Black Fungus in the Corona Virus
Oxidative Stress in Wistar Albino Rats

Formulation Method of Nanoparticles
Evaluation of the Anthelmintic Activity

Highlights

Discovering Thoughts, Inventing Future
## Editorial Board

**Global Journal of Medical Research**

<table>
<thead>
<tr>
<th>Name</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dr. Apostolos Ch. Zarros</strong></td>
<td>DM, Degree (Psycho) holder in Medicine, National and Kapodistrian University of Athens, MRes, Master of Research in Molecular Functions in Disease, University of Glasgow FRNS, Fellow, Royal Numismatic Society Member, European Society for Neurochemistry Member, Royal Institute of Philosophy Scotland, United Kingdom</td>
</tr>
<tr>
<td><strong>Dr. William Chi-shing Cho</strong></td>
<td>Ph.D., Department of Clinical Oncology, Queen Elizabeth Hospital, Hong Kong</td>
</tr>
<tr>
<td><strong>Dr. Alfio Ferlito</strong></td>
<td>Professor Department of Surgical Sciences, University of Udine School of Medicine, Italy</td>
</tr>
<tr>
<td><strong>Dr. Michael Wink</strong></td>
<td>Ph.D., Technical University Braunschweig, Germany, Head of Department Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Germany</td>
</tr>
<tr>
<td><strong>Dr. Jixin Zhong</strong></td>
<td>Department of Medicine, Affiliated Hospital of Guangdong Medical College, Zhanjiang, China, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH 43210, US</td>
</tr>
<tr>
<td><strong>Dr. Pejčić Ana</strong></td>
<td>Assistant Medical Faculty Department of Periodontology and Oral Medicine University of Nis, Serbia</td>
</tr>
<tr>
<td><strong>Rama Rao Ganga</strong></td>
<td>MBBS, MS (University of Health Sciences, Vijayawada, India), MRCS (Royal College of Surgeons of Edinburgh, UK), United States</td>
</tr>
<tr>
<td><strong>Dr. Ivandro Soares Monteiro</strong></td>
<td>M.Sc., Ph.D. in Psychology Clinic, Professor University of Minho, Portugal</td>
</tr>
<tr>
<td><strong>Dr. Izzet Yavuz</strong></td>
<td>MSc, Ph.D., D Ped Dent. Associate Professor, Pediatric Dentistry Faculty of Dentistry, University of Dicle Diyarbakir, Turkey</td>
</tr>
<tr>
<td><strong>Sanguansak Rerksuppaphol</strong></td>
<td>Department of Pediatrics Faculty of Medicine, Srinakharinwirot University, Nakorn Nayok, Thailand</td>
</tr>
<tr>
<td><strong>Dr. Sanjay Díxit, M.D.</strong></td>
<td>Director, EP Laboratories, Philadelphia VA Medical Center Cardiovascular Medicine - Cardiac Arrhythmia, Univ of Penn School of Medicine, Web: pennmedicine.org/wagform/MainPage.aspx?</td>
</tr>
<tr>
<td><strong>Antonio Simone Laganà</strong></td>
<td>M.D. Unit of Gynecology and Obstetrics, Department of Human Pathology in Adulthood and Childhood “G. Barresi” University of Messina, Italy</td>
</tr>
<tr>
<td>Name</td>
<td>Position/Department</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| **Dr. Han-Xiang Deng**      | MD., Ph.D  
Associate Professor and Research Department  
Division of Neuromuscular Medicine  
Davee Department of Neurology and Clinical Neurosciences  
Northwestern University Feinberg School of Medicine  
Web: neurology.northwestern.edu/faculty/deng.html |
| **Dr. Roberto Sanchez**     | Associate Professor  
Department of Structural and Chemical Biology  
Mount Sinai School of Medicine  
Ph.D., The Rockefeller University  
Web: mountsinai.org/ |
| **Dr. Feng Feng**           | Boston University  
Microbiology  
72 East Concord Street R702  
Duke University  
United States of America |
| **Dr. Hrushikesh Aphale**   | MDS- Orthodontics and Dentofacial Orthopedics.  
Fellow- World Federation of Orthodontist, USA. |
| **Gaurav Singhal**          | Master of Tropical Veterinary Sciences, currently pursuing Ph.D in Medicine |
| **Dr. Pina C. Sanelli**     | Associate Professor of Radiology  
Associate Professor of Public Health  
Weill Cornell Medical College  
Associate Attending Radiologist  
NewYork-Presbyterian Hospital  
MRI, MRA, CT, and CTA  
Neuroradiology and Diagnostic Radiology  
M.D., State University of New York at Buffalo, School of Medicine and Biomedical Sciences  
Web: weillcornell.org/pinasanelli/ |
| **Dr. Michael R. Rudnick**  | M.D., FACP  
Associate Professor of Medicine  
Chief, Renal Electrolyte and Hypertension Division (PMC)  
Penn Medicine, University of Pennsylvania  
Presbyterian Medical Center, Philadelphia  
Nephrology and Internal Medicine  
Certified by the American Board of Internal Medicine  
Web: uphs.upenn.edu/ |
| **Dr. Seung-Yup Ku**        | M.D., Ph.D., Seoul National University Medical College, Seoul, Korea Department of Obstetrics and Gynecology  
Seoul National University Hospital, Seoul, Korea |
| **Santhosh Kumar**          | Reader, Department of Periodontology,  
Manipal University, Manipal |
<p>| <strong>Dr. Aarti Garg</strong>          | Bachelor of Dental Surgery (B.D.S.) M.D.S. in Pedodontics and Preventive Dentistry Pursuing Phd in Dentistry |</p>
<table>
<thead>
<tr>
<th><strong>Sabreena Safuan</strong></th>
<th><strong>Arundhati Biswas</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph.D (Pathology) MSc (Molecular Pathology and Toxicology) BSc (Biomedicine)</td>
<td>MBBS, MS (General Surgery), FCPS, MCh, DNB (Neurosurgery)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Getahun Asebe</strong></th>
<th><strong>Rui Pedro Pereira de Almeida</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Veterinary medicine, Infectious diseases, Veterinary Public health, Animal Science</td>
<td>Ph.D Student in Health Sciences program, MSc in Quality Management in Healthcare Facilities</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Dr. Suraj Agarwal</strong></th>
<th><strong>Dr. Sunanda Sharma</strong></th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Osama Alali</strong></th>
<th><strong>Shahanawaz SD</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>PhD in Orthodontics, Department of Orthodontics, School of Dentistry, University of Damascus. Damascus, Syria. 2013 Masters Degree in Orthodontics.</td>
<td>Master of Physiotherapy in Neurology PhD- Pursuing in Neuro Physiotherapy Master of Physiotherapy in Hospital Management</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Prabudh Goel</strong></th>
<th><strong>Dr. Shabana Naz Shah</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>MCh (Pediatric Surgery, Gold Medalist), FISPU, FICS-IS</td>
<td>PhD. in Pharmaceutical Chemistry</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Raouf Hajji</strong></th>
<th><strong>Vaishnavi V.K Vedam</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>MD, Specialty Assistant Professor in Internal Medicine</td>
<td>Master of dental surgery oral pathology</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Surekha Damineni</strong></th>
<th><strong>Tariq Aziz</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph.D with Post Doctoral in Cancer Genetics</td>
<td>PhD Biotechnology in Progress</td>
</tr>
</tbody>
</table>
Contents of the Issue

1. Evaluation De L’activité Anthelminthique Des Extraits Aqueux De Striga Hermonthica (Del.) Benth Et Ceratotheca Sésamoïdes Endl Sur Deux Stades Biologiques De Haemonchus Contortus. 1-8
2. Supercritical Fluids as a State-of-the-Art Formulation Method of Nanoparticles for Ocular Drug Delivery. 9-20
3. The Knowledge, Attitude and Practice of Taking Covid-19 Vaccination among the Rural and Urban People in Patuakhali District. 21-26
4. The Impact of Senna Siamea (Lam) Leaves Extracts on 2, 2-Dichlorovinyldimethyl Phosphate Induced Brain Oxidative Stress in Wistar Albino Rats. 27-38
5. Overuse of Steroid Drugs Methylprednisolone and Dexamethasone (Oral) Causes a Diabetic Patient to Become Infected with the Black Fungus in the Corona Virus. 39-45

v. Fellows
vi. Auxiliary Memberships
vii. Preferred Author Guidelines
viii. Index
Evaluation De L’activité Anthelminthique Des Extraits Aqueux De Striga Hermonthica (Del.) Benth Et Ceratotheca Sésamoïdes Endl Sur Deux Stades Biologiques De Haemonchus Contortus


Abstract- Medicinal plants with anthelmintic properties are an alternative to the chemical fight against small ruminant’s gastrointestinal nematodes. In order to broaden the spectrum of medicinal plants with anthelmintic properties, ovicidal activity and L3 larvae exsheathment of C. sesamoïdes Endl and S. hermonthica (Deli) Benth, aqueous extracts has been done on H contortus egg and L3 larvae. Three concentrations, 3.12 mg / ml, 6.25 mg / ml, 12.5 mg / ml of each plant extract were used to assess the inhibition of fresh egg hatching and larval paralysis as well as the inhibition of L3 larvae exsheathment. A negative control (PBS1x) and a positive control (levamisole, 2.5 mg / ml) were constituted for the fresh egg hatching inhibition assessment while only the negative control (PBS1x) was constituted to evaluate the inhibition of L3 Larvae exsheathment. 1 ml of each concentration was contacted with 1 ml of the egg solution and then incubated for 48 hours at 27 °C in petri dishes (60X15 Cm) for hatching test inhibition. 1 ml of each extract concentration was contacted with 1 ml of the embryonated egg solution after 24 hours of incubation at 27 °C for the larval paralysis test.

Keywords: ceratothéca sesamoides endl, striga hermonthica (Del.) benth, H. contortus, egg hatching inhibition, L3 larval exsheathment inhibition.

GJMR-B Classification: NLMC Code: QV 704
Evaluation De L’activité Anthelminthique Des Extraits Aqueux De Striga Hermonthica (Del.) Benth Et Ceratotheca Sésamoïdes Endl Sur Deux Stades Biologiques De Haemonchus Contortus

Amadou Dicko a, Almamy Konate a, Arnaud S. R. Tapsoba 10, L. D. Dahourou Q, Moumouni Sanou y, Adama Kabore b, S. Tembely x, Linda L. Logan x, Amadou Traore 1, Balé Bayala 1 & Hamidou H. Tamboura 1

Résumé- Les plantes médicinales à propriétés anthelminthiques constituent une alternative à la lutte chimique contre les nématodes gastro-intestinaux des petits ruminants. Dans l’objectif d’élargir le spectre des plantes médicinales à propriétés anthelminthiques, une évaluation de l’activité ovicide et d’inhibition du dégainement des larves L3 de H. contortus, des extraits aéreaux de C. sesamoides Endl et S. hermonthica (Del.) Benth a été effectuée. Trois concentrations, 3,12 mg/ml, 6,25 mg/ml, 12,5 mg/ml de chaque extrait de plante ont été utilisées pour évaluer l’inhibition de l’éclosion des œufs frais et de paralysie larvaire ainsi que l’inhibition du dégainement des larves L3. Un Témoin négatif (PBS1x) et un témoin positif (lévamisole, 2,5 mg/ml) ont été constitués pour l’évaluation de l’inhibition de l’éclosion alors que seulement le témoin négatif (PBS1x) a été constitué pour évaluer l’inhibition du dégainement des larves L3. 1 ml de chaque concentration a été mise en contact avec 1 ml de la solution d’œufs puis incubé pendant 48 heures à 27° C dans des boîtes de pétri (60X15 Cm) pour le test de l’inhibition de l’éclosion. 1 ml de chaque concentration d’extrait a été mis en contact avec 1 ml de la solution d’œuf embryonné après 24 heures d’incubation à 27° C pour le test sur la paralysie larvaire. 1 ml de la solution larvaire a été mise en contact avec 4 ml de chaque concentration de la solution d’extrait pendant 3 heures pour l’inhibition du dégainement des larves L3. Les résultats ont montré une inhibition de l’éclosion de 53,89% à la concentration de 12,5 mg/ml avec C. sesamoides Endl approximativement au témoin positif (Lévamisole) 56,49% alors que celui du S. hermonthica n’a été que de 40,72%. Le taux de paralysie larvaire a été de 51,11% et 60,56% respectivement pour S. hermonthica (Del.) Benth et C. sesamoides Endl. L’inhibition du dégainement pour l’extrait aéreaux de C. sesamoides Endl est de 65,33% à 12,5 mg/ml et 64% à la même concentration pour S. hermonthica (Del.) à Benth après 60 minutes.

Mots clés: ceratothéca sesamoides endl, striga hermonthica (Delii) benth, H. contortus, inhibition éclosion œuf, inhibition dégainement larves L3.

Abstract- Medicinal plants with anthelmintic properties are an alternative to the chemical fight against small ruminant’s gastrointestinal nematodes. In order to broaden the spectrum of medicinal plants with anthelmintic properties, ovicidal activity and L3 larvae exsheathment of C. sesamoides Endl and S. hermonthica (Del.) Benth, aqueous extracts has been done on H. contortus egg and L3 larvae. Three concentrations, 3.12 mg / ml, 6.25 mg / ml, 12.5 mg / ml of each plant extract were used to assess the inhibition of fresh egg hatching and larval paralysis as well as the inhibition of L3 larvae exsheathment. A negative control (PBS1x) and a positive control (levamisole, 2.5 mg / ml) were constituted for the fresh egg hatching inhibition assessment while only the negative control (PBS1x) was constituted to evaluate the inhibition of L3 Larvae exsheathment.1 ml of each concentration was contacted with 1 ml of the egg solution and then incubated for 48 hours at 27 °C in petri dishes (60X15 Cm) for hatching test inhibition.1 ml of each extract concentration was contacted with 1 ml of the embryonated egg solution after 24 hours of incubation at 27 °C for the larval paralysis test. 1 ml of the larval solution was contacted with 4 ml of each concentration of the extract solution for L3 larvae exsheathment inhibition test. The results showed a hatching inhibition of 53.89% at the concentration of 12.5 mg / ml with C. sesamoides Endl approximately to the positive control (Levamisole) 56.49% whereas hatching inhibition of S. hermonthica was only of 40.72%. The larval paralysis rate was 51.11% and 60.56% respectively for S. hermonthica (Delii) Benth and C. sesamoides Endl. The inhibition of L3 larvae exsheathment for the aqueous extract of C. sesamoides Endl is 65.33% at 12.5 mg / ml and 64% at the same concentration for S. hermonthica (Delii) Benth after 60 minutes.

Keywords: ceratothéca sesamoides endl, striga hermonthica (Delii) benth, H. contortus, egg hatching inhibition, L3 larval exsheathment inhibition.
I. Introduction

H. contortus est l’un des nématodes gastro-intestinaux des petits ruminants (Mangi et al., 2006). En effet, la faune parasitaire du tractus digestif des petits ruminants est dominée par H. contortus de par sa fréquence et son intensité (Koné et al., 2005; Dedehou et al., 2014; Koffi et al., 2018). Comparativement aux autres espèces de nématodes, H. contortus est le parasite des petits ruminants le plus pathogène capable de provoquer des maladies aiguës et des fortes mortalités dans toutes les catégories des petits ruminants (Akkari et al., 2013; MbogningTayo et al., 2014). Ce parasite hématophage peut causer une anémie conduisant à la réduction de la productivité chez les petits ruminants (Burke et al., 2007).

La gestion des nématodes gastro-intestinaux des petits ruminants repose fortement sur l’utilisation des anthelminthiques de synthèses (Chartier et al., 2014). L’utilisation répétée de ces anthelminthiques de synthèse a entraîné l’apparition des résistances irréversibles des parasites à la majorité des familles des molécules anthelminthiques (Apala et al., 2020). Aussi, en plus de la difficulté d’accès de ces molécules anthelminthiques de synthèse aux éleveurs ruraux, il faut en plus de la difficulté d’accès de ces molécules anthelminthiques de synthèse aux éleveurs ruraux, il faut ajouter l’écotoxicité de ces molécules pour l’environnement (Dedehou et al., 2014).

Face à cette situation différentes alternatives aux molécules anthelminthiques dans le traitement des nématodes gastro-intestinaux sont envisagées. Parmi ces solutions alternatives il y a l’utilisation des plantes fourragères à propriétés anthelminthiques.

L’objectif de cette présente étude est d’évaluer in vitro l’activité ovicide et inhibitrice du dégainement des larves L3 de H. contortus des extraits aqueux du Striga hermontica (Del.) Benth et de C. sésamoides Endl.

II. Matériel et Méthode

a) Milieu d’étude
Les tests in vitro l’évaluation de l’activité ovicide et inhibitrice du dégainement larvaire ont été réalisés au Laboratoire de Biologie et santé Animale (LaBioSA) du Centre de Recherches Environnementales, Agricoles et de Formation (CREAF) de Kamboinsin.

b) Récoltes des plantes
Céramothéca sésamoides Endl et Striga hermontica (Delile) Benth ont été récoltées puis séchées à l’ombre entre fin Septembre et mi-octobre dans une salle à la température ambiante à la Direction Régionale de la Recherche Environnementale et Agricoles (DRREA-Sahel). Les plantes ont été identifiées à l’Herbier National du Burkina Faso (HNBF) respectivement sous les numéros 8758 et 8759.

c) Préparation des extraits
Les plantes entières des deux espèces ont été récoltées tôt le matin et séchées dans une salle à la DRREA-Sahel à la température ambiante pendant 2 semaines et acheminées au CREAF de Kamboinsin par la suite. Les échantillons de chaque plante ont été par la suite broyés en poudre. Une macération aqueuse (100 g de poudre dans 900 ml d’eau distillée) a été effectuée pendant 24 heures sous agitation mécanique à la température ambiante. Les macérations ont été ensuite filtrées et concentrées au congélateur. Les filtrats concentrés ont été par la suite lyophilisées.

d) Tests biologiques
i. Préparations des solutions mères
Les solutions mères de chaque extrait ont été préparées en diluant 0,25 g d’extrait de chaque plante dans 10ml du PBS 1X pour obtenir 25mg/ml de concentration pour chaque extrait. La solution a été homogénéisée à l’aide d’un sonicateur pendant 3 minutes. A partir de la solution mère trois autres solutions de concentrations inférieures qui seront utilisées à savoir 12,5 mg/ml et 6,25mg/ml et 3,12 mg/ml de chaque extrait ont été réalisées. Le lévamisole (anthelminthique de référence) à 2,5 mg/ml et PBS 1X ont été utilisé respectivement comme témoins positif et négatif.

ii. Récoltes des œufs

iii. Obtenion et Récoltes des larves infestantes L3
Les larves L3 ont été obtenues en cultivant pendant 14 jours à 31° C les œufs dans des fèces d’ovins préalablement stérilisées à l’étuve à 130° C pendant 24 heures et mélangés avec de la sciure du bois. Après 14 jours la culture a été montée dans un gaze puis déposé sur le dispositif de Baermann pour récolter les larves L3. La solution larvaire obtenue a été mise dans un bécher et déposée au congélateur pendant 3 heures. Les larves ayant migrées au fond ont été récupérées et centrifugées trois fois à 2000 trs/mns pendant 10 mns. La solution larvaire a été ensuite réajustée à 2000 larves/ ml.

iv. Test d’inhibition de l’éclosion
Le test d’inhibition de l’éclosion a été réalisé selon la méthode modifié de Kaboré. (2009). 1 ml de la solution larvaire réajustée à 100 œufs/ ml a été mise en contact avec 1 ml de chaque concentration d’extraits dans une boîte de pétri (60X15 Cm) puis incubé...
Pendant 48 heures à 27° C. Après 48 heures quelques gouttes de lugol ont été ajoutées dans chaque boîte de pétri pour stopper l’évolution des œufs.

Le test a été répété trois fois avec trois réplicas pour chaque concentration par répétition.

Le nombre de larve et d’œufs non-éclos ont été évalués en déposant 50 µl entre lame et lamelle et observant au microscope à l’objectif X10.

Le pourcentage d’inhibition de l’éclosion des œufs a été calculé selon la formule suivante (Kaboré, 2009):

Pourcentage d’inhibition (%) = 100(1-X1/X2)

X1: Nombre d’œufs éclos dans les extraits testés
X2: Nombre d’œufs éclos dans le témoin négatif

v. Test d’embryonnement

Le test d’embryonnement a été réalisé selon la méthode modifiée de Waboponé et al. (2006) décrite par Kaboré. (2009). 1ml de la solution d’œuf à 100 œufs/ml a été incubé dans des boîtes de pétri (60X15 Cm) à 27° C. 24 heures après, lorsque les larves L1 ont été transparentes et ont commencé à se mouvoir dans les enveloppes des œufs, 1 ml de chacune des concentration test de chaque extrait a été ajouté dans chacune des boîtes de pétri constituant le lot test. 1 ml du PBS 1X et 1 ml de lévamisol à 2,5 mg/ml ont été ajouté dans les boîtes de pétri pour tenir respectivement de témoin négatif et témoin positif. Après 6 heures de contact des extraits et des œufs embryonnés quelques gouttes de lugol ont été ajoutées dans chaque boîte de pétri pour stopper l’évolution des œufs.

Le taux de paralysie larvaire (TPL) a été déterminé selon la formule de D’Angelo et al. (2014):

TPL = 100- [(1-X1/X2)*100]

vi. Test d’inhibition du dégainement larvaire


En plus des trois concentrations (3,12mg/ml; 6,25mg/ml; 12,5mg/ml) des extraits de chaque plante, et un témoin négatif (PBS) réalisées précédemment, une solution dite de Milton (javel 2,6% dilué dans du NaCl 0,9% au 1/300) a été réalisée.

1 ml de la solution larvaire a été incubé avec 4 ml de la solution d’extrait pendant 3h dans des boîtes de pétri (60X15Cm) et agité chaque 30 minute. Après 3h, 200 µl ont été prélevés de chacune des boîtes de pétri et mis en contact avec 200 µl de la solution de milton dans un tube eppendorf 2 ml. Le test a été répété 3 fois.

La cinétique de dégainement a été suivie au microscope X20 aux temps, T0, T20, T40 et T60mns après contact avec la solution de Milton en mettant 50 µl entre et lamelle et en comptant les larves dégainées et engainées.

Le pourcentage des larves dégainées a été calculé selon la formule suivante:

Pourcentage des larves dégainées (%) = (__________)*100

de l’évolution du dégainement des larves L3 en fonction du temps.

e) Analyse des données

Les données ont été saisies sur le logiciel Excel 2016 qui a servi aux calculs des moyennes et Ecarts Type. Les moyennes du taux d’inhibition des éclosions ainsi que celles du taux de paralysies larvaires et du dégainement larvaire ont été comparées à l’aide d’une analyse de variance (ANOVA) à une voie suivi d’une comparaison multiple de moyenne au seuil de 5% avec le Packages Rcmdr version 2.7-1 du logiciel R version 4.1.0.

Une régression linéaire par la méthode probit a été effectuée avec le logiciel SPSS STATISTICA 26 afin de déterminer les concentrations inhibitrices 50% de l’éclosion des œufs et celle qui engendre la paralysie larvaire des œufs embryonnés de 50%.

Le logiciel PRISM GraphPad Software 5.00.288 nous a servi de tracer les graphiques doses-réponses de l’évolution du dégainement des larves L3 en fonction du temps.
### III. Résultats

**Tableau 1:** Effet des extraits aqueux de *Striga hermonthica* (Del.) Benth et *Ceratotheca Sésoamoides* Endl sur l’éclosion des œufs de *Haemonchus contortus*

<table>
<thead>
<tr>
<th>Répétitions</th>
<th>Doses (mg/ml) /Extrait aqueux du <em>S</em> hermonthica (Del.) Benth</th>
<th>Doses (mg/ml) /Extrait aqueux <em>C.</em> sésoamoides Endl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3,12 6,25 12,5 T(-) T(+)</td>
<td>3,12 6,25 12,5 T(-) T(+)</td>
</tr>
<tr>
<td>R1</td>
<td>23,08 46,16 38,46 0 1,86</td>
<td>22,22 50 0 48,16</td>
</tr>
<tr>
<td>R1</td>
<td>5,88 21,58 52,94 0 64,71</td>
<td>17,65 29,41 60,79 0 42,35</td>
</tr>
<tr>
<td>R 3</td>
<td>15,38 23,08 30,76 0 53,84</td>
<td>21,05 22,81 50,88 0 78,95</td>
</tr>
</tbody>
</table>

Moyenne ±σ: 14,78 ±8,61 ab; 30,27 ±13,78 bc; 40,72 ±11,26 cd

R: Répétitions, T (-): Témoin négatif (PBS: Phosphate-Buffered Saline1X), T (+): témoin positif (Lévamisole à 2,5 mg/ml), σ: Ecart-Type, (a, b, c): différences entre les colonnes pour chaque extrait, les colonnes avec les lettres distinctes ont les moyennes de pourcentage d’inhibition statistiquement différentes entre elles au seuil de 5%.

**Tableau 2:** Valeurs des Concentrations entraînant l’inhibition de 50% d’éclosion des œufs obtenues à partir de l’équation de la droite de régression

<table>
<thead>
<tr>
<th>R² d’ajustement</th>
<th>a</th>
<th>b</th>
<th>CI50</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. sésoamoides Endl</td>
<td>0,97</td>
<td>2,15</td>
<td>11,83 mg/ml</td>
</tr>
<tr>
<td>S. hermonthica (Del.) Benth</td>
<td>0,87</td>
<td>1,19</td>
<td>14,93 mg/ml</td>
</tr>
</tbody>
</table>

R²: R² d’ajustement, a: Ordonnée à l’origine, b: pente de la droite de régression, CI50: Concentration qui inhibe 50% de l’éclosion des œufs

Le tableau 1 montre l’effet des extraits aqueux des plantes testées sur l’inhibition de l’éclosion des œufs de *H. contortus*.

L’analyse de ce tableau nous indique que les extraits aqueux des plantes testées ont une activité inhibitrice de l’éclosion des œufs moyenne voire faible. En effet, on remarque une activité inhibitrice légèrement supérieure ou égale à 50% de l’éclosion des œufs seulement à la dose la plus élevée qui est de 12,5 mg/ml. Aussi seulement l’extrait aqueux de *C. sésoamoides* Endl a obtenu un taux moyen d’inhibition de 53,89% alors que celui de l’extrait aqueux du *S. hermonthica* (Del.) Benth est de 40,72%. Ce tableau nous indique aussi une faible activité inhibitrice du lévamisole (témoin positif) qui est de 56,49% en moyenne pour le test avec l’extrait aqueux de *C. sésoamoides* Endl et 57,46% en moyenne avec l’extrait aqueux du *S. hermonthica* (Del.) Benth.

Le tableau 2 nous indique les valeurs de la droite de régression qui nous a permis d’obtenir les concentrations inhibitrices des extraits des deux plantes testées.

**Tableau 3:** Effet des extraits aqueux de *Striga hermonthica* (Del.) Benth et *Ceratotheca sésoamoides* Endl sur la paralysie des larves L1 embryonnées de *Haemonchus contortus*

<table>
<thead>
<tr>
<th>Répétitions</th>
<th>Doses (mg/ml) /Extrait aqueux du <em>S</em> hermonthica (Del.) Benth</th>
<th>Doses (mg/ml) /Extrait aqueux <em>C.</em> sésoamoides Endl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3,12 6,25 12,5 T(-) T(+)</td>
<td>3,12 6,25 12,5 T(-) T(+)</td>
</tr>
<tr>
<td>R1</td>
<td>26,67 26,67 53,34 10 66,67</td>
<td>30 34,34 54,67 5 55</td>
</tr>
<tr>
<td>R1</td>
<td>20 46,67 10 60 36,67</td>
<td>32 60 0 0 60</td>
</tr>
<tr>
<td>R 3</td>
<td>33,34 33,34 53,34 15 73,34</td>
<td>26,67 35 67 10 80</td>
</tr>
</tbody>
</table>

Moyenne ±σ: 26,67 ±3,8; 31,11 ±5,09; 33,78 ±6,12; 60,56 ±5,09; 65,5 ±13,23

R: Répétitions, T (-): Témoin négatif (PBS: Phosphate-Buffered Saline1X), T (+): témoin positif (Lévamisole à 2,5 mg/ml), σ: Ecart-Type, (a, b, c): différences entre les colonnes pour chaque extrait, les colonnes avec les lettres distinctes ont les moyennes de pourcentage de paralysie larvaire statistiquement différentes entre elles au seuil de 5%.
Tableau 4: Valeurs des Concentrations entrainants la Paralysie larvaire de 50% des œufs embryonnées obtenues à partir de l’équation de la droite de régression

<table>
<thead>
<tr>
<th></th>
<th>R² d’ajustement</th>
<th>a</th>
<th>b</th>
<th>CI50</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. sesamoides Endl</td>
<td>0,94</td>
<td>0,84</td>
<td>0,09</td>
<td>9,80 mg/ml</td>
</tr>
<tr>
<td>S. hermonthica (Deli.) Benth</td>
<td>0,97</td>
<td>0,79</td>
<td>0,07</td>
<td>11,79 mg/ml</td>
</tr>
</tbody>
</table>

a: Ordonnée à l’origine b: pente de la droite régression, CI50: Concentration qui entraîne la paralysie larvaire de 50% des œufs embryonnées.

Le tableau 3 nous indique l’effet des extraits aqueux de S. hermonthica (Deli.) Benth et de C. sesamoides Endl sur la paralysie larvaire des œufs embryonnées de H. contortus. Ce tableau nous montre que les extraits aqueux des deux plantes testées ont un taux moyen d’activité de paralysie larvaire des œufs embryonnées de H. contortus. En effet, ce tableau nous indique un taux moyen de paralysie larvaire de 51,11% et 60,56% respectivement pour l’extrait aqueux du S. hermonthica (Deli.) Benth et de celui de C. sesamoides Endl.

Nous remarquons un taux de paralysie larvaire de 11, 66% du lot témoin négatif (PBS1X) pour le test avec l’extrait aqueux du S. hermonthica (Deli.) Benth et 5% pour le test avec l’extrait aqueux de C. sesamoides Endl. Cependant, l’activité du témoin positif (lévamisole) n’a été que de 66,67% et 65% respectivement pour test avec S. hermonthica (Del.) Benth et C. sesamoides Endl. Le tableau 4 nous indique les valeurs de la droite de régression qui nous a permis d’obtenir les concentrations des extraits des deux plantes testées qui engendrent la paralysie larvaire de 50% des œufs embryonnées.

![Extrait aqueux de C.sesamoïdes Endl](image)

**Figure 1:** Evolution du dégainement des larves L3 avec l’extrait aqueux de Ceratotheca sesamoïdes Endl en fonction du temps

La figure 1 nous montre l’évolution du dégainement en fonction du temps après contact avec la solution de Milton des larves L3 mises en contact avec les différentes concentrations de l’extrait aqueux de C. sesamoides Endl. La figure 1 nous montre une évolution similaire des courbes du dégainement des larves mises en contact avec le témoin négatif (PBS 1X) et celles mises en contact avec la dose 3,12 mg/ml. L’analyse de ce graphique nous indique aussi que les doses 6,25 mg/ml et 12,5 mg/ml de l’extrait aqueux de C. sesamoides Endl ont eu une inhibition de dégainement respectivement faible et moyenne des larves.

La comparaison multiple des moyennes du pourcentage de dégainement par la méthode de Tukeys montre une forte significativité entre la moyenne de pourcentage du dégainement des larves L3 au temps (T60) et les temps (T0) ainsi qu’au temps (T20) avec une probabilité p < 0,001 alors que la moyenne du pourcentage du dégainement des larves au temps (T60) et celle au temps (T40) n’a pas été très significative p < 0,011.
Figure 2: Évolution du dégainement des larves L3 avec l’extrait aqueux de Striga hermonthica (Deli.) Benth en fonction du temps

La figure 2 nous montre l’évolution du dégainement des larves L3 en fonction du temps après contact à la solution de Milton des larves L3 mises en contact avec les différentes concentrations de l’extrait aqueux du S. hermonthica (Deli) Benth. Ce graphique nous indique une évolution identique des courbes en fonction du temps de la moyenne de dégainement des larves L3 mises en contact avec les concentrations 3, 12 mg/ml, 6,25 mg/ml et celle mises en contact avec le témoin négatif (PBS 1X).

La comparaison multiple des moyennes par la méthode de Tukey montre une forte significativité entre les moyennes du pourcentage de dégainement du temps (T60) et (T20) ainsi qu’aux temps (T60) et temps (T0) P< 0,001. Par contre les moyennes du pourcentage du déagienement du temps (T20) et temps (T0) ainsi que temps (T40) et temps (T60) n’ont pas été très significatifs avec respectivement P< 0,011 et P< 0,028.

IV. Discussion

Les résultats de cette étude nous montrent que l’inhibition de l’éclosion des œufs de H. contortus est doses-dépendante avec les extraits aqueux de C. sesamoides Endl et S. hermonthica (Deli.) Benth. En effet, l’extrait aqueux de C. sesamoides Endl, a obtenu un taux d’inhibition évolutif en fonction de la concentration qui atteint une moyenne de 53,89% à la dose de 12, 5 mg/ml. L’extrait aqueux du S. hermonthica (Deli.) Benth a aussi eu un effet inhibiteur évolutif en fonction de la concentration mais de moyenne faible n’atteignant pas 50% à la dose de 12,5 mg/ml. Par contre les extraits des deux plantes testées ont eu un effet inhibiteur et évolutif en fonction de la concentration qui atteint respectivement 51,11% pour S. hermonthica (Deli.) Benth et 60,56% pour C. sesamoides Endl. Koffi et al. (2018), ont eu des résultats similaires avec les extraits hydro-méthanoliques à 2000 µg/ml de sept (07) espèces parmi huit (08) dont seulement l’extraits hydro-méthanoliques du Morus mesozyna a eu un de taux d’inhibition de l’éclosion de 84, 08% alors que les sept (07) autres plantes ont eu des taux faibles allant de 5,65% avec Ficus lutea à 28, 08% pour Antiaris africana. Cette dissemblance de résultats avec l’extrait du Morus mesozyna serait due à la nature du solvant d’extraction car les métabolites secondaires extraites des plantes dépendent du solvant d’extraction. Ainsi, l’extraction aqueuse de nos plantes testées pourrait ne pas contenir les métabolite secondaire pouvant inhiber l’éclosions des œufs jusqu’à un tel pourcentage que celui du Morus mesozyna. Par contre le fait que les sept (07) autres plantes testées par ces auteurs n’ont pas eu 50% d’inhibition de l’éclosions pourrait aussi s’expliquer par la nature leurs solvant d’extractions et aussi par la résistance à des œufs de H. contortus qui sont naturellement très résistante. D’Angelo et al. (2014) ont aussi eu des résultats différents au notre avec les extraits methylene Choloro-méthanolique de l’écocé du tronc de Annona senegalensis à différentes doses sur Heligmosomoides Bakeri. En effet, ces auteurs, ont obtenu un taux d’inhibition de l’éclosion des œufs de 16, 10% alors que le taux de paralysie embryonnaire des œufs embryonnées était de 20,80% à la dose la plus élevée qui est de 5000µg/ml. Cette différence de résultats serait due à la différence de l’espèce de nématode étudiée car notre présente étude porte sur H. contortus alors que leur étude à porte sur
**Heligmosoides Bakeri.** Aussi, cette différence pourrait être due à la différence des plantes utilisées et à la différence des solvants d’extraction qui favorisent la présence de certains métabolites secondaires que d’autres. Les extraits des feuilles de A. leiocarpus et des écorces du tronc de D. oliveri ont eu un effet dose-dépendant sur la paralysie des œufs embryonnés avec respectivement une CI50 de 411,4 µg/ml pour A. leiocarpus et 362,3 µg/ml pour D. oliveri et une inhibition de l’éclosion des œufs de 100% pour les extraits aqueux de ces deux plantes (Kaboré, 2009). La différence d’efficacité de ces plantes avec celles des plantes utilisées dans notre présente étude pourrait être due à la nature des plantes utilisées. En effet, notre présente étude a porté sur des herbacées entières alors que cette étude a porté sur des feuilles et écorces de ligneux.

Cette présente étude nous montre à travers les résultats obtenus que les extraits aqueux de C. sesamoïdes Endl et ceux du S. hermonthica (Del.) Benth ont une inhibition qui est dose-dépendante sur le dégainement des larves L3 de *H. contortus*. En effet, les figure 1 et 2 nous montrent une évolution similaire de la cinétique du dégainement des larves mises en contact avec le témoin négatif (PBS1x) et celles mises en contact avec la dose de 3,12 mg/ml. Par contre l’évolution des courbes des larves mises en contact avec le témoin négatif (PBS1x) et celles des larves mises en contact avec la dose de 3,12 mg/ml est différente de celle des larves mises en contact avec les doses de 6,25 mg/ml et de celles des larves mises en contact avec la dose de 12,5 mg/ml dont l’évolution de l’inhibition du dégainement est juste au-dessus de 50%. Les extraits aqueux et méthanolique d’*Acacia nilotica* ainsi que A. raddiana ont obtenu une inhibition du dégainement des larves L3 mise en contact avec les doses de 1,2 mg/ml et 0,6 mg/ml qui atteint 100% d’inhibition (Zabré, 2018). La différence avec les résultats obtenus dans cette présente étude pourrait être due à la nature des plantes utilisées car notre présente étude a porté sur les herbacées entières alors qu’il s’agit des feuilles de ligneux dans l’autre étude.

Cette efficacité moyenne des extraits aqueux de C. sesamoïdes Endl et S. hermonthica (Del.) Benth serait due à la présence de certains métabolites secondaires tels que les tanins et les flavonoïdes dans ces extraits aqueux. Cependant au vu de résultats d’inhibition de l’éclosion des œufs frais et des œufs embryonnés (paralysie larvaire) ainsi que celui du dégainement des larves L3 nous pouvons émettre l’hypothèse que la solution aqueuse ne permet pas d’extraire la dose suffisante à inhiber l’évolution des œufs et des larves à 100%.

**V. Conclusion**

La présente étude confirme que les extraits aqueux de C. sesamoïdes Endl, et S. hermonthica (Del.) Benth contiennent des composés bioactifs qui inhibe l’évolution des œufs et le dégainement des larves L3 de *H. contortus*. Cependant, du fait que l’inhibition de l’évolution des œufs et du dégainement des larves L3 n’atteint pas 100% nous laisse penser que la présence de ces composés bioactifs serait optimisée par un autre solvant d’extraction. Toutefois les extraits aqueux de C. sesamoïdes Endl et S. hermonthica (Del.) Benth pourraient être employés comme désinfectant sur les pâturages pour interrompre le cycle évolutif des nématodes et diminuer ainsi la charge parasitaire des pâturages.

**Bibliographie**

1. **Adama Kabore, 2009:** Activités anthelminthiques de deux plantes tropicales testées *in vivo* et *in vitro* sur les strongyles gastro-intestinaux des ovin s mosis du Burkina Faso. Thèse de doctorat unique en développement rural, Université polytechnique de Bobo-Dioulasso. 167p.


Supercritical Fluids as a State-of-the-Art Formulation Method of Nanoparticles for Ocular Drug Delivery

By Naida Omerović & Edina Vranić

University of Sarajevo

Abstract- Conventional ophthalmic dosage forms, although being simple to apply and presenting great patients' compliance, display poorer drug bioavailability and retention time on the eye surface. To cope with these problems, one must formulate novel drug delivery systems, such as nanosystems, for ocular drug delivery. Different formulation methods of nanoparticles have been developed, but some of them, such as the supercritical fluid method, have not reached their full potential in ocular drug delivery. This article aims to present the possibilities of the supercritical fluid method when preparing nanosystems for ocular drug delivery. This method could be used more frequently and efficiently because it is environmentally friendly and produces nanoparticles of the desired physicochemical properties, which is especially important in ocular drug delivery considering its peculiarities. Modifications of the supercritical fluid method can be used when a drug has some specific properties, which is an additional benefit in ocular drug delivery.

Keywords: nanosystems, nanoparticles, ocular drug delivery, supercritical fluids.

GJMR-B Classification: NLMC Code: QV 785
Supercritical Fluids as a State-of-the-Art Formulation Method of Nanoparticles for Ocular Drug Delivery

Supercritical Fluids in Nano-Ophthalmology

Naida Omerović & Edina Vranić

Abstract- Conventional ophthalmic dosage forms, although being simple to apply and presenting great patients' compliance, display poorer drug bioavailability and retention time on the eye surface. To cope with these problems, one must formulate novel drug delivery systems, such as nanosystems, for ocular drug delivery. Different formulation methods of nanoparticles have been developed, but some of them, such as the supercritical fluid method, have not reached their full potential in ocular drug delivery. This article aims to present the possibilities of the supercritical fluid method when preparing nanosystems for ocular drug delivery. This method could be used more frequently and efficiently because it is environmentally friendly and produces nanoparticles of the desired physicochemical properties, which is especially important in ocular drug delivery considering its peculiarities. Modifications of the supercritical fluid method can be used when a drug has some specific properties, which is an additional benefit in ocular drug delivery.

Keywords: nanosystems, nanoparticles, ocular drug delivery, supercritical fluids.

I. INTRODUCTION

Different eye barriers present a challenge for drug delivery to the eye (Figure 1). These barriers are [1,2]:

- Anatomical (conjunctiva, sclera, choroid, retina, with the blood-retinal barrier (BRB) comprising retinal capillary endothelial cells and retinal pigment epithelium (RPE)).
- Physiological (removal of the solution by blinking, dilution of the solution by tearing).

![Figure 1: Different eye layers](image-url)
They are an obstacle during the development of different ophthalmic dosage forms [3,4]. Conventional ophthalmic dosage forms include eye drops, ointments, suspensions, and emulsions. These formulations have proven their advantages, such as great patients' compliance and easy applicability, but their limitations include more frequent administration to get the therapeutic drug concentration, as well as poor drug bioavailability. Conventional ophthalmic dosage forms are more powerful in the treatment of the diseases of anterior rather than posterior eye segment. Previously mentioned dosage forms can also cause the occurrence of eye irritation, eye redness, and visual impairment [5,6].

To cope with these disadvantages of conventional ophthalmic dosage forms, novel drug delivery systems for ocular drug delivery are being developed, wherein nanosystems get special attention. Nanosystems that can be used as ocular drug delivery systems include nanoparticles, nanomicelles, liposomes, niosomes, dendrimers, nanosuspensions, nanoemulsions, nanocrystals, etc (Figure 2). Their advantages are [7,8]:

- Higher: drug permeability, bioavailability, and retention on the eye surface
- Lower: drug degradation, risk of visual impairment, required drug doses, and occurrence of side effects.

There are two classifications of nanoparticles, the first being nanospheres and nanocapsules, and the second being polymeric nanoparticles and lipid nanoparticles. Nanospheres are matrix systems, in which a drug is adsorbed on the surface or evenly dispersed in the matrix. They have a solid core with a dense polymer around it, with a size from 100 to 1000 nm. Nanocapsules are vesicular systems, in which a drug is usually dissolved in the particle's nucleus, but it can also be adsorbed on the surface. They have an oil-filled cavity with a firm polymeric envelope around it, with a size from 10 to 1000 nm [9,10]. In polymeric nanoparticles, a drug is dissolved or encapsulated in different polymers. These nanoparticles have a size from
10 to 100 nm, are biodegradable, non-toxic, biocompatible, and mucoadhesive. Polymers can be made either by the direct process of polymerisation of different monomers or from derived polymers [11]. Lipid nanoparticles comprise lipids that are solid at room and body temperature, unlike water/oil (w/o) emulsions that have liquid lipids. These nanoparticles have a size from 150 to 300 nm, are non-toxic and long-term stable, protect a drug from its degradation, present controlled drug release and improved drug bioavailability. There are two groups of lipid nanoparticles: solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) [12]. The SLN comprise a solid lipid with an incorporated drug, and water, co-emulsifiers, and emulsifiers as excipients [13]. The NLC incorporate liquid and solid lipids as the basic matrix [14].

Factors that must be considered when designing an ocular drug delivery nanosystem are [15]:
• Size of nanoparticles
• Blood and lymphatic circulations
• Melanin pigmentation
• Diseases
• Functionalization of nanoparticles
• Charge of nanoparticles.

These factors are discussed in the following paragraphs.

Amrite et al. conducted a study to determine the distribution of carboxylate-modified, negatively charged polystyrene (PS) nanoparticles in the eye. Nanoparticles, sizes of 20 nm and 200 nm, were applied subconjunctivally to Sprague Dawley rats. Nanoparticles of 200 nm were detected two months post-application in the periocular tissue of the eye, whereas nanoparticles of 20 nm were not, as they were fastly eliminated from that tissue. Subconjunctivally applied nanoparticles of 200 nm and larger could be kept at the site of application for at least two months, which meant that nanosystems of this size could be useful as controlled drug delivery nanosystems for periocular application [16]. Amrite et al. conducted another study to investigate the transscleral permeability of celecoxib-loaded nanoparticles, sizes of 20 nm and 200 nm, in the presence and absence of blood and lymphatic circulations in Sprague Dawley rats. Various clearance rates showed that the clearance of nanoparticles affected the concentrations of celecoxib in the retina. Larger nanoparticles showed sustained drug delivery in the retina better compared with the smaller ones, but these differences were reduced with an increase in the release rate of celecoxib from nanoparticles. Blood and lymphatic circulations played an important role in the clearance of the 20 nm-sized nanoparticles, which were transported across the sclera in a minimal amount, but with significant transport across the sclera-choroid-RPE, because of their fast disposition in the presence of blood and lymphatic circulations. The 200 nm-sized nanoparticles displayed low clearance by blood and lymphatic circulations and were suitable for controlled transscleral drug delivery [17].

Cheruvu et al. conducted a study to determine the effect of the melanin pigmentation on the transscleral delivery of celecoxib-loaded nanoparticles in Sprague Dawley (non-pigmented, albino) and Brown Norway (pigmented) rats. The affinity and the extent of the binding of celecoxib to natural melanin were not significantly different compared with those to synthetic melanin. Transscleral retinal and vitreal drug deliveries were significantly lower in Brown Norway rats compared with those in Sprague Dawley rats, which could be attributed to the significant binding of celecoxib to melanin and its accumulation in the melanin-rich choroid-RPE [18]. Cheruvu et al. conducted another study to investigate the effect of diabetes on the transscleral delivery of celecoxib-loaded nanoparticles in Sprague Dawley and Brown Norway rats. Transscleral retinal and vitreal drug deliveries were significantly higher in diabetic animals of both Sprague Dawley and Brown Norway rats, which could be attributed to the disruption of the BRB because of diabetes. Transscleral delivery of celecoxib was higher in Brown Norway rats compared with that in Sprague Dawley rats [19].

Kompella et al. conducted a study to determine whether topical ocular drug delivery of nanoparticles of size <100 nm could be enhanced by their coating with peptides or proteins in the bovine eye. The uptake of nanoparticles was in the following order: corneal epithelium>stroma>endothelium, with concentrations in the aqueous humour being undetectable. Transport across the cornea was enhanced because of the functionalization of PS nanoparticles of 20 nm with deslorelin, a luteinizing hormone-releasing hormone (LHRH) agonist, as well as transferrin. Surface modification of nanoparticles by conjugating a LHRH agonist or transferrin was useful to provide fast and efficient delivery of nanoparticles across the cornea [20]. Eljarrat-Binstock et al. conducted a study to determine the penetration of positively and negatively charged fluorescent nanoparticles in rabbits. Positively charged nanoparticles showed better penetration into the inner ocular tissues compared with the negatively charged ones [21]. Kim et al. conducted a study to investigate the movement of intravitreally applied human serum albumin (HSA) nanoparticles concerning the surface charge of nanoparticles. Either cationic or anionic HSA nanoparticles were injected to determine the effect of surface charge on the intravitreal movement of nanoparticles. Anionic nanoparticles with a surface charge of -33.3 ± 6.1 mV diffused more easily through the collagen fibres compared with the cationic ones with a surface charge of 11.7 ± 7.2 mV. Anionic HSA nanoparticles presented a promising drug delivery nanosystem to the subretinal ocular tissue and RPE.
Supercritical Fluids as a State-of-the-Art Formulation Method of Nanoparticles for Ocular Drug Delivery

II. Formulation Methods of Nanoparticles for Ocular Drug Delivery: Classification and Steps

The most important methods of formulating the nanoparticles that are used in ocular drug delivery can be classified as [24,25]:
- Polymerisation of a monomer:
  - Emulsion polymerisation
  - Interfacial polymerisation
  - Interfacial polycondensation
- Production from pre-formed polymers:
  - Solvent removal
  - Interfacial deposition
  - Emulsification – solvent evaporation
  - Emulsification – solvent diffusion
  - Salting-out
- Production from natural macromolecules:
  - Ionic gelation
  - Desolvation of macromolecules
  - Newer methods: supercritical fluid
  - Other methods: homogenisation and milling.

a) Supercritical fluid

A supercritical fluid is a substance under a condition above its critical points, which are the highest temperature and pressure at which a substance exists as the gas and the liquid in equilibrium. Thus, a supercritical fluid shows the properties of both gases (such as penetration) and liquids (such as dissolution) simultaneously. Gases, such as carbon dioxide (CO₂), ammonia (NH₃), or water (H₂O), can be converted to supercritical fluids under particular conditions. But only supercritical CO₂ can be economically useful because its critical points (temperature = 31.1 °C, pressure = 73.8 bar) are relatively easy to reach [26].

Supercritical fluids have great potential in the production of nanosystems but have not been used frequently in ocular drug delivery. Studies conducted so far include the preparation of dexamethasone [27] and griseofulvin [28] nanoparticles. This method can replace the use of organic solvents while preparing nanoparticles for advanced drug delivery and formulation systems, such as the ocular ones. What makes a difference between conventional formulation methods, such as freeze- or spray-drying, where larger particles are primarily formed and then reduced to the desired size, is the fact that the supercritical fluid method instantly forms particles in a manner to get the desired properties. This is the way to avoid the unwanted effects caused by the energy transfer in the system, which is needed to reduce the size of particles. Particles formed this way do not have to undergo additional treatment, which is a significant advantage [29,30].

The most important characteristic of this method is the fact that there is no phase boundary between the gas and the liquid, so there is continuity in the physicochemical properties of the fluid. With small variations in pressure and temperature of the system, the properties of supercritical fluids are alterable from the gas to the liquid, therefore the viscosity and diffusivity are closer to those of the gas and the density is closer to that of the liquid [31].

The most important types of the supercritical fluid method include [24,32]:
- Supercritical anti-solvent (SAS)
- Rapid expansion of the supercritical solution (RESS).
  
  i. Supercritical anti-solvent (SAS)

The SAS method includes the precipitation of a solute in a compressed fluid under supercritical conditions. The precipitation can be done in two ways [33]:
1. An anti-solvent can be added to a solution (normal-addition precipitation)
2. A solution can be added to an anti-solvent (reverse-addition precipitation).

A supercritical anti-solvent must be miscible with a solution solvent, whereas a solute must not be soluble in a supercritical anti-solvent [33].

In normal-addition precipitation, first, a solute is dissolved in a liquid solvent, and second, a supercritical anti-solvent is added to a solution. The latter is done in a partially filled container at the ambient pressure. With the addition of a supercritical anti-solvent, both the pressure of a closed container and the volume of a solution/anti-solvent mixture increase. With an increase in the anti-
solvent fraction in the mixture and a decrease in solubility of a solute, the precipitation of a solute occurs. To get nanoparticles of the desired physicochemical properties, the precipitate is washed with an anti-solvent [33].

In reverse-addition precipitation, first, a liquid solution is sprayed into a supercritical anti-solvent. The precipitation of a solute occurs because of the fast diffusion of a solvent from the solution sprayed into a supercritical anti-solvent. Second, the precipitate is washed with an anti-solvent and filtered to get nanoparticles of the desired physicochemical properties [33].

No matter whether the precipitation of a solute is normal or reverse, both the size and size distribution of nanoparticles depend on [33]:
- The selection of a solution/anti-solvent mixture
- The concentration of a solute
- The relative quantities of a solution and an anti-solvent
- The rate of the anti-solvent addition
- The degree of mixing.

In a study conducted by Subramaniam et al., the size of nanoparticles depended on the pressure because smaller nanoparticles are formed when the pressure of an added anti-solvent increases. This was attributed to the faster nucleation of a solute [34]. In a study conducted by Rantakylä et al., the size of nanoparticles depended on both temperature and pressure, but was independent of the nozzle exit velocity and diameter. The initial size of droplets did not affect the size of nanoparticles [35].

The SAS method is presented in Figure 3.

![Figure 3: The supercritical anti-solvent (SAS) method](image)

1. An active ingredient and a coating material are dissolved in an organic solvent.
2. The organic solution is sprayed into supercritical CO₂.
3. Supercritical CO₂, as an anti-solvent, leads to supersaturation and precipitation of nanoparticles.
4. When the expansion occurs, CO₂ reverts to its gaseous state, while an organic solvent is being removed.

One advantage of the SAS method is the fact that supercritical fluids are environmentally safe, thus this method could be used for preparing biodegradable nanoparticles, which is recommended in ocular drug delivery. Nevertheless, there are some new modifications of this method, which include the nebulisation of a solution by supercritical fluids that also behave as anti-solvents to precipitate a solute. Here, a solution and a supercritical fluid are mixed and sprayed into a drying atmosphere. Examples are [36–39]:
- CO₂-assisted nebulisation with a bubble dryer (CAN-BD)
- Supercritical fluid-assisted atomisation (SAA).

A big disadvantage of the SAS method is the fact that the formation of nanoparticles is followed by a long drying period, which can lead to their agglomeration and aggregation. Still, this problem is manageable by intensive mixing of a solution with a supercritical anti-solvent. This can lead to a more efficient and enhanced mass transfer caused by the sonic waves in an energising gas stream, as well as the smaller size of droplets, by using an ultrasonic nozzle. The preparation of nanoparticles at low temperature is...
important for drugs that are thermally labile or shock-sensitive, such as some drugs used in the treatment of ocular diseases. Still, many substances have a higher solubility in liquid solvents compared with low-temperature supercritical fluids, and the SAS method allows a higher flow rate [40].

ii. Rapid expansion of the supercritical solution (RESS)

The RESS method differs from the SAS method because, in the RESS method, first, a solute is dissolved in a supercritical fluid to form a solution, and second, a solution is quickly expanded into an area of lower pressure or ambient air [41–43]. A rapid decrease in pressure and density causes the precipitation of a solute. A supercritical solution can be generated in two ways, by [44]:

- Heating and pressurizing a solution at room temperature (expansion is performed at a constant concentration)
- Continuously extracting a solute with an extraction column (useful for solutes insoluble in solvents).

The temperature of a solvent can differ from or be the same as the temperature at which the rapid expansion is performed. Both the extraction temperature and flow rate control the concentration of a solute. Rapid expansion from supercritical to ambient pressure leads to supersaturation because the solubility of supercritical fluids can be over 100 times higher compared with that under ideal gas conditions. Fast reduction of pressure results in the homogeneous nucleation of a solute but narrow size distribution of nanoparticles [44].

The RASS method can be used for preparing organic, inorganic, or polymeric nanomaterials. Fibres of polymeric nanomaterials can be prepared under the controlled expansion conditions, therefore variations in these conditions allow modifications of physicochemical properties of nanoparticles. Pre-expansion temperatures that are significantly higher or lower compared with the melting point of a polymer are used to create particles, whereas those that are close to the melting point of a polymer are used to create fibres [44].

The RASS method could also create nanoparticles with the controlled release of drugs, which could be very useful in ocular drug delivery [29]. The physicochemical properties of nanoparticles depend on various processing conditions, which allow control over them. Microparticles are generally obtained as primary products, but nanoparticles (100–300 nm) can also be produced in the RESS method by using the appropriate nozzles [45].

The RESS method requires minimum to no organic solvent. The precipitation process is enhanced because of the decrease in pressure and temperature that a solute goes through during the rapid expansion [46]. Caution should be taken regarding the occurrence of agglomeration and aggregation during the rapid expansion [47,48].

A big disadvantage of the RESS method with supercritical CO₂ is the low solubility of polar drugs. This method only applies to solutes that show good solubility in supercritical CO₂. Poorly soluble substances with high molecular weights and polar bonds are great candidates for preparing nanoparticles, but many of them have low to negligible solubility in supercritical CO₂ at temperatures less than 60 °C and pressures less than 300 bar. Co-solvents could be added to CO₂ to enhance drug solubility [46]. To cope with this problem, a modified method of RESS with solid co-solvent (RESS-SC) was proposed. A solid co-solvent provides an obstacle for coagulation, enhances the solubility, and can be later removed by lyophilisation (sublimation) [49–51]. Rapid expansion from supercritical to aqueous solution (RESAS) is another modification, which produces nanoparticles of water-insoluble drugs [52–54].

The RESS method is presented in Figure 4.
1. An active ingredient and a coating material are dissolved in supercritical CO₂.
2. When the expansion occurs, CO₂ reverts to its gaseous state.
3. Nanoparticles are formed because of the decrease in solubility and precipitation of an active ingredient and a coating material.

iii. Operating parameters affecting the properties of nanoparticles formed by the supercritical fluid method

Various thermodynamic and aerodynamic factors, as well as properties of the material, affect the morphology, shape, size, and size distribution of nanoparticles. Thermodynamic factors include temperature, pressure, rate of addition of substances, as well as phase and composition changes during the expansion. Aerodynamic factors include nozzle geometry, mechanical shear, and impact distance of the jet against a surface [46].

The supercritical fluid method allows the production of tiny particles (such as nanoparticles) and experiments conducted so far have shown that the size of particles, as well as some other properties, depend on a solvent, a solute, and the pre-expansion conditions. Still, there are some dilemmas regarding the relationship between the conditions of the process and the mechanism of the formation of particles. The interaction between nucleation, condensation, and coagulation is also important to understand. Thus, there is a need to model the supercritical fluid method and its conditions to better understand how different operating parameters affect the properties of the final product, such as nanoparticles. Some of the most cited studies conducted regarding the operating parameters affecting the properties of nanoparticles formed by the supercritical fluid method are presented in Table 1, along with their most important remarks.

Table 1: Studies conducted regarding the operating parameters affecting the properties of nanoparticles formed by the supercritical fluid method

<table>
<thead>
<tr>
<th>STUDY</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>[55]</td>
<td>Mathematical modelling of particle growth and nucleation during the supercritical fluid method was presented. The flow rate was steady and one-dimensional. Partial expansion of supercritical solutions was proven to be an effective way to form powders of desired size and other properties. The size of particles responded to the extraction temperature (the temperature at which a solute is dissolved in a supercritical fluid), as well as the pre-expansion temperature (the temperature at which the saturated mixture is isobarically pre-heated before the expansion). A decrease in solubility upon isobaric heating embodied three trends predicted by calculations: an increase in the size of particles upon the increase in pre-expansion temperature and a decrease in the size of particles upon the increase in extraction temperature and pressure.</td>
</tr>
<tr>
<td>[56]</td>
<td>Hydrodynamic modelling of the RESS method was presented. The expansion occurred at the nozzle inlet, along with the nozzle, and in the expansion chamber, and the thermodynamic transformations for each step were calculated, along with the temperature and pressure profiles. Flow rate at the nozzle exit was measured for two supercritical fluids, CO₂ and fluoroform (CHF₃), at different pre-expansion temperatures and pressures. Computed values were comparable with the experimental data.</td>
</tr>
</tbody>
</table>
A one-dimensional flow modelling of the RESS method was presented. The emphasis was on the wall friction in the nozzle and heat interchange with the surrounding region. Calculations were performed on the ibuprofen–supercritical CO₂ system and supersaturation, as well as nucleation, were considered. Results showed that very high supersaturation and nucleation rates can be obtained when the fluid leaves the nozzle exit. A decrease in the size of particles was also visible. Sensitivity analyses showed that pre-expansion temperatures and pressures, as well as the nozzle length, did not significantly influence the flow rate.

Modelling of the thermodynamic behaviour of supercritical solutions during the RESS method was presented and an impact of the solubility and surface tension of different solutes in supercritical fluids on nucleation rates under typical RESS conditions was investigated. Calculations showed that the nucleation rate is a solubility and surface tension function. It was shown that it is not possible to investigate coagulation and homogeneous nucleation separately. Also, the information about the properties of a solute needs to be more reliable.

Experiments were carried out with CO₂ and CHF₃ as supercritical fluids, as well as with cholesterol, benzoic acid, and griseofulvin. It was shown that it is possible to produce particles of the desired size, which leads to improved dissolution. Flow rate and the formation and growth of particles during the RESS method were modelled numerically. The flow rate was steady and one-dimensional. Results showed that the formation of particles mainly occurs in the supersonic free jet and that the primary mechanism of particle growth is coagulation.

The size distribution of fine powders formed during the RESS method depends on the operating parameters and the geometry of the expansion utensil. Homogeneous nucleation, condensation, and coagulation during the expansion of a non-volatile solute in a supercritical fluid were modelled. Calculations showed that the RESS method is very successful in the formation of particles in the diameter range of 10–50 nm. Delayed nucleation, precipitation of a small solute fraction, and narrow size distribution of particles were mostly displayed.

In this study, it was shown that the aerodynamic factors remain constant over a wide range of pressures. The formation of particles was probably a result of gas-phase nucleation and growth within the expansion utensil, rather than within discrete liquid droplets.

In this study with the toluene (solvent)–CO₂ (anti-solvent) system, it was displayed that the size of droplets depends on densities of both solvent and anti-solvent. It is recommended to work in a single-phase zone at high pressure because higher mass transfer and supersaturation ratio are then achieved. Mathematical modelling of mass transfer between an organic solvent and an anti-solvent was presented when two phases were miscible. The mass transfer behaviour of droplets could affect the morphology of particles. Under supercritical conditions of the mixture, a solvent and an anti-solvent were miscible. Thus, the interface between the droplets and their environment was not defined. By defining a radius of droplets based on the difference in density between a solvent and an anti-solvent, the mass transfer can be calculated, as well as an impact of the operating parameters. Calculations showed droplets swell when a solvent has a higher density compared with an anti-solvent, and shrink when an anti-solvent has a higher density compared with a solvent. Near the critical points of the mixture, droplets swelled and were more sensitive to changes in temperature and pressure. The lifetime of droplets was significantly shorter under miscible conditions. When the critical points of the mixture are close, droplets swelled less. The extent of droplet swelling or shrinking was a temperature and pressure function correlated to the difference in density and diffusivity between a solvent and an anti-solvent. Supercritical conditions resulted in faster mass transfer, causing higher supersaturation and nucleation rates but the smaller size of particles.

The authors of these two studies investigated whether the spherical shape of particles results from the drying of droplets after the atomisation of a liquid solution. Above the critical pressure, the surface tension influences the formation of particles. When atomisation is not obtained, the precipitation from the fluid phase produces particles. They also investigated the relationship between the solute concentration, the vessel temperature and pressure, as well as the fraction of CO₂. They concluded that the fraction of CO₂ greater than 0.95–0.97 is the most useful for the formation of smaller particles. Critical points of the mixture of CO₂ and a liquid organic solvent were then achieved.

### III. Conclusion

Novel drug delivery systems are continuously being developed for ocular drug delivery because of their many advantages compared with conventional ophthalmic dosage forms. Nanoparticles have especially been receiving attention. The most important formulation methods of nanoparticles for ocular drug delivery include polymerisation of a monomer (emulsion polymerisation, interfacial polymerisation, interfacial polycondensation), production from pre-formed polymers (solvent removal, interfacial deposition, emulsification – solvent evaporation, emulsification – solvent diffusion, salting-out), production from natural macromolecules (ionic gelation), desolvation of macromolecules, newer formulation methods (supercritical fluid), and other formulation methods (homogenisation, milling). The choice of the formulation method depends on an active pharmaceutical ingredient and other substances used, as well as the desired physicochemical properties of nanoparticles. New formulation methods are constantly being developed to get the most out of nanotechnology, such as the supercritical fluid method. The SAS method uses a liquid solvent and a supercritical fluid, which are miscible with each other, but a solute is not soluble in a...
Supercritical Fluids as a State-of-the-Art Formulation Method of Nanoparticles for Ocular Drug Delivery

Supercritical fluid. Instantaneous precipitation of a solute, which is formed because of the extraction of a liquid solvent by a supercritical fluid, causes the formation of nanoparticles. In the RESS method, a supercritical fluid dissolves a solute. Consequently, the solvent power of a supercritical fluid is reduced and a solute precipitates because of its rapid expansion into an area of lower pressure. These methods can create nanoparticles of adequate size, size distribution, and other properties for ocular drug delivery, and should be applied more regularly.

Conflict of interest

The authors declare that there was no conflict of interest that could be perceived as prejudicing the impartiality of this research.

Originality

The authors confirm that this manuscript is their original work and has not been previously published nor is sent to another journal for consideration.

Funding

The authors declare that there was no financial support and sponsorship for this research and its publication and that it did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors’ contributions

Naida Omerović: concept/design, data collection, data analysis/interpretation, drafting the manuscript, critical revision of the manuscript, approval of the manuscript; Edina Vranić: concept/design, data analysis/interpretation, critical revision of the manuscript, approval of the manuscript.

Consent

Not applicable.

Ethical approval

Not applicable.

References Références Referencias


61. Lengsfeld CS, Delplanque JP, Barocas VH, Randolph TW. Mechanism governing microparticle morphology during precipitation by a compressed antisolvent: atomization vs nucleation and growth. J...


Abbreviations
BRB – blood-retinal barrier
CAN-BD – CO2-assisted nebulisation with a bubble dryer
HSA – human serum albumin
LHRH – luteinizing hormone-releasing hormone
NLC – nanostructured lipid carriers
PEG – poly(ethylene glycol)
PS – polystyrene
RESAS – rapid expansion from supercritical to aqueous solution
RESS – rapid expansion of the supercritical solution
RESS-SC – rapid expansion of the supercritical solution with solid co-solvent
RPE – retinal pigment epithelium
SAA – supercritical fluid-assisted atomisation
SAS – supercritical anti-solvent
SLN – solid lipid nanoparticles
w/o – water/oil
The Knowledge, Attitude and Practice of Taking Covid-19 Vaccination among the Rural and Urban People in Patuakhali District

By Sultan Ahmed Sikder, Sharmin Jahan Shubarna, Sahedul Islam Bhuiyan & Mohoshina Karim

Abstract- Objective: In this study our main goal is to evaluate the Knowledge, Attitude and Practice of taking Covid-19 vaccination among the rural and urban people in Patuakhali District.

Method: This cross-sectional study was carried out at Tertiary medical College Hospital, Patuakhali District. Bangladesh. Where data were collected from July 2020 to May 2021. A total of 120 participants where in rural 60 participants and in urban 60 participants were included in the study.

Results: In this study total respondents were 120. In rural group highest 23 (38.33%) respondents belong to 32-43 years and in urban group highest 31 (51.66%) respondents belong to 44-55 years. Most of the respondents were male. In rural area highest 30 (50%) respondents were HSC passed followed by primary 13 (21.7%) and in urban area highest 25 (41.25%) respondents were HSC followed by graduate 18 (30%).

Keywords: Covid-19, vaccine, KAP study.

GJMR-B Classification: NLMC Code: WA 115

© 2021. Sultan Ahmed Sikder, Sharmin Jahan Shubarna, Sahedul Islam Bhuiyan & Mohoshina Karim. This research/review article is distributed under the terms of the Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0). You must give appropriate credit to authors and reference this article if parts of the article are reproduced in any manner. Applicable licensing terms are at https://creativecommons.org/licenses/by-nc-nd/4.0/.
The Knowledge, Attitude and Practice of Taking Covid-19 Vaccination among the Rural and Urban People in Patuakhali District

Sultan Ahmed Sikder a, Sharmin Jahan Shubarna a, Sahedul Islam Bhuiyan a & Mohoshina Karim a

Abstract: Objective: In this study our main goal is to evaluate the Knowledge, Attitude and Practice of taking Covid-19 vaccination among the rural and urban people in Patuakhali District.

Method: This cross-sectional study was carried out at Tertiary medical College Hospital, Patuakhali District. Bangladesh. Where data were collected from July 2020 to May 2021. A total of 120 participants where in rural 60 participants and in urban 60 participants were included in the study.

Results: In this study total respondents were 120. In rural group highest 23 (38.33%) respondents belong to 32-43 years and in urban group highest 31 (51.66%) respondents belong to 44-55 years. Most of the respondents were male. In rural area highest 30 (50%) respondents were HSC passed followed by primary 13 (21.7%) and in urban area highest 25 (41.25%) respondents were HSC followed by graduate 18 (30%). The association of Covid-19 infected status between rural & urban respondents were highly significant (P= 0.00), similarly knowledge regarding first vaccine in Bangladesh also highly significant (p=0.02) between rural & urban respondents. Another findings regarding knowledge related factor regarding the lowest age range for this vaccination was moderately significant (p=0.03), finally knowledge related factor regarding the way to take the vaccine was also significantly associated (p=0.04) between the respondents of rural & urban area, beside the knowledge related factors the other variables were not significantly associated. The association of Covid-19 vaccine taken status between rural & urban respondents were highly significant (P= 0.00), similarly the opinion regarding so many rumors about corona vaccine also moderately significant (p=0.03) others attitude and practice related factors were not significantly associated.

Conclusion: The COVID-19 pandemic is still experiencing worldwide disasters and lives, but a possible ray of hope for the future can be found with the COVID-19 vaccine. The findings recommend immediate programs of health education and that the respective health authorities should provide more accurate information. In order to decrease vaccine relief enabled and promoted by disinformation in the media, policymakers should take efforts to provide appropriate understanding, favorable attitudes and views of COVID-19 immunization.

Keywords: Covid-19, vaccine, KAP study.

I. Introduction

As SARS-CoV-2 is highly infectious virus that impacts communities around the globe, vaccines are the most significant measure of public health and the most effective approach for protecting the population against COVID-19. The competitive effort for the discovery and development of COVID-19 vaccines against the spread and disastrous consequences of the illness is ongoing with the creation of new, more effective vaccinations as the pandemic passes. 1-2 The distribution of vaccines is in the process and the acceptance of COVID-19 immunizations by the community must be investigated. 3

On 27 January 2021 the Bangladeshi authorities decided to utilize the Indian vaccination Covidshield; Runu (A Nurse) was the first COVID-19 receiver. 4

Bangladeshi officials approved. However, there is a large debate among the general people of Bangladesh over COVID-19 vaccines. A fraction of the people of Bangladesh hesitate to take the Indian vaccine so that they are not infected. 5 A worldwide COVID-19 research showed that 48 percent of the study population had misunderstandings with the COVID-19 vaccines and were doubtful about their vaccination. 6

In this study our main goal is to evaluate the Knowledge, Attitude and Practice of taking Covid-19 vaccination among the rural and urban people in Patuakhali District.

II. Objective

- To assess the Knowledge, Attitude and Practice of taking Covid-19 vaccination among the rural and urban people.

III. Methodology

a) Types of study
- It was a cross sectional study.

b) Place and period of the study
- The study place was carried out at Tertiary medical College Hospital, Patuakhali District. Bangladesh. Where data were collected from July 2020 to May 2021.
c) **Study population**
- A total of 120 participants where in rural 60 participants and in urban 60 participants were included in the study. Sample were collected through purposive sampling as per inclusion criteria.

d) **Method**
- Data were collected by using a pre designed questionnaire. The questionnaire was prepared reviewing literature and consulting with medical research experts.

e) **Data analysis**
- All collected data were coding and input in SPSS-25 for further analysis. Both descriptive and inferential statistics done. Descriptive statistics included frequency distribution, percent, mean, standard deviation; graph, tables, figures and inferential statistics.

### IV. **Results**

In table-1: Shows distribution of the respondents by age where in rural group highest 23 (38.33%) respondents belong to 32-43 years and in urban group highest 31 (51.66%) respondents belong to 44-55 years. The following table is given below in detail:

**Table-1: Distribution of the respondents by age (n=120)**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Rural Frequency (n=60) (%)</th>
<th>Urban Frequency (n=60) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-31 years</td>
<td>13 (21.67)</td>
<td>06 (10)</td>
</tr>
<tr>
<td>32-43 years</td>
<td>23 (38.33)</td>
<td>16 (26.67)</td>
</tr>
<tr>
<td>44-55 years</td>
<td>18 (30)</td>
<td>31 (51.66)</td>
</tr>
<tr>
<td>55 above years</td>
<td>06 (10)</td>
<td>7 (11.67)</td>
</tr>
<tr>
<td>Total</td>
<td>60 (100%)</td>
<td>60 (100%)</td>
</tr>
</tbody>
</table>

In figure-1 shows gender distribution of the study group where in both group majority of the cases were male. The following figure is given below in detail:

**Figure-1:** Gender distribution of the study group.

It was found from the table no. 2 that in rural area highest 30 (50%) respondents were HSC passed followed by primary 13 (21.7%) and in urban area highest 25 (41.25%) respondents were HSC followed by graduate 18 (30%). The bellow table is given in details.
Table-2: Distribution of the respondents by Education (n=120)

<table>
<thead>
<tr>
<th>Education</th>
<th>Rural Frequency (n=60) (%)</th>
<th>Urban Frequency (n=60) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>13 (21.7)</td>
<td>05 (8.33)</td>
</tr>
<tr>
<td>SSC</td>
<td>05 (8.33)</td>
<td>12 (20)</td>
</tr>
<tr>
<td>HSC</td>
<td>30 (50)</td>
<td>25 (41.67)</td>
</tr>
<tr>
<td>Graduate</td>
<td>12 (20)</td>
<td>18 (30)</td>
</tr>
<tr>
<td>Total</td>
<td>60 (100%)</td>
<td>60 (100%)</td>
</tr>
</tbody>
</table>

Table-4 Explores the distribution of the respondents by knowledge related factors and association with gender of rural & urban area. The association of Covid-19 infected status between rural & urban respondents were highly significant (P= 0.00), similarly knowledge regarding first vaccine in Bangladesh also highly significant (p=0.02) between rural & urban respondents. Another findings regarding knowledge related factor regarding the lowest age range for this vaccination was moderately significant (p=0.03), finally knowledge related factor regarding the way to take the vaccine was also significantly associated (p=0.04) between the respondents of rural & urban area, beside the knowledge related factors the other variables were not significantly associated. The following table is given below in detail:

Table-4: Distribution of the respondents by knowledge related factors and association with gender (n=120)

<table>
<thead>
<tr>
<th>Knowledge items</th>
<th>Rural</th>
<th>Urban</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male Frequency (n=46) (%)</td>
<td>Female Frequency (n=14) (%)</td>
<td>Male Frequency (n=38) (%)</td>
</tr>
<tr>
<td>Have you ever been infected to covid-19?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>22 (47.8)</td>
<td>8 (57.14)</td>
<td>22 (57.89)</td>
</tr>
<tr>
<td>• No</td>
<td>24 (52.2)</td>
<td>6 (42.86)</td>
<td>16 (42.11)</td>
</tr>
<tr>
<td>Any of your family member ever got infected to covid-19?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>25 (54.35)</td>
<td>7 (50)</td>
<td>20 (52.63)</td>
</tr>
<tr>
<td>• No</td>
<td>21 (45.65)</td>
<td>7 (50)</td>
<td>18 (47.37)</td>
</tr>
<tr>
<td>Do you have any Knowledge of covid-19 vaccination?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>38 (80.61)</td>
<td>10 (71.73)</td>
<td>34 (89.47)</td>
</tr>
<tr>
<td>• No</td>
<td>8 (19.29)</td>
<td>4 (28.27)</td>
<td>4 (10.53)</td>
</tr>
<tr>
<td>When did Bangladesh started corona vaccination?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• January 2021</td>
<td>33 (71.73)</td>
<td>8 (57.14)</td>
<td>30 (78.95)</td>
</tr>
<tr>
<td>• February 2021</td>
<td>13 (28.27)</td>
<td>6 (42.86)</td>
<td>8 (21.05)</td>
</tr>
<tr>
<td>What is the name of first corona vaccine in Bangladesh?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• COVISHIELD-Oxford</td>
<td>30 (65.22)</td>
<td>8 (57.14)</td>
<td>30 (78.94)</td>
</tr>
<tr>
<td>• Sputnik V-Russia</td>
<td>6 (13.04)</td>
<td>3 (21.42)</td>
<td>8 (21.06)</td>
</tr>
<tr>
<td>• Pfizer-USA</td>
<td>8 (17.39)</td>
<td>2 (14.28)</td>
<td>00</td>
</tr>
<tr>
<td>• Sinovac-China</td>
<td>2 (4.33)</td>
<td>1 (7.16)</td>
<td>00</td>
</tr>
<tr>
<td>How many corona vaccines dose need to take in this country?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 1 dose</td>
<td>5 (10.86)</td>
<td>4 (28.57)</td>
<td>3 (7.89)</td>
</tr>
<tr>
<td>• 2 doses</td>
<td>41 (89.14)</td>
<td>10 (71.43)</td>
<td>35 (92.11)</td>
</tr>
<tr>
<td>After the first dose of vaccination how many days it takes for the second dose?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 60 days</td>
<td>40 (86.95)</td>
<td>12 (85.71)</td>
<td>35 (92.10)</td>
</tr>
<tr>
<td>• 45 days</td>
<td>06 (3.05)</td>
<td>02 (14.29)</td>
<td>03 (7.90)</td>
</tr>
</tbody>
</table>

P value
What is the lowest age range for this vaccination?
- 25 years
- 40 years

<table>
<thead>
<tr>
<th>Age Range</th>
<th>Rural</th>
<th>Urban</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 years</td>
<td>20 (43.47)</td>
<td>4 (28.57)</td>
<td>8 (21.05)</td>
</tr>
<tr>
<td>40 years</td>
<td>26 (56.53)</td>
<td>10 (71.43)</td>
<td>30 (78.95)</td>
</tr>
</tbody>
</table>

What is the way to take this vaccine?
- Present directly to the health center
- Firstly, to register on the website
- I don’t know

<table>
<thead>
<tr>
<th>Way to Take</th>
<th>Rural</th>
<th>Urban</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>8 (17.39)</td>
<td>04 (28.57)</td>
<td>8 (21.06)</td>
</tr>
<tr>
<td>Register</td>
<td>34 (73.91)</td>
<td>08 (57.14)</td>
<td>30 (78.94)</td>
</tr>
<tr>
<td>Don’t Know</td>
<td>04 (8.70)</td>
<td>02 (14.29)</td>
<td>00</td>
</tr>
</tbody>
</table>

Note: p ≤ 0.05 considered as significant value

Table-5: Distribution of each attitude and practice item and gender difference (n=120)

<table>
<thead>
<tr>
<th>Description</th>
<th>Rural</th>
<th>Urban</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you taken the vaccine?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>16 (34.78)</td>
<td>5 (35.71)</td>
<td>25 (65.79)</td>
</tr>
<tr>
<td>• No</td>
<td>30 (65.22)</td>
<td>9 (64.29)</td>
<td>13 (34.21)</td>
</tr>
<tr>
<td>Is there any side effect of corona vaccination?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>14 (30.43)</td>
<td>4 (28.57)</td>
<td>8 (21.05)</td>
</tr>
<tr>
<td>• No</td>
<td>32 (69.57)</td>
<td>10 (71.43)</td>
<td>30 (78.95)</td>
</tr>
<tr>
<td>After taking the vaccine is there any side effect seen?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Fever</td>
<td>09 (19.56)</td>
<td>02 (14.29)</td>
<td>06 (15.79)</td>
</tr>
<tr>
<td>• Body ache</td>
<td>15 (32.61)</td>
<td>03 (21.43)</td>
<td>10 (26.32)</td>
</tr>
<tr>
<td>• Pain in inject area</td>
<td>20 (43.48)</td>
<td>08 (57.14)</td>
<td>22 (57.89)</td>
</tr>
<tr>
<td>• Others</td>
<td>02 (4.35)</td>
<td>01 (7.14)</td>
<td>00</td>
</tr>
<tr>
<td>There are so many rumors about corona vaccine, do you believe those?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>12 (26.09)</td>
<td>5 (35.71)</td>
<td>03 (7.89)</td>
</tr>
<tr>
<td>• No</td>
<td>34 (73.91)</td>
<td>9 (64.29)</td>
<td>35 (92.11)</td>
</tr>
<tr>
<td>Are you tensed about the side effect of corona vaccine?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>12 (26.09)</td>
<td>5 (35.71)</td>
<td>05 (13.16)</td>
</tr>
<tr>
<td>• No</td>
<td>34 (73.91)</td>
<td>9 (64.29)</td>
<td>33 (86.84)</td>
</tr>
<tr>
<td>Do you think the related person should be more concern about corona vaccine?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>32 (69.57)</td>
<td>9 (64.29)</td>
<td>33 (86.84)</td>
</tr>
<tr>
<td>• No</td>
<td>14 (30.43)</td>
<td>5 (35.71)</td>
<td>33 (86.84)</td>
</tr>
<tr>
<td>Would you share the right information to your family friends and society?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>46 (100)</td>
<td>14 (100)</td>
<td>38 (100)</td>
</tr>
<tr>
<td>• No</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
</tbody>
</table>

Note: p ≤ 0.05 considered as significant value
V. Discussion

During the study, knowledge was significantly associated with education, monthly income of a family, and previous vaccine uptake experience. However, attitudes were significantly associated with only gender and earlier vaccine administration experience. Importantly, the majority of participants showed positive attitude towards COVID-19 vaccine.

Knowledge regarding COVID-19 vaccinations negative correlation observed in terms of participants’ gender. This finding is similar to studies concerning knowledge towards COVID-19 (not vaccinations) conducted in Bangladesh which reported that males had marginally higher scores in knowledge regarding COVID-19 than females.7

However, this finding is inconsistent to studies concerning knowledge towards COVID-19 (not vaccinations) conducted in Bangladesh which reported that males had marginally higher scores in knowledge regarding COVID-19 than females. 8

These discrepancies of knowledge found in our study on COVID-19 vaccinations are possibly due to limited government exposures to information or publicity on COVID-19 vaccinations since the vaccine rollout started. In addition, the potential under-reporting or misinformation of data on the seriousness of incidence and mortality of COVID-19 may reduce concerns about vaccine safety or indeed make residents of Bangladesh reluctant to seek information on either COVID-19 or related vaccinations. Thus, it is essential to support community members by providing easy access to trusted, evidence-based vaccine information. 9

According to our study, participants with a higher level of education were found to have more knowledge about COVID-19 vaccinations, which is also supported by previous research. Similar scenarios were found in other earlier studies in Bangladesh, illustrating that individuals with a higher educational background showed more knowledge regarding COVID-19. 10

It may be the case that more educated people are more knowledgeable and concerned about their health and well-being, through access to more information sources, and become more engaged in life events that could impact them, such as COVID-19 vaccinations.11

People who have received any vaccine earlier were found to have more knowledge regarding COVID-19 vaccinations in this study. A recent study in China evaluating COVID-19 vaccine acceptance found that people who were previously vaccinated against influenza were more likely to accept the COVID-19 vaccine, which was also demonstrated in a study in Hong Kong.12,3

This tendency among people may be due to previous positive experiences from vaccination. The level of knowledge about COVID-19 vaccinations were significantly higher among people living in the urban areas, compared to rural areas. This is supported by an earlier study in Bangladesh which demonstrated significant correlation between COVID-19 knowledge and urban location.7 However, our finding is inconsistent with a recent study which found more accurate knowledge about COVID-19 among people in rural areas in Bangladesh.8

In the present study, over 80% of participants had more positive attitudes towards COVID-19 vaccine. This association is in line with a previous study on attitudes towards dengue vaccination conducted in Indonesia [35] and attitudes towards COVID-19 carried out in Bangladesh. 8

In our study, in participants assumed that the recently discovered COVID-19 vaccine (the vaccine currently being used in Bangladesh) could have some side-effect, which is similar to a study in the US.13 A study in China found that 48% of respondents postponed vaccination before confirmation of the safety of the vaccine, which shows their doubt regarding vaccine safety.14 Worryingly, the exceptionally rapid pace of vaccine development, the skepticism of certain groups of science and health experts might elevate doubt about COVID-19 vaccine. 15

VI. Conclusion

The COVID-19 pandemic is still experiencing worldwide disasters and lives, but a possible ray of hope for the future can be found with the COVID-19 vaccine. The findings recommend immediate programs of health education and that the respective health authorities should provide more accurate information. In order to decrease vaccine relief enabled and promoted by disinflation in the media, policymakers should take efforts to provide appropriate understanding, favorable attitudes and views of COVID-19 immunization.

References Références Referencias

The Knowledge, Attitude and Practice of Taking Covid-19 Vaccination among the Rural and Urban People in Patuakhali District


The Impact of *Senna Siamea* (Lam) Leaves Extracts on 2, 2-Dichlorovinylidimethyl Phosphate Induced Brain Oxidative Stress in Wistar Albino Rats

By Oni, Olaiya Peter, Oseni, Olatunde Abass & Okoh, Olayinka Sunday

_Ekiti-State University_

**Abstract** - Organophosphate compounds have been a common source of mortality in recent times. A typical example is dichlorvos [2, 2-Dichlorovinylidimethylphosphate (DDVP)]; an important agricultural pesticide. This study sought to investigate the phytochemical screening, in-vitro antioxidant property of the leaf solvents extracts and ameliorating effects of *Senna siamea* in DDVP-induced brain oxidative stress in Wistar rats. The aqueous, methanol and ethanol extracts of the leaves were obtained and analyzed for their in-vitro antioxidant parameters. Thirty-two healthy Wistar albino rats were grouped into eight of 4 rats each weighing between 140-150g. The animals were orally administered with 6.6mg/kg body weight of DDVP except groups 1 and 2 followed by treatments with the three solvents extracts respectively that lasted for four weeks.

**Keywords:** *Senna siamea; brain toxicity; oxidative stress; antioxidant properties; γ-glutamyl transferase.*

**GJMR-B Classification:** NLMC Code: WB 340

---

© 2021. Oni, Olaiya Peter, Oseni, Olatunde Abass & Okoh, Olayinka Sunday. This research/review article is distributed under the terms of the Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0). You must give appropriate credit to authors and reference this article if parts of the article are reproduced in any manner. Applicable licensing terms are at https://creativecommons.org/licenses/by-nc-nd/4.0/.
The Impact of Senna Siamea (Lam) Leaves Extracts on 2, 2-Dichlorovinylidimethyl Phosphate Induced Brain Oxidative Stress in Wistar Albino Rats

Oni, Olaya Peter *, Oseni, Olatunde Abass ° & Okoh, Olayinka Sunday ρ

Abstract- Organophosphate compounds have been a common source of mortality in recent times. A typical example is dichlorvos [2, 2-Dichlorovinylidimethylphosphate (DDVP)]; an important agricultural pesticide. This study sought to investigate the phytochemical screening, in-vitro antioxidant property of the leaf solvents extracts and ameliorating effects of Senna siamea leaf solvents extracts on DDVP-induced brain oxidative stress in Wistar rats. The aqueous, methanol and ethanol extracts of the leaves were obtained and analyzed for their in-vitro antioxidant parameters. Thirty-two healthy Wistar albino rats were grouped into eight of 4 rats each weighing between 140-150g. The animals were orally administered with 6.6mg/kg body weight of DDVP except groups 1 and 2 followed by treatments with the three solvents extracts respectively that lasted for four weeks. The in-vitro antioxidant potentials of the plant extracts and malondialdehyde (MDA); reduced glutathione (GSH) concentrations; γ-glutamyl transferase (GGT); catalase (CAT); superoxide dismutase (SOD); glutathione peroxidase (GPx); glutathione transferase (GST) and glutathione reductase (GR) activities were determined on the serum and brain homogenate. The three plant extracts were found to possess significant in-vitro antioxidant potentials while treatments of the DDVP induced brain toxicity with the extracts caused significant reduction in the raised values of MDA concentration and GGT activity with increased antioxidant enzymes activities when compared to the DDVP-induced group. The results obtained from the study thus showed that the leaves extracts contained important phytochemicals, possessed in-vitro and in-vivo antioxidant potentials responsible for protective and curative effects against DDVP-induced brain oxidative stress of the rats.

Keywords: Senna siamea; brain toxicity; oxidative stress; antioxidant properties; γ-glutamyl transferase.

1. Introduction

Exposure of human beings to poisons and some other toxic materials has been responsible for many mortality cases in our generation most of which are accidental. Reports indicate that nothing less than 200,000 were dead as a result of organophosphate compound, one of which is 2, 2- Dichlorovinylidimethylphosphate (DDVP). The organophosphate compounds is unarguably one of the toxic and adequately chronic organophosphates that is of detrimental effect to the health of humans and animals. It was said that the continuous exposure to humans and animals to DDVP has been identified as one of the leading causes of acetyl cholinesterase (AChE) inhibition especially at the presynaptic cleft, thus leading to the accumulation of acetylcholine as well as the triggering of postsynaptic neurons in animals, and ultimately to death. Notably, the exposure of humans and animals have been fingered to be a key player in respiratory problems including that of discomfort in the chest, bloody or running nose, severe and sometimes dry coughing, difficulty in breathing and increased fluid in the bronchial tubes. Oxidative stress arising from free radicals like reactive oxygen species (ROS) now appears to be a fundamental mechanism underlying many degenerative diseases such as diabetes, viral infection, auto-immune pathologies and probably aging. Evidence suggests that ROS can be scavenged through chemoprevention utilizing antioxidant compounds present in foods and medicinal plants. Plants play a significant role in maintaining human health and improving the quality of human life. They serve humans as valuable components of food, cosmetics, dyes, and medicines. The World Health Organization estimated that 80% of the earth’s inhabitants rely on traditional medicine for their primary healthcare needs, and most of this therapy involves the use of plant extracts and their active components. Senna siamea has a long history of use as a folk-medicine and its therapeutic efficacy is well recognized. Different parts of S. siamea can be used for various medical purposes. The fruit is used to charm away intestinal worms and to prevent convulsions in children. The heartwood is said to be a laxative, and a decoction is used against scabies. Senna siamea also known as Siamese cassia, kassod tree, cassod tree and cassia tree is a legume in the subfamily Caesalpinioideae. It is native to South and Southeast Asia, widespread in Africa, although its exact origin is unknown. This plant has proven to contain some important biochemical components like alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes. Senna siamea is a medicinal plant acknowledged to be rich in phenolics, consisting of condensed tannin and phlobatannin, Gallic acid, protocatechuc acid,
pyrocatechol, (+)-catechin, (-) epi-gallocatechin-7-gallate and (-) epigallocatechin-5, 7-digallate. Its antioxidative property and ameliorating effects on organophosphate toxicity has not been done. This current study however tends to investigate the effects of Senna siamea leaf extracts on some biochemical indices in 2, 2-dichlorovinylidemethyl phosphate (DDVP) induced brain toxicity using Wistar albino rats.

II. MATERIALS AND METHODS

a) Collection and Extraction of the Senna siamea leaf

The fresh leaves of Senna siamea (Lam) Irwin & Barneby (Fabaceae) were obtained from Ifaki-Ekiti community, Ekiti State and was authenticated at Department of Plant Science, Ekiti State University, Ado-Ekiti and the plant specimen was preserved with Herbarium numbers (UHAE 2020055). The leaves were rinsed with water and then air-dried by spreading them on a clean surface at room temperature in the laboratory. The air-dried leaves were then pulverized and three major separate extractions were carried out with two hundred grams portions each of the dried powdered leaves soaked in 500mL each of water, ethanol and methanol as solvents to obtain three extracts for analyses.

b) Phytochemical screening and in-vitro anti-oxidant parameters determination of the leaf’s extracts

The qualitative phytochemical screening [flavonoids, saponin, phlobatannins, terpenoids, Salkowski test for cardiac glycosides (steroidal ring or terpenoids), Keller-Killani test for cardiac glycosides (deoxysugar), Lieberman’s test for steroidal nucleus and test for tannins] of aqueous, methanol and ethanol extracts of the leaves were carried out according to the methods of to identify the active constituents while the in-vitro antioxidants properties were determined by the following methods:

a. Hydrogen Peroxide Scavenging Effects: The ability of the leaf extracts to scavenge hydrogen peroxide was assessed by the method of Ruch et al.

b. ABTS Scavenging Effects: The antioxidant effect of the leaf extracts was studied using ABTS (2,2’-azinobis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation discoloration assay according to the method of Shirwaikar et al.

c. Measurement of Nitric Oxide Scavenging Activity: The extent of inhibition of nitric oxide radical generation in vitro was followed as per the method reported by Green et al.

d. DPPH spectrophotometric assay: The free radical scavenging activities of the samples by DPPH method were determined according to the method reported by Brand-Williams et al.

e. Measurement of Superoxide Scavenging Activity: The superoxide scavenging ability of the extracts was assessed by the method of Winter bourn et al.

f. Estimation of Total Phenols: The total phenolic content was determined according to a well-cited protocol.

g. Estimation of Flavonoids: The total flavonoid contents in the samples were determined following the method reported by Zhishen et al.

c) Animal management

Thirty-two (32) healthy albino Wistar rats were obtained and housed in the animal house of the College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria. The animals were acclimatized for two weeks before administration of DDVP. The acclimatization was done under standard environmental conditions of good lighting, moderate temperature and adequate ventilation. They were also fed on standard rat feed containing adequate proteins, carbohydrate, fats, vitamins, minerals back up with clean and adequate water. The animals were handled under standard laboratory protocols as stipulated by the Institutional Animal Care and Use Committee.

d) Experimental design

The animals were divided into six groups according to their weights with Groups 2 having 3 subgroups, one for each of the three extracts. Each group had four animals. The animals were orally administered with 0.5mL of 6.6mg/kg body weight of 500 folds dilution of DDVP solution for two weeks except for Groups 1 and 2 followed by treatments with 0.5mL of 3.3mg/kg body weight of each extract of the plant for another two weeks of the four weeks study.

Group 1 Normal Control

group 2 Extract control (Each subgroup animal was given 0.5mL of 3.3mg/kg body weight of 0.5g/100mL of each solvent (aqueous, methanolic and ethanolic) extract of the plant)

Group 3 DDVP control (animals were administered orally with 0.5mL of 6.6mg/kg body weight of 500 folds dilution of DDVP solution to induce brain toxicity)

Group 4 DDVP induced animals + 0.5mL of 3.3mg/kg body weight of 0.5g/100mL aqueous extract of the plant.

Group 5 DDVP induced animals + 0.5mL of 3.3mg/kg body weight of 0.5g/100mL methanolic extract of the plant.

Group 6 DDVP induced animals + 0.5mL of 3.3mg/kg body weight of 0.5g/100mL ethanolic extract of the plant.

All animals in the groups were also given rats feed and drinking water ad libitum.
**Preparation of serum and brain homogenate**

At the end of the experiment, the rats were chloroform anesthetized and quickly dissected with their blood samples and brain removed. 10% of the brain homogenate was prepared in 6.7nM potassium phosphate buffer (pH 7.4) using the Top drive n electric homogenizer. The homogenate was centrifuged at 3,000rpm for 10 minutes at 4°C to obtain a clear supernatant while serum sample was prepared from the whole blood collected from the heart into the plain sample bottle and centrifuged at 3,000 rpm after coagulation. The individual serum and brain homogenate were used for measurement of the studied biochemical parameters. The lipid peroxidation was done by measuring the TBARS in accordance with the modified method of Utley et al. GGT activity was determined using standard Sigma-Aldrich kit from USA while the antioxidants enzymes activities (Catalase (CAT), superoxide dismutase (SOD), Glutathione-S-transferase (GST), Glutathione reductase (GR) and Glutathione peroxidase (GPx)); reduced glutathione (GSH) were determined by the methods described by Chance and Maehly as calculated by Von Euler and Josephson, Misra and Fridovich, Habig et al, Carlberg and Mannervik, Mohandas et al and Jollow et al respectively.

**Statistical analyses**

The results obtained were evaluated using the statistical test of Means triplicates results of four animals per group.

### III. Results

#### Determination of Phytochemicals

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tapenoid</td>
<td>300</td>
</tr>
<tr>
<td>Tannins</td>
<td>200</td>
</tr>
<tr>
<td>Saponin</td>
<td>100</td>
</tr>
<tr>
<td>Saskowski - C. glycoside</td>
<td>300</td>
</tr>
<tr>
<td>Phlobatannin</td>
<td>200</td>
</tr>
<tr>
<td>Phenolic Compounds</td>
<td>100</td>
</tr>
<tr>
<td>Leeberman's steroid</td>
<td>300</td>
</tr>
<tr>
<td>Keller-killani glycosides</td>
<td>100</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>300</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>100</td>
</tr>
</tbody>
</table>

*Figure 1:* Phytochemical screening of the composition of aqueous, methanol and ethanol extracts of *Senna siamea* leaf. Less than 100 means trace of the phytochemicals, 100 means “+”; 200 means “++”; while 300 means “+++”.
Figure 2: *In-vitro* anti-oxidants determination of the aqueous, methanol and ethanol extracts of *Senna siamea* leaves at 0.25, 0.50, 0.75 and 1.00mg/mL concentrations of the extracts.
Figure 3: Effect aqueous, methanol and ethanol extracts of *Senna siamea* leaves on MDA (µmol TBARS/h/mg tissue protein) in 2, 2-Dichlorovinyldimethylphosphate (DDVP)-induced toxicity in Wistar albino rats. Normal Group animals were given water and feed only, Extract Group animals were given the extracts, while the DDVP Group animals were induced with DDVP and the Treatment Group animals were treated with the *Senna siamea* leaves extracts after being induced with DDVP.
Figure 4: Effect of Senna siamea extracts on Brain $\gamma$-glutamyl transferase -GGT (nmole/min/mL) in 2,2-Dichlorovinylidimethylphosphate (DDVP) induced toxicity in Wistar albino rats. $p < 0.01 (**), p < 0.001 (***)$, $p < 0.0001< (****)$. Normal Group rats received feeds and water only; Extract Group rats received one of aqueous, methanol or ethanol extract of Senna siamea in addition to feeds and water; DDVP Group rats were induced with DDVP while the Treatment Group rats received one of aqueous, methanol or ethanol extract of Senna siamea after exposure to DDVP.
**Figure 5a:** The effect of DDVP - induced toxicity on Catalase, Superoxide Dismutase, Glutathione Peroxidase and Glutathione Transferase in Wistar albino rats.
The effect of DDVP – induced toxicity on Glutathione reductase and Reduced glutathione in Wistar albino rats.

**IV. Discussion**

The Phytochemical assessment of the three solvents extracts of the leaves of *Senna siamea* contained some plant chemicals of interest and important medicinal potentials as shown in Figure 1.0. This important plant phytochemicals are known to support bioactive activities in medicinal plants and may...
Therefore be responsible for the medicinal properties of these leaves extracts which was similar to many other reported studies by29 in protective effects of the ethanolic extract of *Alstonia boonii* stem bark and30 in in-vitro compositional investigations of antioxidants, phytochemicals, nutritional and minerals in the fruit of *Kigelia africana* (Lam.) Benth. Figure 2.0 related the in-vitro antioxidant properties of the plants extracts studied within 0.25 and 1.0mg/mL concentrations range for both extracts. The % H2O2, %ABTS, %NO, %DPPH and %SO scavenging activities which occurred in a concentration dependent fashion similar to what was obtained for total phenol and total flavonoids concentrations which are the bioactive compounds responsible for the antioxidant potentials of the *Senna siamea* various solvents extracts. The three extracts of the leaves showed appreciable scavenging potentials for nitric oxide, the nitric oxide scavenging property of any plant extract has been said to help in the arrest of various chains of reactions initiated by excess generation of NO that are detrimental to the wellbeing of the body. The results obtained in this study however corroborated the earlier obtained for the nitric oxide scavenging activity of *Ceropsdecandra*34. The three extracts of the leaves showed appreciable scavenging potentials for DPPH radicals in a concentration dependent manner. indicating that the higher the concentration used, the higher the scavenging activity with the highest scavenging activity found in methanol extract. The ability of the plant to freely scavenge DPPH radicals may be due to the presence of flavonoids35. The scavenging of DPPH radical by antioxidants agents is due to the reaction between antioxidant molecules and radical progress which results in the scavenging of the radicals by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. Hence, DPPH is usually used as a substance to evaluate the antioxidant potential of medicinal plants36. The Ethanolic extract of *Senna siamea* displayed a more superior superoxide scavenging activity at the 1mg/mL concentration which is far greater than the results for both aqueous and methanolic extracts at the same concentration. The super oxide scavenging activity of *Senna siamea* can be seen in its ability to scavenge super oxide radical ions to form stable radicals and by such can help in the termination of radical chain reaction37. In the hydrogen peroxide scavenging activity, the ethanolic extracts possess the highest activity than those aqueous and methanol. However, Shalu et al38 reported a similar observation for the plant in their study. Total phenols and total flavonoids were also observed to be considerably present in all the plant extracts. The total phenol aqueous extract had the highest value followed closely by the ethanolic and methanolic extracts concentrations. These results obtained for *Senna siamea* showed resemblance to the work of Jyoti et al39 on *Acacia nilotica*. Phenols are said to contribute to the quality and nutritional value in terms of modifying aroma, color, taste and flavor40. Phenolic compounds could be a major determinant of antioxidant potentials of food plants and could therefore be a natural source of antioxidants41. In this study, the results obtained for total flavonoids showed that ethanolic extract contained the highest level of flavonoids than aqueous and methanolic extracts. Flavonoids are part of the secondary metabolites present in plants as part of its arsenal and has been reported by Choudhary et al40 that flavonoids show some antioxidant activity and that it has considerable effects on both human’s health and nutrition. He described its mechanisms as the one with either scavenging or chelating process. They are said to possess hydroxyl groups which prompted their radical scavenging effects in the plant. Figure 3.0 showed the effects of the leaf extracts on the level of lipid peroxidation both in the serum and brain of the studied animals which revealed that the DDVP induced control group 3 caused almost tenfold elevation in the malondialdehyde concentration of both serum and brain tissue. The treatment of the rats with the three solvents extracts showed that both extracts produced ten-fold reduction effects in the concentration of malondialdehyde in both serum and brain tissues when compared with the DDVP-induced group. It has been reported that enhanced lipid peroxidation leads to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals41. It is clearly evidenced in this study that treatments of the induced animals with the aqueous, methanolic and ethanolic extracts of the *Senna siamea* leaf respectively caused a substantive decrease in the level of lipid peroxidation of both serum and brain tissues. This result however corroborated the observation of Ojo et al42 who postulated that DDVP induction would lead to an increased MDA value and the treatment with antioxidant containing extracts would lead to the reduction of the MDA value. The induction of DDVP in group 3 from Figure 4.0 showed a significantly increase in the value of GGT which thus implies that the exposure caused a damage to the brain including increased permeability, possible neurosis in the brain. This result also showed that the treatment with the various solvents extracts caused a significant decrease in the level of the GGT indicating that the extracts of *Senna siamea* caused reversal to the detrimental DDVP induction in the animals. This is in line with the results of Ojo et al42 who also postulated that DDVP induction will lead to an increased GGT value and the treatment with a good antioxidant plant like *Senna siamea* will reverse back the damage caused by the exposure to DDVP; it should also be noted in this study that all the solvents extracts of the plant produced tremendous attenuating effect of the brain toxicity. Figures 5a and 25b presented the results of some antioxidant enzymes of (catalase, superoxide dismutase, glutathione peroxidase,
glutathione transferase, glutathione reductase) and reduced glutathione. Oxidative stress plays a major role in the pathogenic of many disorders including aging, cancer, diabetes, Alzheimer’s, strokes, viral infections (that cause airway epithelial inflammation), neurodegenerative processes (including cell death, motor neuron diseases and axonal injury) and infraction, and brain edema. Antioxidant enzyme plays an important role in protecting oxidative injury to the body. One of the therapeutic approaches by which these disorders can be prevented is to increase the levels of these antioxidant enzymes\textsuperscript{42}. The catalase activity was significantly increased at \((p<0.05)\) in the aqueous, methanolic and ethanolic treatments groups towards the control and extract treated groups when compared with the DDVP induced group. Other researchers have also reported an upward trend in catalase level using various plants extract treatments in other complications as we observed in this study\textsuperscript{43,44,45,46}. Catalase are heme-containing enzymes that convert hydrogen peroxide \((H_2O_2)\) to water and \(O_2\), and they are largely localized in subcellular organelles such as peroxisomes\textsuperscript{47}. The %SOD activity observed in this study showed a significant \((p<0.05)\) increase in the various treatment’s groups after a gross reduction by the DDVP induced group when compared with the normal control and extracts treated groups. Similar observations were also reported in earlier studies of \(\text{Oseni et al}\textsuperscript{43}; \text{Uroko et al}\textsuperscript{44}; \text{Onoja et al}\textsuperscript{45} \text{and Sani et al}\textsuperscript{46}\). SOD is the antioxidant enzyme that catalysed the dismutation of the highlyreactive superoxide anion to \(O_2\) and to the less reactive species \(H_2O_2\); the peroxide can then be destroyed by Catalase or glutathione peroxidase reactions as reported by\textsuperscript{48,49,50}. Glutathione peroxidase, glutathione transferase and glutathione reductase in addition with SOD are antioxidants enzymes that work in synergy to protect the organism from reactive oxygen species (ROS). These enzymes were observed to be significantly \((p<0.05)\) increased in all the treatment groups when compared with the induced group to reverse the effects of induction towards the normal control group. Our observation is in consonance with what was reported by\textsuperscript{51,52}in their various studies on amelioration of thioacetamide-induced oxidative stress and hepatic damage in albino rats by Solanum trilobatum and antioxidant effect of grapevine leaf extract on the oxidative stress induced by a high-fat diet in rats respectively. Reduced glutathione (GSH) is another compound that play a vital role as an antioxidant. \textit{Senna siamea} solvents extracts were found to significantly \((p<0.05)\) reverse the reduced GSH in DDVP induced rats to the normal control as observed in this study. Reduced glutathione is found in high concentrations in cellular systems and plays a major role in detoxication of various electrophilic compounds, deficiency of which puts the cell at risk for oxidative damage. Previous works have also shown that medicinal plants extracts have abilities to enhance glutathione concentration to reverse the effects of oxidative stress\textsuperscript{51,53}.

\section*{V. Conclusion}

This study has reasonably showed that the oral exposure of the rats to DDVP ( Dichloros) caused the brain oxidative stress in the Wistar albino rats as indicated by the increased level of lipid peroxidation, increased GGT activity, reduced antioxidant potentials both in the serum and brain while the aqueous, methanolic and ethanolic extracts of \textit{Senna siamea} showed a significant and protective effects against the action of DDVP induced oxidative stress in the rats.

The plant studied here will in no doubt do well as a neurotoxicity protective agents and further researches needed to be carried out to explore it for raw materials needed for the treatment of neurodegenerative diseases like Alzheimer’s diseases and other complications.

\textit{Ethics approval and consent to participate:} All necessary National and International ethical considerations were fully followed in handling the animals.

\textit{Consent for publication:} The consent for publication was given by all the Authors.

\textit{Availability of data and material:} All data and material regarding the manuscript are available and not under any restriction elsewhere.

\textit{Competing interests:} The Authors declare that no Competing interests exist in any-form

\textit{Funding:} The research was self-funding by the authors

\textit{Authors’ contributions:} The Authors OOA and OOP designed the work concept, involved in laboratory Analyses, and write up of the manuscript. Author OOS was involved in the statistical analysis and proof-reading of the manuscript.

\textbf{References Références Referencias}


4. Chedi BAZ, Aliyu M. Effect and management of Acute Dichloros poisoning in wistar rats, Bayero
The Impact of \textit{Senna Siamea} (Lam) Leaves Extracts on 2, 2-Dichlorovinyldimethyl Phosphate Induced Brain Oxidative Stress in Wistar Albino Rats

Journal of pure and applied sciences, 2010; 3(2): 1-3


11. USDA United State Department of Agriculture. “\textit{Senna siamea}”. Natural Resources Conservation Service PLANTS Database. accessed May 09 2020


19. IACUC. International Animal Care and Use Committee Lab Animal. 2010; 19(6).


29. Oseni OA and Williams OD. In-vitro compositional investigations of antioxidants, phytochemicals, nutritional and minerals in the fruit of \textit{Kigelia africana} (Lam.) Benth. International Journal of Contemporary Research and Review. 2018; 9(8): 20529-20628


42. Krishnamurthy P, Wadhwani A. Antioxidant Enzymes and Human Health. InTech open access chapter distributed under the terms of the Creative Commons Attribution License. 2012; 13-15.


Overuse of Steroid Drugs Methylprednisolone and Dexamethasone (Oral) Causes a Diabetic Patient to Become Infected with the Black Fungus in the Corona Virus

By Ashwin Singh Chouhan, Bharat Parihar, Bharti Rathod & Ramprasad Prajapat

Jai Narain Vyas University

Abstract- Background: Overuse of both methylprednisolone and dexamethasone drug on a corona patient can result in serious side effects and new infections may appear during their use. Infection with any pathogen, including viral, bacterial, fungal, protozoan, or helminthic infections at any site of the body, may be associated with the use of methylprednisolone or dexamethasolone in combination with other immunosuppressive agents that increase cellular immunity, humoral immunity, or Suppress neutrophils. The function they affect.

These infections can be mild, but can be serious and sometimes fatal. With increasing doses of methylprednisolone and dexamethasone, the rate of occurrence of infectious complications increases. When methylprednisolone and dexamethasone are used, there may be reduced resistance and inability to localize the infection.

Keywords: methylprednisolone, dexamethasone, side effect, drug interaction, diabetes patients, black fungus, eye diseases etc.

GJMR-B Classification: NLMC Code: QV 745

© 2021. Ashwin Singh Chouhan, Bharat Parihar, Bharti Rathod & Ramprasad Prajapat. This research/review article is distributed under the terms of the Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0). You must give appropriate credit to authors and reference this article if parts of the article are reproduced in any manner. Applicable licensing terms are at https://creativecommons.org/licenses/by-nc-nd/4.0/.
Overuse of Steroid Drugs Methylprednisolone and Dexamethasone (Oral) Causes a Diabetic Patient to Become Infected with the Black Fungus in the Corona Virus

Ashwin Singh Chouhan °, Bharat Parihar °, Bharti Rathod ° & Ramprasad Prajapat °

Abstract- Background: Overuse of both methylprednisolone and dexamethasone drug on a corona patient can result in serious side effects and new infections may appear during their use. Infection with any pathogen, including viral, bacterial, fungal, protozoan, or helminthic infections at any site of the body, may be associated with the use of methylprednisolone or dexamethasone in combination with other immunosuppressive agents that increase cellular immunity, humoral immunity, or Suppress neutrophils. The function they affect.

These infections can be mild, but can be serious and sometimes fatal. With increasing doses of methylprednisolone and dexamethasone, the rate of occurrence of infectious complications increases. When methylprednisolone and dexamethasone are used, there may be reduced resistance and inability to localize the infection.

Prolonged use of methylprednisolone and dexamethasone may produce posterior subcapsular glaucoma, glaucoma with potential damage to the optic nerves, and may accelerate the establishment of secondary ocular infections caused by fungi or viruses.

It has also been observed that more methylprednisolone and dexamethasone drug increases the level of glucose in the body, leading to normal corona patients who do not have any disease and diabetes after recovering from excessive consumption of methylprednisolone and dexamethasone drug.

Materials and Methods: A cross sectional study was conducted among 50 COVID doctors from the department's outpatient pool of COVID patients, distributing questionnaires to all subjects of different age groups. The questionnaire included information related to the name, age, gender and various factors that affect the doctor's choice of methylprednisolone and dexamethasone drug.

Result and Discussion: A total of 50 doctors and some medical stores from across India were included in the survey. Doctors prescribed more methylprednisolone and dexamethasone medicine than steroid medicines to corona patients. In our research, most side effects were observed for corona patients taking methylprednisolone and dexamethasone drug.

Conclusion: This research had shown that overdose of methylprednisolone and dexamethasone drug take diabetes patient he has serious eye effect and cause black fungus.

Keywords: methylprednisolone, dexamethasone, side effect, drug interaction, diabetes patients, black fungus, eye diseases etc.

I. Background

a) Introduction

Methylprednisolone (Depo-Medrol, Medrol, Solu-Medrol) is a synthetic glucocorticoid, primarily prescribed for its anti-inflammatory and immunosuppressive effects.[1],[2],[3] It is either used at low doses for chronic illnesses or used concomitantly at high doses during acute flares. Methylprednisolone and its derivatives can be administered orally or parenterally.[4]

Regardless of route of administration, methylprednisolone integrates systemically as exhibited by its effectiveness to quickly reduce inflammation during acute flares.[5] It is also associated with many adverse reactions that require tapering off the drug as soon as the disease is under control.[6] Serious side effects include iatrogenic Cushing's syndrome, hypertension, osteoporosis, diabetes, infection, and skin atrophy.[6]

Chemically, methylprednisolone is a synthetic pregnane steroid hormone derived from hydrocortisone and prednisolone. It belongs to a class of synthetic glucocorticoids and more generally, corticosteroids. It acts as a mineralocorticoid and glucocorticoid receptor agonist. In comparison to other exogenous glucocorticoids, methylprednisolone has a higher affinity to glucocorticoid receptors than to mineralocorticoid receptors.

Glucocorticoid’s name was derived after the discovery of their involvement in regulating carbohydrate metabolism.[6] The cellular functions of glucocorticoids, such as methylprednisolone, are now understood to regulate homeostasis, metabolism, development, cognition, and inflammation.[6] They play a critical role in adapting and responding to environmental, physical and emotional stress.[6]

Methylprednisolone was first synthesized and manufactured by The Upjohn Company (now Pfizer) and FDA approved in the United States on October 2, 1957.[7] In 2018, it was the 153rd most commonly
prescribed medication in the United States, with more than 4 million prescriptions.\textsuperscript{[8][9]} Methylprednisolone has been a prescribed therapy amidst the COVID-19 pandemic, but there is no evidence it is either safe or effective for this purpose.\textsuperscript{[10][11]}

Dexamethasone is a glucocorticoid medication\textsuperscript{[12]} used to treat rheumatic problems, a number of skin diseases, severe allergies, asthma, chronic obstructive lung disease, croup, brain swelling, eye pain following eye surgery, and along with antibiotics in tuberculosis\textsuperscript{[12]}

In adrenocortical insufficiency, it may be used in combination with a mineralocorticoid medication such as fludrocortisone.\textsuperscript{[12]} In preterm labor, it may be used to improve outcomes in the baby.\textsuperscript{[12]} It may be given by mouth, as an injection into a muscle, as an injection into a vein, as a topical cream or ointment for the skin or as a topical ophthalmic solution to the eye.\textsuperscript{[12]} The effects of dexamethasone are frequently seen within a day and last for about three days.\textsuperscript{[12]}

The long-term use of dexamethasone may result in thrush, bone loss, cataracts, easy bruising, or muscle weakness.\textsuperscript{[12]} It is in pregnancy category C in the United States, meaning that it should only be used when the benefits are predicted to be greater than the risks.\textsuperscript{[10]} In Australia, the oral use is category A, meaning it has been frequently used in pregnancy and not been found to cause problems to the baby.\textsuperscript{[14]} It should not be taken when breastfeeding.\textsuperscript{[12]}

Dexamethasone has anti-inflammatory and immunosuppressant effects.\textsuperscript{[12]}

Dexamethasone was first synthesized in 1957 by Philip Showalter Hench and was approved for medical use in 1961.\textsuperscript{[15][16][17]} It is on the World Health Organization’s List of Essential Medicines.\textsuperscript{[18]} In 2017, it was the 321st most commonly prescribed medication in the United States, with more than one million prescriptions.\textsuperscript{[19]}

\textbf{Aim:} To study the factors that influence doctor’s choice of methylprednisolone and dexamethasone and to understand the most preferred options in selection with respect to the methylprednisolone and dexamethasone.

\section{Materials and Methods}

A cross-sectional study was conducted among 50 COVID-19 patients from the outpatient pool of the Department of COVID-19 patients. The study was approved by the ethical committee of the University. The questionnaire included information related to age, gender, and various factors that influence a doctor’s choice of methylprednisolone and dexamethasone.

\subsection*{a) Description}

MEDROL Tablets contain methylprednisolone which is a glucocorticoid. Glucocorticoids are adrenocortical steroids, both naturally occurring and synthetic, which are readily absorbed from the gastrointestinal tract. Methylprednisolone occurs as a white to practically white, odorless, crystalline powder. It is sparingly soluble in alcohol, in dioxane, and in chloroform, and very slightly soluble in ether. It is practically insoluble in water.

The chemical name for methylprednisolone is \textit{pregna - 1, 4 - diene - 3, 20-dione, 11, 17, 21-trihydroxy-6-methyl-, (6α, and 11β)}-and the molecular weight is 374.48. The structural formula is represented below:

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{methylprednisolone.png}
\caption{Chemical Structure of Methylprednisolone}
\end{figure}

Each MEDROL (methylprednisolone) Tablet for oral administration contains 2 mg, 4 mg, 8 mg, 16 mg or 32 mg of methylprednisolone.

\textbf{DEXAMETHASONE}, a synthetic adrenocortical steroid, is a white to practically white, odorless, crystalline powder. It is stable in air. It is practically insoluble in water. The molecular formula is $\text{C}_{22}\text{H}_{29}\text{FO}_{5}$.
The molecular weight is 392.47. It is designated chemically as 9-fluoro-11β, 17, 21-trihydroxy-16α-methylpregna-1, 4-diene, 3, 20-dione and the structural formula is:

![Chemical Structure of Dexamethasone](image)

**Figure No. 2: Chemical Structure of Dexamethasone.**

Dexamethasone provides relief for inflamed areas of the body. It is used to treat a number of different conditions, such as inflammation (swelling), severe allergies, adrenal problems, arthritis, asthma, blood or bone marrow problems, kidney problems, skin conditions, and flare-ups of multiple sclerosis. Dexamethasone is a corticosteroid (cortisone-like medicine or steroid). It works on the immune system to help relieve swelling, redness, itching, and allergic reactions.

**b) Side Effects of Methylprednisolone**

- **Fluid and Electrolyte Disturbances**
  - Sodium retention, Congestive heart failure in susceptible patients, Hypertension, Fluid retention, Potassium loss, Hypokalemic alkalosis etc.

- **Musculoskeletal**
  - Muscle weakness, Loss of muscle mass, Steroid myopathy, Osteoporosis, Tendon rupture, particularly of the Achilles tendon, Vertebral compression fractures, Aseptic necrosis of femoral and humeral heads, Pathologic fracture of long bones etc.

- **Gastrointestinal**
  - Peptic ulcer with possible perforation and hemorrhage, Pancreatitis, Abdominal distention, Ulcerative esophagitis, Increases in alanine transaminase (ALT, SGPT), aspartate transaminase (AST, SGOT), and alkaline phosphatase have been observed following corticosteroid treatment. These changes are usually small, not associated with any clinical syndrome and are reversible upon discontinuation etc.

- **Dermatologic**
  - Impaired wound healing Petechiae and ecchymoses, May suppress reactions to skin tests, Thin fragile skin, Facial erythema, Increased sweating etc.

- **Neurological**
  - Increased intracranial pressure with papilledema (pseudo-tumor cerebri) usually after treatment, Convulsions, Vertigo, Headache etc.

- **Endocrine**
  - Development of Cushingoid state, Suppression of growth in children, Secondary adrenocortical and pituitary unresponsiveness, particularly in times of stress, as in, trauma, surgery or illness, Menstrual irregularities, Decreased carbohydrate tolerance.

- **Manifestations of latent diabetes mellitus**
  - Increased requirements of insulin or oral hypoglycemic agents in diabetics

- **Ophthalmic**
  - Posterior subcapsular cataracts, Increased intraocular pressure, Glaucoma, Exophthalmos.

- **Metabolic**
  - Negative nitrogen balance due to protein catabolism

  The following additional reactions have been reported following oral as well as parenteral therapy: Urticaria and other allergic, anaphylactic or hypersensitivity reactions.22

**c) Side Effects of Dexamethasone**

The following side effects have been reported with dexamethasone or other corticosteroids:

- **i. Allergic Reactions**
  - Anaphylactoid reaction, anaphylaxis, angioedema.

- **ii. Cardiovascular**
  - Bradycardia, cardiac arrest, cardiac arrhythmias, cardiac enlargement, circulatory collapse, congestive heart failure, fat embolism, hypertension, hypertrophic, cardiomyopathy in premature infants, myocardial rupture following recent myocardial infarction, edema, pulmonary edema, syncope, tachycardia, thromboembolism, thrombophlebitis, vasculitis etc.
iii. **Dermatologic**

Acne, allergic dermatitis, dry scaly skin, ecchymoses and petechiae, erythema, impaired wound healing, increased sweating, rash, striae, suppression of reactions to skin tests, thin fragile skin, thinning scalp hair, urticaria etc.

iv. **Endocrine**

Decrease carbohydrate and glucose tolerance, development of cushingoid state, hyperglycemia, glycosuria, hirsutism, hypertrichosis, increased requirements for insulin or oral hypoglycemic agents in diabetes, manifestations of latent diabetes melitus, menstrual irregularities, secondary adrenocortical and pituitary unresponsiveness (particularly in times of stress, as in trauma, surgery, or illness), suppression of growth in pediatric patients.

v. **Fluid and Electrolyte Disturbances**

Congestive heart failure in susceptible patient’s fluid retention, hypokalemic alkalosis, potassium loss, sodium retention etc.

vi. **Gastrointestinal**

Abdominal distention, elevation in serum liver enzyme levels (usually reversible upon discontinuation), hepatomegaly, increased appetite, nausea, pancreatitis, peptic ulcer with possible perforation and hemorrhage, perforation of the small and large intestine (particularly in patients with inflammatory bowel disease), ulcerative esophagitis.

vii. **Metabolic**

Negative nitrogen balance due to protein catabolism.

viii. **Musculoskeletal**

Aseptic necrosis of femoral and humeral heads, loss of muscle mass, muscle weakness, osteoporosis, and pathologic fracture of long bones, steroid myopathy, tension rupture, and vertebral compression fractures.

ix. **Neurological/Psychiatric**

Convulsions, depression, emotional instability, euphoria, headache, increased intracranial pressure with papilledema (pseudotumor cerebri) usually following discontinuation of treatment, insomnia, mood swings, neuritis, neuropathy, paresthesia, personality changes, psychotic disorders, vertigo etc.

x. **Ophthalmic**

Exophthalmos, glaucoma, increased intraocular pressure, posterior subcapsular cataracts.

d) **Drugs Interaction**

**DECADRON** may interact with aminoglutethimide, potassium-depleting agents (e.g., amphotericin B, diuretics), macrolide antibiotics, anticholinesterases, oral anticoagulants, antidiabetics, antitubercular drugs, cholestyramine, cyclosporine, dexamethasone suppression tests (DST), digoxisides, ephedrine, estrogens and oral contraceptives, barbiturates, phenytoin, carbamazepine, rifampin, ketoconazole, aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs), phenytoin, skin tests, thalidomide, and live or inactivated vaccines. Tell your doctor all medications and supplements you use and all vaccines you recently received. Decadron has been used in pediatric patients. Such use should be done in most patients in conjunction with a pediatric specialist.

**MEDROL** may interact with aspirin (taken on a daily basis or at high doses), diuretics (water pills), blood thinner, cyclosporine, insulin or oral diabetes medications, ketoconazole, rifampin, seizure medications, or “live” vaccines. Tell your doctor all medications and supplements you use and all vaccines you recently received.

Corticosteroids may mask some signs of infection, and new infections may appear during their use. Infections with any pathogen including viral, bacterial, fungal, protozoan or helminthic infections, in any location of the body, may be associated with the use of corticosteroids alone or in combination with other immunosuppressive agents that affect cellular immunity, humoral immunity, or neutrophil function.

These infections may be mild, but can be severe and at times fatal. With increasing doses of corticosteroids, the rate of occurrence of infectious complications increases. There may be decreased resistance and inability to localize infection when corticosteroids are used.

Prolonged use of corticosteroids may produce posterior subcapsular cataracts, glaucoma with possible damage to the optic nerves, and may enhance the establishment of secondary ocular infections due to fungi or viruses.

e) **Black Fungus Infection (Mucormycosis)**

- Black fungus, also known as Mucormycosis, is a rare but dangerous infection. Black fungus is caused by getting into contact with fungus spores in the environment. It can also form in the skin after the fungus enters through a cut, scrape, burn, or another type of skin trauma.

- Fungi live in the environment, particularly in soil and decaying organic matter such as leaves, compost piles, rotten wood, and so on. This fungal infection is caused by a type of mould known as ‘mucormycetes’. It should be noted that this rare fungal infection affects persons who have health issues or who use drugs that weaken the body’s ability to fight the infections.
i. **Black Fungus Causes**
- Mucormycetes are a type of mould that causes fungal infections. These moulds can be found everywhere in the environment, including soil, air, and food. They enter the body via the nose, mouth, or eyes and can have an impact on the brain if it is not treated on time. According to medical experts, the main cause of black fungus (mucormycosis) is steroid overuse during COVID treatment.
- Black fungus (mucormycosis) primarily affects people who have health problems or who take medications that reduce the body's ability to fight germs and illness. The person's immunity is low after covid treatment, which makes them vulnerable to black fungus infection. People with diabetes and COVID-19 patients are at greater risk of developing an infection. 27

ii. **Black Fungus Risks**
- People who fall into the following categories are more likely to develop black fungus:
  - Uncontrolled diabetes, diabetic ketoacidosis, and diabetics taking steroids or tocilizumab.
  - Patients taking immunosuppressant’s or receiving anticancer treatment, as well as those suffering from a chronic debilitating illness.
  - Patients taking high doses of steroids or tocilizumab for an extended period.
  - Cases of COVID-19 Severity
  - Patients on oxygen who required nasal prongs, a mask, or a ventilator support
  - Patients who get COVID treatment within six weeks are more likely to develop black fungus. 27

### III. Result and Discussion

A total of 50 doctors and some medical stores from across India were included in the survey. Doctors prescribed more methylprednisolone and dexamethasone drug medicine than steroid medicines to corona patients.

In our research, most side effects were observed for corona patients taking methylprednisolone and dexamethasone drug.

Due to over-prescription of doctors, we came to know from other studies that diabetics who were cured of taking methylprednisolone and dexamethasone medicine when they had corona, got a disease called black fungus after some time. And more deaths from black fungus disease were seen in diabetic patients.

We also found in our survey that Methylprednisolone drug is prescribed more by the doctor in corona patients. Compared to methylprednisolone, doctor prescribed dexamethasone drug is less given in corona patients.

---

**Figure 3:** Names of the Cities we Placed in Our Survey
IV. Conclusion

This research had shown that overdose of methylprednisolone and dexamethasone drug take diabetes patient he has serious eye effect and cause black fungus.

It has been found from research that three things have been detected by giving high amount of steroid drugs to corona patients.

1) Patients who have been cured of corona, who did not have any disease before corona, after recovering from corona in their body, after going home, they have diabetes.

2) Those corona patients who already have diabetes, after being cured by giving more steroid medicine, they got a complaint of black fungus disease.

3) These two things have shown that more steroids are being given to corona patients than giving more side effects because giving more steroids reduces immunity in the body, due to which the black fungus present in the environment is easily found in patients with low immunity. It goes away and the infection increases in the patient's eyes, if the patient does not take treatment on time, then his life is also lost.

However very less work has been on this drug & there is further more scope of scientific investigation.

Acknowledgment

We grateful thanks to all the sincere and extremely helping jai narain vyas university ratanada students for their support and help for the completion of work. Last but not the least, we thankful to all those who cooperated and helped us directly or indirectly to carry out this work.

References Références Referencias


14. "Prescribing medicines in pregnancy database". Australian Government. 3 March 2014. Archived from the original on 8 April 2014. Retrieved 22 April 2014. Drugs which have been taken by a large number of pregnant women and women of childbearing age without any proven increase in the frequency of malformations or other direct or indirect harmful effects on the fetus having been observed.


**MEMBERSHIPS**

**FELLOWS/ASSOCIATES OF MEDICAL RESEARCH COUNCIL**

**FMRC/AMRC MEMBERSHIPS**

---

**INTRODUCTION**

FMRC/AMRC is the most prestigious membership of Global Journals accredited by Open Association of Research Society, U.S.A (OARS). The credentials of Fellow and Associate designations signify that the researcher has gained the knowledge of the fundamental and high-level concepts, and is a subject matter expert, proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice. The credentials are designated only to the researchers, scientists, and professionals that have been selected by a rigorous process by our Editorial Board and Management Board.

Associates of FMRC/AMRC are scientists and researchers from around the world are working on projects/researches that have huge potentials. Members support Global Journals’ mission to advance technology for humanity and the profession.

---

**FMRC**

**FELLOW OF MEDICAL RESEARCH COUNCIL**

FELLOW OF MEDICAL RESEARCH COUNCIL is the most prestigious membership of Global Journals. It is an award and membership granted to individuals that the Open Association of Research Society judges to have made a 'substantial contribution to the improvement of computer science, technology, and electronics engineering.

The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Fellows are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Fellow Members.
**Benefit**

**To the institution**

**Get letter of appreciation**

Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

**Exclusive Network**

**Get access to a closed network**

A FMRC member gets access to a closed network of Tier 1 researchers and scientists with direct communication channel through our website. Fellows can reach out to other members or researchers directly. They should also be open to reaching out by other.

**Certificate**

**Certificate, LoR and Laser-Momento**

Fellows receive a printed copy of a certificate signed by our Chief Author that may be used for academic purposes and a personal recommendation letter to the dean of member’s university.

**Designation**

**Get honored title of membership**

Fellows can use the honored title of membership. The “FMRC” is an honored title which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., FMRC or William Walldroff, M.S., FMRC.

**Recognition on the Platform**

**Better visibility and citation**

All the Fellow members of FMRC get a badge of “Leading Member of Global Journals” on the Research Community that distinguishes them from others. Additionally, the profile is also partially maintained by our team for better visibility and citation. All fellows get a dedicated page on the website with their biography.

© Copyright by Global Journals | Guidelines Handbook
**Future Work**

**Get discounts on the future publications**
Fellows receive discounts on the future publications with Global Journals up to 60%. Through our recommendation programs, members also receive discounts on publications made with OARS affiliated organizations.

**GJ Internal Account**

**Unlimited forward of emails**
Fellows get secure and fast GJ work emails with unlimited storage of emails that they may use them as their primary email. For example, john [AT] globaljournals [DOT] org.

**Premium Tools**

**Access to all the premium tools**
To take future researches to the zenith, fellows receive access to all the premium tools that Global Journals have to offer along with the partnership with some of the best marketing leading tools out there.

**Conferences & Events**

**Organize seminar/conference**
Fellows are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent. Additionally, they get free research conferences (and others) alerts.

**Early Invitations**

**Early invitations to all the symposiums, seminars, conferences**
All fellows receive the early invitations to all the symposiums, seminars, conferences and webinars hosted by Global Journals in their subject.

© Copyright by Global Journals | Guidelines Handbook
PUBLISHING ARTICLES & BOOKS

EARN 60% OF SALES PROCEEDS
Fellows can publish articles (limited) without any fees. Also, they can earn up to 70% of sales proceeds from the sale of reference/review books/literature/publishing of research paper. The FMRC member can decide its price and we can help in making the right decision.

REVIEWS

GET A REMUNERATION OF 15% OF AUTHOR FEES
Fellow members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

ACCESS TO EDITORIAL BOARD

BECOME A MEMBER OF THE EDITORIAL BOARD
Fellows and Associates may join as a member of the Editorial Board of Global Journals Incorporation (USA) after successful completion of three years as Fellow and as Peer Reviewer.

AND MUCH MORE

GET ACCESS TO SCIENTIFIC MUSEUMS AND OBSERVATORIES ACROSS THE GLOBE
All members get access to 5 selected scientific museums and observatories across the globe. All researches published with Global Journals will be kept under deep archival facilities across regions for future protections and disaster recovery. They get 10 GB free secure cloud access for storing research files.
ASSOCIATE OF MEDICAL RESEARCH COUNCIL

ASSOCIATE OF MEDICAL RESEARCH COUNCIL is the membership of Global Journals awarded to individuals that the Open Association of Research Society judges to have made a ‘substantial contribution to the improvement of computer science, technology, and electronics engineering.

The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Associate membership can later be promoted to Fellow Membership. Associates are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Associate Members.
**Benefit**

**To the Institution**
**Get Letter of Appreciation**
Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

**Exclusive Network**
**Get Access to a Closed Network**
A AMRC member gets access to a closed network of Tier 2 researchers and scientists with direct communication channel through our website. Associates can reach out to other members or researchers directly. They should also be open to reaching out by other.

**Certificate**
**Certificate, LoR and Laser-Momento**
Associates receive a printed copy of a certificate signed by our Chief Author that may be used for academic purposes and a personal recommendation letter to the dean of member's university.

**Designation**
**Get Honored Title of Membership**
Associates can use the honored title of membership. The “AMRC” is an honored title which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., AMRC or William Waldroff, M.S., AMRC.

**Recognition on the Platform**
**Better Visibility and Citation**
All the Associate members of AMRC get a badge of "Leading Member of Global Journals" on the Research Community that distinguishes them from others. Additionally, the profile is also partially maintained by our team for better visibility and citation.
**FUTURE WORK**

**GET DISCOUNTS ON THE FUTURE PUBLICATIONS**

Associates receive discounts on future publications with Global Journals up to 30%. Through our recommendation programs, members also receive discounts on publications made with OARS affiliated organizations.

---

**GJ ACCOUNT**

**UNLIMITED FORWARD OF Emails**

Associates get secure and fast GJ work emails with 5GB forward of emails that they may use them as their primary email. For example, john [AT] globaljournals [DOT] org.

---

**PREMIUM TOOLS**

**ACCESS TO ALL THE PREMIUM TOOLS**

To take future researches to the zenith, fellows receive access to almost all the premium tools that Global Journals have to offer along with the partnership with some of the best marketing leading tools out there.

---

**Conferences & Events**

**Organize seminar/conference**

Associates are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent. Additionally, they get free research conferences (and others) alerts.

---

**Early Invitations**

**Early invitations to all the symposiums, seminars, conferences**

All associates receive the early invitations to all the symposiums, seminars, conferences and webinars hosted by Global Journals in their subject.
Publishing Articles & Books

Earn 60% of Sales Proceeds

Associates can publish articles (limited) without any fees. Also, they can earn up to 30-40% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.

Reviewers

Get a Remuneration of 15% of Author Fees

Associate members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

And Much More

Get Access to Scientific Museums and Observatories Across the Globe

All members get access to 2 selected scientific museums and observatories across the globe. All researches published with Global Journals will be kept under deep archival facilities across regions for future protections and disaster recovery. They get 5 GB free secure cloud access for storing research files.
<table>
<thead>
<tr>
<th>ASSOCIATE</th>
<th>FELLOW</th>
<th>RESEARCH GROUP</th>
<th>BASIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$4800 lifetime designation</td>
<td>$6800 lifetime designation</td>
<td>$12500.00 organizational</td>
<td>APC per article</td>
</tr>
<tr>
<td>2 discounted publishing/year</td>
<td>Unlimited discounted publishing/year</td>
<td>Momento</td>
<td></td>
</tr>
<tr>
<td>Gradation of Research</td>
<td>Gradation of Research</td>
<td>Unlimited free</td>
<td></td>
</tr>
<tr>
<td>10 research contacts/day</td>
<td>Unlimited research</td>
<td>publishing/year</td>
<td></td>
</tr>
<tr>
<td>1 GB Cloud Storage</td>
<td>research contacts/day</td>
<td>Unlimited research</td>
<td></td>
</tr>
<tr>
<td>GJ Community Access</td>
<td>5 GB Cloud Storage</td>
<td>contacts/day</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Online Presence Assistance</td>
<td>Unlimited Cloud Storage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GJ Community Access</td>
<td>Online Presence</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Assistance</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GJ Community Access</td>
<td></td>
</tr>
</tbody>
</table>
We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe InDesign, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

**Before and during Submission**

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and agree to Global Journals' ethics and code of conduct, along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author’s email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s)’ names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted must not have been submitted or published elsewhere and all authors must be aware of the submission.

**Declaration of Conflicts of Interest**

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

**Policy on Plagiarism**

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors’ institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures

© Copyright by Global Journals | Guidelines Handbook
Authorship Policies

Global Journals follows the definition of authorship set up by the Open Association of Research Society, USA. According to its guidelines, authorship criteria must be based on:

1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

Copyright

During submission of the manuscript, the author is confirming an exclusive license agreement with Global Journals which gives Global Journals the authority to reproduce, reuse, and republish authors’ research. We also believe in flexible copyright terms where copyright may remain with authors/employers/institutions as well. Contact your editor after acceptance to choose your copyright policy. You may follow this form for copyright transfers.

Appealing Decisions

Unless specified in the notification, the Editorial Board’s decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.
**Manuscript Style Instruction (Optional)**

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27” x 11’’, left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word “Abstract” in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

**Structure and Format of Manuscript**

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.

b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.

c) Up to 10 keywords that precisely identify the paper’s subject, purpose, and focus.

d) An introduction, giving fundamental background objectives.

e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.

f) Results which should be presented concisely by well-designed tables and figures.

g) Suitable statistical data should also be given.

h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.

j) There should be brief acknowledgments.

k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.
Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title
The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details
The full postal address of any related author(s) must be specified.

Abstract
The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords
A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods
Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations
Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations
Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends
Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.
Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Electronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for Writing a Good Quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.
6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.
20. **Think technically:** Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. **Adding unnecessary information:** Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn’t be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. **Report concluded results:** Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. **Upon conclusion:** Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium through which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

**Informal Guidelines of Research Paper Writing**

**Key points to remember:**

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

**Final points:**

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

**The introduction:** This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

**The discussion section:**

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

**General style:**

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

**To make a paper clear:** Adhere to recommended page limits.
Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don’t address the reviewer directly. Don’t use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract:

This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.
The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

**Approach:**

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

**Procedures (methods and materials):**

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

**Materials:**

*Materials may be reported in part of a section or else they may be recognized along with your measures.*

**Methods:**

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

**Approach:**

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

**What to keep away from:**

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.
Results:
The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective
details of the outcome, and save all understanding for the discussion.
The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to
present consequences most efficiently.
You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data
or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if
requested by the instructor.

Content:
- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if
appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or
manuscript.

What to stay away from:
- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:
As always, use past tense when you submit your results, and put the whole thing in a reasonable order.
Put figures and tables, appropriately numbered, in order at the end of the report.
If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:
If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached
appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and
include a heading. All figures and tables must be divided from the text.

Discussion:
The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded
based on problems with the discussion. There is no rule for how long an argument should be.
Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the
paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results
and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The
implication of results should be fully described.
Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain
mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have
happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the
data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded
or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

**Approach:**

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

**The Administration Rules**

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

*Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.*

**Segment draft and final research paper:** You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

**Written material:** You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.


**CRITERION FOR GRADING A RESEARCH PAPER (Compilation)
BY GLOBAL JOURNALS**

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

<table>
<thead>
<tr>
<th>Topics</th>
<th>Grades</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A-B</td>
</tr>
<tr>
<td><strong>Abstract</strong></td>
<td>Clear and concise with appropriate content, Correct format. 200 words or below</td>
</tr>
<tr>
<td><strong>Introduction</strong></td>
<td>Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited</td>
</tr>
<tr>
<td><strong>Methods and Procedures</strong></td>
<td>Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads</td>
</tr>
<tr>
<td><strong>Result</strong></td>
<td>Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake</td>
</tr>
<tr>
<td><strong>Discussion</strong></td>
<td>Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited</td>
</tr>
<tr>
<td><strong>References</strong></td>
<td>Complete and correct format, well organized</td>
</tr>
<tr>
<td>Index</td>
<td>Page References</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------</td>
</tr>
<tr>
<td>A</td>
<td>Anthelmintic · 1, 8</td>
</tr>
<tr>
<td></td>
<td>Scavenging · 33, 34, 41, 44, 45, 46</td>
</tr>
<tr>
<td></td>
<td>Supernatant · 35</td>
</tr>
<tr>
<td></td>
<td>Susceptible · 50, 51</td>
</tr>
<tr>
<td>C</td>
<td>Cataracts · 49, 50, 51, 52</td>
</tr>
<tr>
<td></td>
<td>Compliance · 9, 10</td>
</tr>
<tr>
<td></td>
<td>Tapering · 48</td>
</tr>
<tr>
<td>D</td>
<td>Decoction · 32</td>
</tr>
<tr>
<td></td>
<td>Deviation · 27</td>
</tr>
<tr>
<td></td>
<td>Dismutation · 42</td>
</tr>
<tr>
<td></td>
<td>Dispersed · 10</td>
</tr>
<tr>
<td></td>
<td>Dissected · 35</td>
</tr>
<tr>
<td></td>
<td>Morphology · 18</td>
</tr>
<tr>
<td></td>
<td>Nucleation · 14, 16, 18, 23, 24</td>
</tr>
<tr>
<td></td>
<td>Precipitation · 12, 14, 16, 18, 20, 21, 23, 25</td>
</tr>
<tr>
<td>I</td>
<td>Emulsifiers · 11</td>
</tr>
<tr>
<td></td>
<td>Iatrogenic · 48</td>
</tr>
<tr>
<td></td>
<td>Viscosity · 12</td>
</tr>
<tr>
<td>M</td>
<td>Nucleation · 14, 16, 18, 23, 24</td>
</tr>
<tr>
<td></td>
<td>Precipitation · 12, 14, 16, 18, 20, 21, 23, 25</td>
</tr>
<tr>
<td>N</td>
<td>Ruminant’s · 1</td>
</tr>
<tr>
<td>R</td>
<td>Scavenging · 33, 34, 41, 44, 45, 46</td>
</tr>
<tr>
<td></td>
<td>Supernatant · 35</td>
</tr>
<tr>
<td></td>
<td>Susceptible · 50, 51</td>
</tr>
<tr>
<td></td>
<td>Tapering · 48</td>
</tr>
<tr>
<td></td>
<td>Viscosity · 12</td>
</tr>
</tbody>
</table>