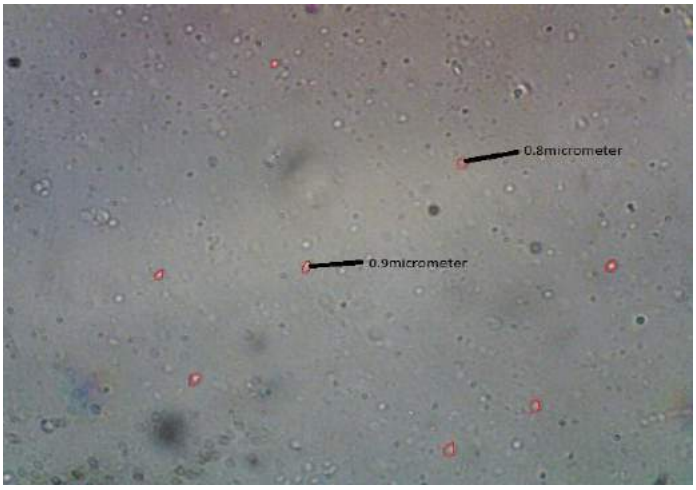

Fig 6: Globule size of Formulation 1

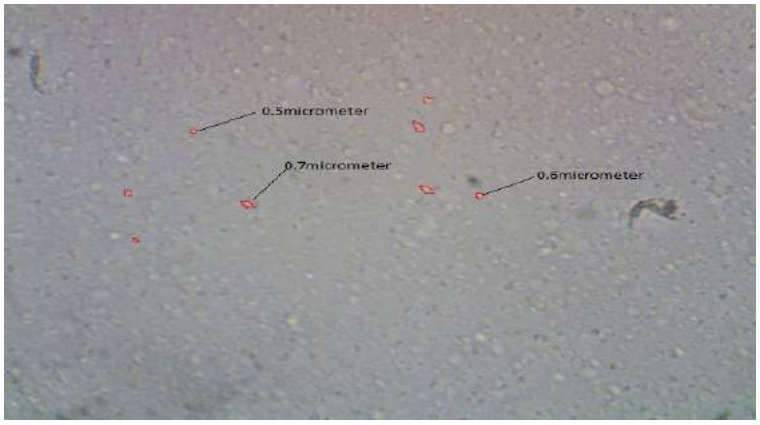

Fig 7 : **Globule size of Formulation 2**

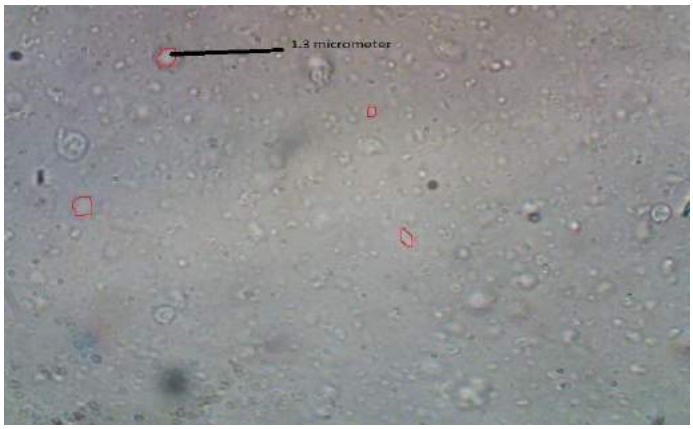

Fig 8 :Globule size of Formulation 3

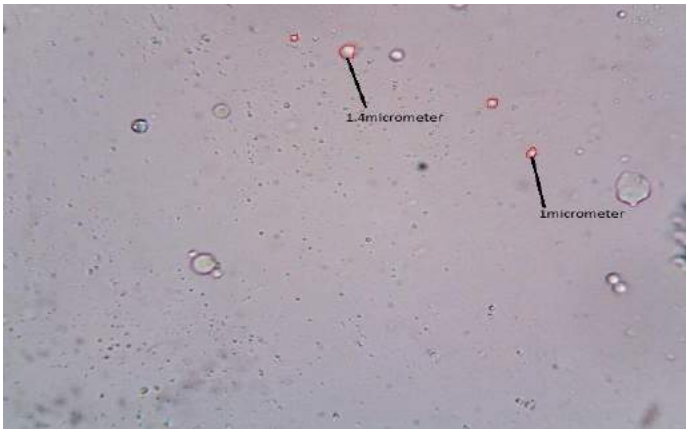

Fig 9: Globule size of Formulation 4

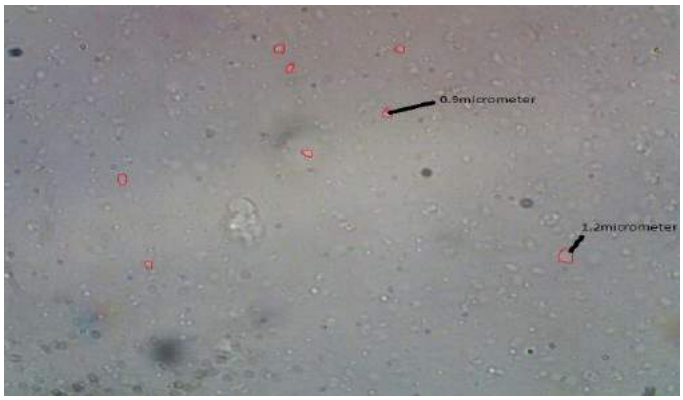

Fig 10: Globule size of Formulation 5

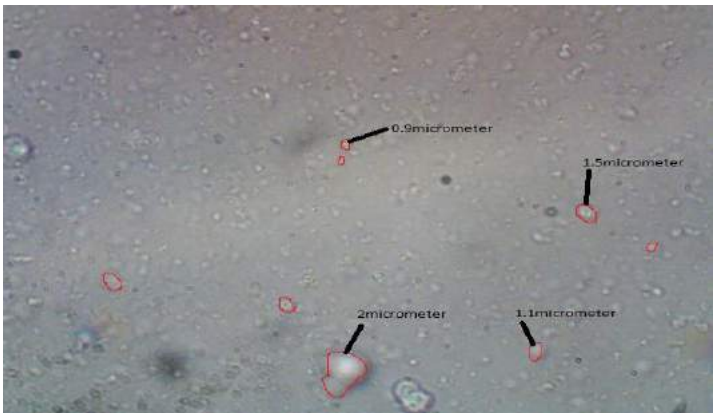

Fig 11: Globule size of Formulation 6

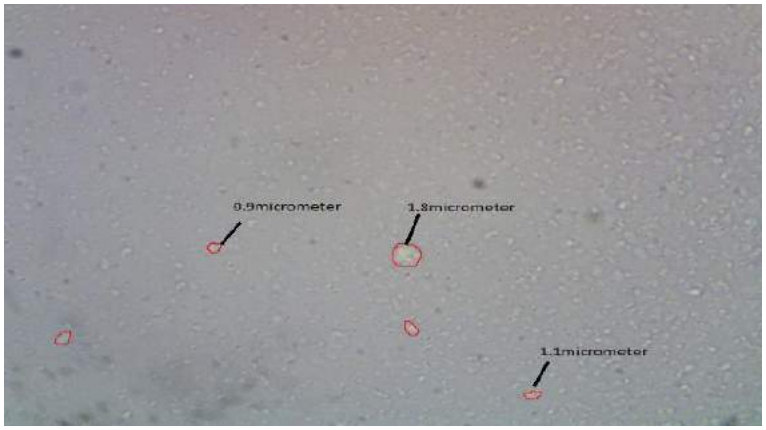

Fig 12: Globule size of Formulation 7

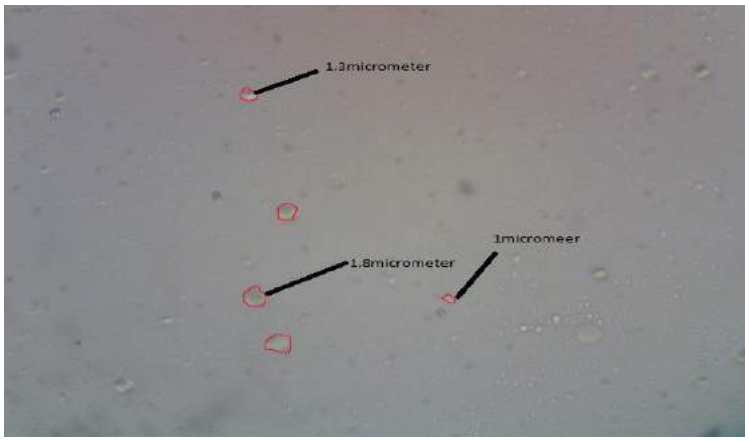

Fig 13: Globule size of Formulation 8

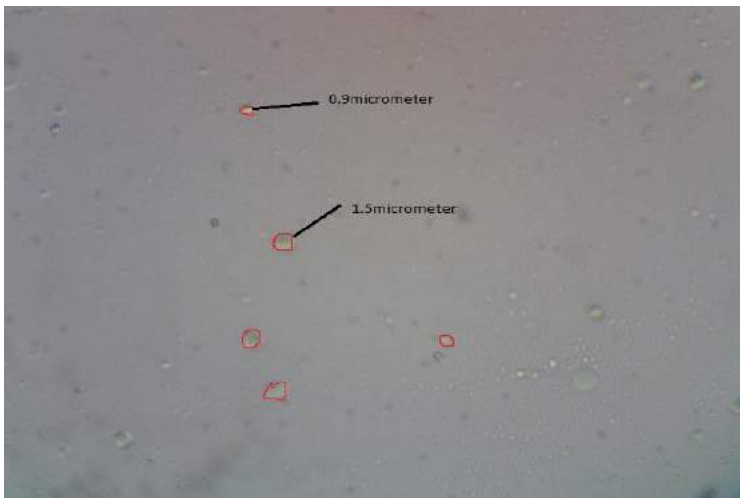

Fig 14: Globule size of Formulation 9

Formulation F2 was having better globule size than other formulations. Globule size for Formulation F2 of was found to be in Range of 500nm - 800nm

Table 4: *Invitro* Drug Release

S.NO	TIME	F1	F2	F4	F5	F8	DRUG IN SOLUTION
1	0	0	0	0	0	0	0
2	5	31.2	23.4	21.4	26.4	25.2	12.5
3	15	52.8	36.6	48.1	37.1	51.6	36.5
4	30	65.9	59.2	59.5	62.9	62.3	55.7
5	45	78.2	79.8	65.9	70.5	70.4	68.4
6	60	89..6	95.7	74.4	84.5	78.1	80.2

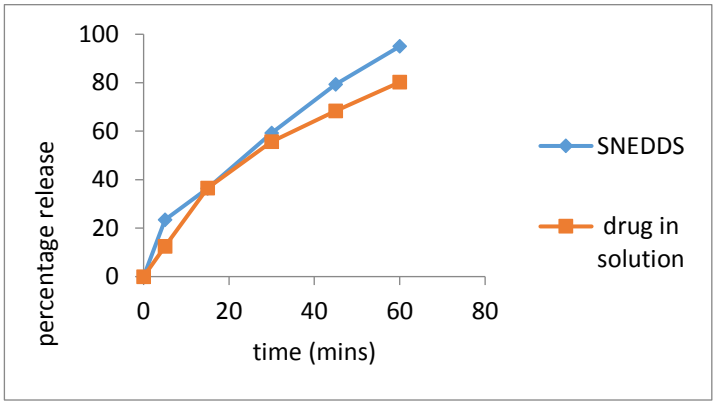

Fig 15: Comparative drug release study of SNEDDS and Drug solution

Out of nine formulations, F2 having better release of 95% when compared with dru in solution (80%) *in vitro* release pattern of formulation F2 showed The rapid drug release and as the time increases it was found to be controlled. In case of drug in solution the initial release was less when compared with SNEDDS formulation.

CONCLUSION

Self nanoemulsifying drug delivery system (SNEDDS) serves as an ideal carrier for the delivery of drugs belonging to BCS class II and class IV. The present study has clearly demonstrated the potential utility of self-nano emulsifying drug delivery system for formulating model drug resversatrol with improved solubility and dissolution rate. The formulation of Resveratrol loaded self-Nano Emulsifying drug delivery system consisting of Eucalyptus oil are oil phase kolliphor RH 40 are surfactant, propylene glycolant are co-surfact and drug. The formulations were evaluated for self emulsification time, dilution test, globule size and *in vitro* drug release. The result showed that the Self emulsification time of F2 formulation was less time of 10 seconds when compared with other formulations, globule size of all the formulations was found to be in optimum. Formulation F1, F2, and F3 gave clear solution for 24 hrs in the visual clarity in distilled water while other F4, F5, F6, F7, F8 and F9 turned hazy, unclear after 24 hrs. *In vitro* release of different formulation was performed in phosphate saline buffer pH 7.4 and compared with drug in solution and F2 formulation maximum drug release of (95%). The result revealed the comparative *in vitro* study. Hence we conclude that the prepared SNEDDS with promising *in vitro* characteristics hopes to solve the oral delivery problems encountered for BCS class II and IV drugs.

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GENOTYPE AND PHENOTYPE OF COVID-19-A REVIEW

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Abstract

COVID-19 is a novel coronavirus with an outbreak of unusual viral pneumonia in Wuhan, China, and then pandemic. Based on its phylogenetic relationships and genomic structures the COVID-19 belongs to genera Betacoronavirus. Human Betacoronaviruses (SARS-CoV-2, SARS-CoV, and MERS-CoV) have many similarities, but also have differences in their genomic and phenotypic structure that can influence their pathogenesis. COVID-19 is containing single stranded (positive-sense) RNA associated with a nucleoprotein within a capsid comprised of matrix protein. A typical CoV contains at least six over reading frame {ORFs} in its genome.

Key words: COVID-19, genome, phenotype

Coronavirus genome structure

COVID-19 is a spherical or pleomorphic enveloped particles containing single-stranded (positive-sense) RNA associated with a nucleoprotein within a capsid comprised of matrix protein (Fig-1). The envelope bears club-shaped glycoprotein projections. Some coronaviruses also contain a hem agglutinin-esterase protein (HE) (1-3)

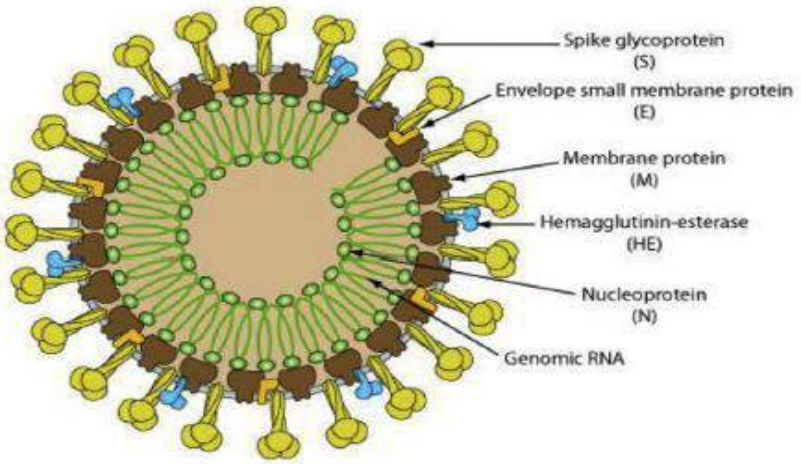

Fig-1 schematic of a coronavirus

Coronaviruses possess the largest genomes among all known RNA viruses. Variable numbers of small ORFs are present between the various conserved genes (ORF1ab, spike, envelope, membrane and nucleocapsid) and, downstream to the nucleocapsid gene in different coronavirus lineages. The viral genome contains distinctive features, including a unique N-terminal fragment within the spike protein. Genes for the major structural proteins in all coronaviruses occur in the 5'-3' order as S, E, M, and N (Fig-2).

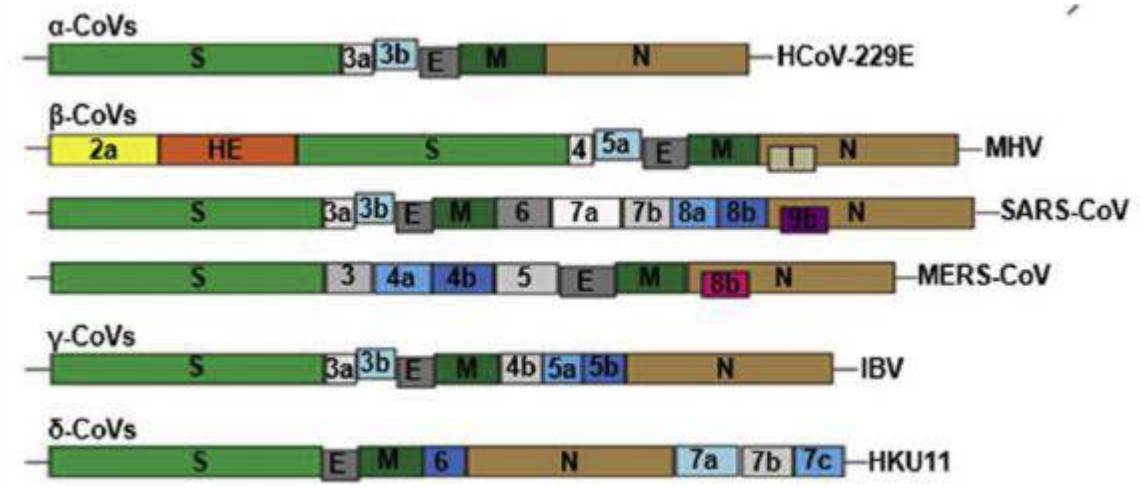

Fig-2 S, E, M, and N are represented of the four structural proteins spike, envelope, membrane, and nucleocapsid. CoVs, coronavirus; Viral names: HKU, coronaviruses identified by Hong Kong University; HCoV, human coronavirus; IBV, infectious bronchitis virus; MHV, murine hepatitis virus.

A typical CoV contains at least six ORFs in its genome. Except for Gammacoronavirus that lakes nsp1, the first ORFs (ORF1a/b), about two-thirds of the whole genome length, encode 16 nsps (nsp1-16). ORF1a and ORF1b contain a frameshift in between which produces two polypeptides: pp1a and pp1ab. These polypeptides are processed by virally encoded chymotrypsin-like protease (3CLpro) or main protease (Mpro) and one or two papain-like protease into 16 nsps.

Four main structural proteins contain spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins are encoded by ORFs 10, 11 on the one-third of the genome near the 3-terminus. Besides these four main structural proteins, different CoVs encode special structural and accessory proteins, such as HE protein, 3a/b protein, and 4a/b protein. These mature proteins are responsible for several important functions in genome maintenance and virus replication.

There are three or four viral proteins in the coronavirus membrane. The most abundant structural protein is the membrane (M) glycoprotein; it spans the membrane bilayer three times, leaving a short NH2-terminal domain outside the virus and a long COOH terminus (cytoplasmic domain) inside the virion. The spike protein (S) as a type I membrane glycoprotein constitutes the peplomers. In fact, the main inducer of neutralizing antibodies is S protein. Between the envelope proteins with exist a molecular interaction that probably determines the formation and composition of the coronaviral membrane (4-6).

Replication Process in Pathogenicity

SARS-CoV-2 (COVID-19) binds to ACE2 (the angiotensinconverting enzyme 2) by its Spike and allows COVID-19 to enter and infect cells. In order for the virus to complete entry into the cell following this initial process, the spike protein has to be primed by an enzyme called a protease.

After the virus enters the host cell and uncoats, the genome is transcribed and then translated. Coronavirus genome replication and transcription takes place at cytoplasmic membranes and

involve coordinated processes of both continuous and discontinuous RNA synthesis that are mediated by the viral replicate, a huge protein complex encoded by the replicase gene. Besides RNA dependent RNA polymerase, RNA helicase, and protease activities, which are common to RNA viruses, The coronavirus replicase was recently predicted to employ a variety of RNA processing enzymes that are not (or extremely rarely) found in other RNA viruses (4).

Conclusion

At present, there is no specific treatment for COVID-19. Given the high rate of transmission of this virus between humans and its pandemics, it is important to identify the basis of its replication, structure, and pathogenicity for discovering a way to the special treatment or the prevention. Due to the high similarity of the virus to its families, efforts have been made to provide medicines and vaccines for COVID-19. Differences in the length of the spike as it is longer in COVID-19 are likely to play an important role in the pathogenesis and treatment of this virus. However, identifying the specific molecular details of the virus is helpful in achieving treatment goals.

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COMPARATIVE EFFICACY OF HERBAL METHIONINE (METSAKTI) AND DL-METHIONINE ON PERFORMANCE OF BROILER CHICKEN

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Abstract

Objective: A trial was conducted to determine the comparative efficacy of herbal methionine feed supplement (MetSakti) and synthetic DL-methionine on performance of broiler chickens. **Method:** Day old commercial broilers of total 1800 Nos. were randomly divided into 3 groups (Group A, B and C) of 600 birds each. Each group was further subdivided into three replicates of 200 broilers per replicate. Group A was control group and fed with control feed (Standard feed); Group B was control plus 1kg DL-methionine/tonne diet; Group C was control plus 1kg herbal methionine (MetSakti, supplied by Sakti Agrotec) / tonne diet. Duration of dietary supplementation was 41 days. The study was conducted to observe the effect of feed intake, feed conversion ratio, body weight gain and survivability. **Result:** Effect of dietary supplementation on body weight, body weight gain, feed intake and feed conversion ratio at 0 to 41 day, was significant. The broilers fed with herbal methionine for 41 days showed significant increase in body weight and body weight gain than other treatment groups. Similarly, herbal methionine treated groups demonstrated significant feed conversion ratio than synthetic methionine supplied groups. Neither DL-methionine nor herbal methionine supplementation had a significant effect on broiler mortality. **Conclusion:** The study demonstrates that herbal methionine can replace DL- methionine more efficiently at the rate 1kg/tonne diet of commercial broiler chicken.

Key words: Herbal methionine, DL- methionine, Performance, Broiler chicken

I. INTRODUCTION

Amino acids, basic units of protein, can exist as D- or L isomers or mixture of two products. Animal cells can utilize only L-amino acids for protein synthesis which naturally exists in animal and plant tissues. Whereas, D- amino acids, if ingested, need to be converted to L-Methionine to be biologically available (Leeson and Summers, 2001). Methionine an indispensible amino acid, must be supplied in the feed of chicken, as chick cannot synthesis of its own in order to sustain its growth. Methionine may act as a lipotropic agent through its role as an amino acid in balancing protein or through its role as a methyl donor and involvement in choline, betaine, folic acid and vitamin B12 metabolism (Chen *et al*., 1993). Chronic deficiencies of certain nutrients impair immune response and increases susceptibility to infectious diseases affecting growth and performance. One such nutrient is methionine which is required at higher level when bird is predisposed to fast growth along with high production performance and must be supplied in the diet of the chicken, as the poultry birds are unable to synthesize it in the amounts necessary to sustain life and growth.

The increase in demand for cheap meat has given rise to the use of synthetic methionine in feed. But the use of synthetic analogues has serious adverse effects to the birds as they are

metabolized into highly toxic compounds such as methyl propionate (Bender, 1975). Moreover, synthetic methionine is listed among the prohibited synthetic substances and its usage has been questioned in organic farming practices (Anonymous, 1999). Therefore, there is a trend in developing natural alternative supplements to maintain animal performance and well being. Herbal methionine is claimed to be potential alternative as optimum agent for protein accretion in poultry birds. In this perspective, a study was conducted to evaluate the effects of herbal methionine in commercial broiler chicken in comparison with synthetic methionine.

II. MATERIALS AND METHODS

a. Birds and experimental design: A farm trial on DL- methionine and herbal methionine supplementation in commercial broiler chicken (Vencobb) was conducted in Jyothi poultry farm, Namakkal. One thousand eight hundred, a day old commercial broilers were purchased from hatcheries and were randomly divided into three dietary treatment groups of 600 broilers each. Each treatment group was further subdivided into three replicates of 200 birds. Commercial brooding and management procedures were followed, and all broilers were fed a typical commercial broiler starter diet for the first 3 weeks of the experiment followed by finisher diet up to 6 weeks. The treatment groups were control diet without methionine (Group A); control plus 1kg DL- methionine / tonne diet (Group B), and control plus 1kg herbal methionine (MetSakti, supplied by Sakti Agrotec) /tonne diet (Group C).

b. Housing and management: The broilers were housed in floor pens and rice husk served as litter material. The house was cleaned thoroughly with formaldehyde and potassium permanganate solution three days prior to arrival of birds. The day old chicks were offered electrolyte solution upon arrival. Birds were maintained on a 24 hours constant light schedule. The brooding temperature was maintained close to their requirement, first by heating device for 3 days following arrival of chicks. The birds were vaccinated against Ranikhet disease and Infectious Bursal Disease on day 7, 14 and 21 and provided antibiotic for the first 5 days as per recommendation.

c. Details of the feeding regimens: The chicks were offered broiler starter and broiler finisher in mash form. These diets were formulated to meet (Table 1) or exceed the Bureau of Indian Standard for broiler chickens (1992) nutritional requirement of broiler chicken. The diets were fortified with mineral and vitamin premix as per the standard stipulated by BIS (1992). Total amount of feed offered during 24 hours to a replicate under a specific treatment groups was divided into 3 equal proportions. The amount and timing of feed was adjusted in such a way that the birds consume the whole of the diet offered at any one time. As a result hardly any residue can be obtained from the replicate after a day's feeding. The standard techniques of the proximate analysis were used to determine nutrient content of experimental diets (AOAC, 1995). Individual body weight and feed consumption of broilers from all pens were measured at the 0, 21 and 42 day of age. Mortality of each pen was recorded on a daily basis. Feed conversion ratio was adjusted according to the feed consumption of the dead broilers. Body weight was recorded before offering feed.

Table 1: Ingredients and chemical composition of starter and finisher diet

Name	Starter	Finisher
Ingredients (as air dried basis), kg/1000 kg		
Maize	554.6	615.0
Rice polish	40.0	50.5
Soybean	335.0	250.4
Fish meal	50.0	50.0
Calcite	4.2	5.5
Di-Calcium Phosphate	11.0	11.0
Soybean oil	-	12.0
Salt	0.2	0.95
Trace mineral mixture†	1.0	1.0
Vitamin premix‡	0.3	0.45
Choline chloride	0.8	0.8
Maduramycin	0.5	0.5
Sodium bicarbonate	1.0	1.0
Zinc oxide	0.2	0.2
Manganese sulfate	0.2	0.2
Natuzyme	0.5	0.5
Lincomycin (1%)	0.5	0.4

Chemical composition		
Dry matter, %[a]	89.23	89.76
Crude Protein, %[a]	22.87	20.00
Metabolizable Energy, Kcal/kg[b]	2850	2960
Calcium, %[a]	0.92	0.90
Available Phosphorous, %[b]	0.46	0.45
Methionine, %[b]	0.42	0.29
Lysine, %[b]	1.20	1.06

† Composition of each Kg trace mineral mixture : Cu, 15g.; Co, 02g.; Fe, 60g.; Zn, 80g.; Mn, 80g.; I, 02g.; Se, 0.3g.; Mo, 0.1g. ‡ Each Kg contains : Vitamin A- 80 MIU; Vitamin D3- 12 MIU; Vitamin E- 70g; Vitamin K3 -8g; Vitamin B1- 6.4g; Vitamin B2- 40g; Vitamin B6- 12.8g; Vitamin B12-160mg; Nicotinic acid- 80g; Vitamin B5- 115g; Folic acid-4g; Biotin- 24mg. ¶ Each Kg contains: Cellulase-6,000,000 U; Xylanase- 10,000,000 U; "glucanase- 700,000 U; " amylase-700,000 U; Pectinase-70,000 U; Protease-3,000,000 U; Phytase-400,000 U. [a]Assayed value [b]Calculated on the basis of standard values applicable under Indian condition (Singh and Panda, 1996).

III. RESULTS AND DISCUSSION

The effect of DL- methionine and herbal methionine supplementation on the performance of broiler is shown in Table 2. Herbal methionine (1kg /tonne of feed) increased ($P<0.01$) the body weight and body weight gain at day 21 and 42 compared to other groups. Control group weighed 1808g at the end of 42 days of supplementation. DL-methionine treated group birds, at the end of 42 day treatment, weighed 1821.5g as average weight, while herbal methionine supplement weighed 1854.4g. Body weight gain of birds supplemented with herbal methionine (1kg/tonne of feed) was 1815.8±9.10g while the performance of DL- methionine supplemented birds was 1773.6±12.6g. Herbal methionine supplemented birds performed better than DL-methionine at 1kg/tonne of feed, which could be attributed to its active methionine form (L-form) and availability of herbal methionine to the birds. If Methionine can be supplemented entirely as L-Methionine, it may enhance the utilization of Methionine in the tissues to help the redox status compared with the use of DL-Methionine (Luo and Levine, 2009; Shen *et al*., 2015), because only L-Methionine can be metabolized to glutathione and taurine to function as antioxidants in the gut and the liver (Finkelstein, 1990). Feed intake of control birds at the end of day 21 and 42 were higher ($P<0.05$) than methionine (DL-or herbal) supplemented groups, which indicate both synthetic and herbal methionine did not improve the feed intake.

Table 2: Performance of broiler chicken fed DL-methionine and herbal methionine

Attributes	Age, day	Control (A)	DL-methionine supplementation (B)	Herbal methionine (MetSakti) supplementation (C)	P value
Body weight, g	0	38.09±1.5	38.66±1.23	38.54±0.8	*
	21	469.21±4.81	461.87±6.11	488.5±6.18	*
	42	1808.3±16.5	1821.5±11.4	1854.4±12.5	**
Body weight gain, g	0-21	431.1±7.71	458.0±14.4	454.19±6.8	*
	0-42	1770.2±8.1	1773.6±12.6	1815.8±9.10	**
Feed intake, g	0-21	849.2±5.45	849.5±9.16	885.1±8.82	*
	0-42	3500.7±46.1	3238.6±51.3	3292.8±58.6	NS
Feed conversion ratio (FCR), g/g	0-21	1.96±0.02	1.85±0.04	1.817±0.02	NS
	0-42	1.98±0.01	1.82±0.03	1.77±0.02	**
Survivability (%)	0-42	99.4	99.5	99.8	

A-Control without methionine; B-Control plus 1kg DL- methionine /tonne diet; C-Control plus 1kg herbal methionine /tonne diet; * $p<0.05$; ** $p<0.01$; NS- Not significant.

Supplementation of DL- methionine or herbal methionine improved ($P<0.01$) feed conversion ratio (FCR) compared to control. FCR was not significant between the DL- methionine and herbal methionine supplemented birds during the period 0-21 days, but there was significant FCR observed during the period 0-42 days ($P<0.01$). Reported studies show that the herbal methionine supplementation is more beneficial towards cost-benefit ratio also viz., Huyghebaert (1993) reported that increase in methionine level promoted an increase of 14% in weight gain compared to methionine deficient basal diet. A similar result was described by Rostagno and Barbosa (1995) who observed that the increase in methionine levels promoted an increase of 13% in weight gain for birds fed a basal diet without methionine. Source or level did not affect feed intake during the growing period and no interaction was seen between the two factors. Previous reported works revealed that supplementation of methionine in the diet improved performance in terms of FCR but feed intake was similar between methionine supplemented and non-supplemented groups. Similarly in the present investigation supplementation of DL- methionine improved FCR compared to control whereas supplementation of herbal methionine at the rate of 1kg/tonne showed better FCR compared to DL- methionine supplemented birds. The survivability of birds during the experimental period did not differ significantly between the treatments.

CONCLUSION:

Herbal methionine is required at optimum level when bird is predisposed to fast growth along with high production performance. Herbal methionine at 1kg/tonne of feed can successfully replace the synthetic methionine analogues from broiler ration.

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