Effects of 'dedo de moça' pepper extract administration on wistar rat pregnancy and fetal development

Efeitos da administração do extrato de pimenta dedo de moça na gravidez e no desenvolvimento fetal de ratas wistar

Efectos de la administración de extracto de pimienta 'dedo de moça' en la gestación y el desarrollo fetal de la rata wistar

DOI: 10.54022/shsv5n3-022

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ABSTRACT

Introduction: The dedo de moça pepper is spicy due to capsaicin, which activates receptors in sensory neurons, generating a burning sensation. Studies suggest that capsaicin-sensitive neurons, by releasing neuropeptides, may affect uterine contraction. Objective: To evaluate the effect of chronic administration of capsaicin extract (Cps) during pregnancy in Wistar rats and their fetuses. Methodology: Pregnant female Wistar rats were divided into 4 groups (n = 5/group): Group I – Negative Control; Group II – Positive Control; Group III – Cps 1 – 0.25 mL; Group IV – Cps 2 – 0.5 mL. Exposure was initiated after embryo implantation (3rd day of pregnancy) until full term (20th day of pregnancy), with gavage performed three times per week. Results: On the 20th day, pregnant rats were euthanized, and outcomes evaluated included weight gain, placental morphology, reproductive
capacity, and embryonic/fetal development. Ingestion of capsaicin extract resulted in negative impacts on fetal intrauterine growth parameters and placental alterations, with deposition of atypical hyaline material for gestational age and changes in vascular structures. **Conclusion:** The results indicate that capsaicin extract interfered with placental exchange, leading to intrauterine growth restriction of the fetuses.

**Keywords:** Placenta. Pregnancy. Capsaicin. Uterine contraction. *Dedo de Moça* Pepper.

**RESUMO**
Introdução: A pimenta dedo de moça é picante devido à capsaicina, que ativa os receptores nos neurônios sensoriais, gerando uma sensação de queimação. Estudos sugerem que os neurônios sensíveis à capsaicina, ao liberarem neuropeptídeos, podem afetar a contração uterina. Objetivo: Avaliar o efeito da administração crónica de extrato de capsaicina (Cps) durante a gravidez em ratas Wistar e seus fetos. Metodologia: Ratas Wistar fêmeas grávidas foram divididas em 4 grupos (n = 5/grupo): Grupo I – Controle Negativo; Grupo II – Controle Positivo; Grupo III – Cps 1 – 0,25 mL; Grupo IV – Cps 2 – 0,5 mL. A exposição foi iniciada após a implantação do embrião (3º dia de gestação) até o termo (20º dia de gestação), com gavagem realizada três vezes por semana. Resultados: No 20º dia, as ratas prenhes foram sacrificadas e os resultados avaliados incluíram ganho de peso, morfologia da placenta, capacidade reprodutiva e desenvolvimento embrionário/fetal. A ingestão de extrato de capsaicina resultou em impactos negativos nos parâmetros de crescimento intrauterino fetal e alterações placentárias, com deposição de material hialino atípico para a idade gestacional e alterações nas estruturas vasculares. Conclusão: Os resultados indicam que o extrato de capsaicina interferiu na troca placentária, levando à restrição do crescimento intrauterino dos fetos.


**RESUMEN**
Introducción: El pimiento *dedo de moça* es picante debido a la capsaicina, que activa receptores en neuronas sensoriales, generando una sensación de ardor. Los estudios sugieren que las neuronas sensibles a la capsaicina, al liberar neuropeptídeos, pueden afectar a la contracción uterina. Objetivo: Evaluar el efecto de la administración crónica de extracto de capsaicina (Cps) durante la gestación en ratas Wistar y sus fetos. Metodología: Las ratas Wistar hembras preñadas se dividieron en 4 grupos (n = 5/grupo): Grupo I – Control Negativo; Grupo II – Control Positivo; Grupo III – Cps 1 – 0,25 mL; Grupo IV – Cps 2 – 0,5 mL. La exposición se inició después de la implantación del embrión (3er día de gestación) hasta el término (20º día de gestación), con gavage realizado tres veces por semana. Resultados: En el 20º día, se practicó la eutanasia a las ratas preñadas y se evaluaron el aumento de peso, la morfología de la placenta, la capacidad reproductiva y el desarrollo embrionario/fetal. La ingestión de extracto de capsaicina produjo efectos negativos en los parámetros de crecimiento fetal intrauterino y alteraciones placentarias, con deposición de material hialino atípico para la edad gestacional y cambios en las estructuras vasculares. Conclusiones:
Los resultados indican que el extracto de capsaicina interfirió en el intercambio placentario, provocando una restricción del crecimiento intrauterino de los fetos.


1 INTRODUCTION

Peppers are fruits from plants of the genus *Capsicum*, which belong to the Solanaceae family. They are widely cultivated and consumed worldwide due to their characteristic spicy flavor, as well as being used as seasonings and ingredients in many cuisines (Alonso-Villegas *et al.*, 2023).

In terms of the chemical composition of peppers, they feature a diverse array of compounds, with capsaicinoids being the most notable, responsible for their spicy flavor. Capsaicin emerges as the primary capsaicinoid. Additionally, peppers also contain carbohydrates, proteins, fiber, vitamins, and minerals (Bogusz *et al.*, 2018).

The hanging variety of *Capsicum baccatum*, known as "dedo de moça," is renowned for its spiciness, attributed to the presence of capsaicinoids, particularly capsaicin (8-methyl-N-vanillyl-6-nonenamide) (Batiha *et al.*, 2020). In Brazil, it is one of the most popular peppers, especially enjoyed in the South and Southeast regions (Cardoso *et al.*, 2018).

Capsaicinoids are chemical compounds found in peppers that are responsible for their spicy flavor, with capsaicin being the most prominent and well-known. These compounds act by activating pain and heat receptors in the mouth and on the tongue, sending signals to the brain that the region is being burned or heated (Hamed *et al.*, 2019).

Despite their spicy taste, capsaicinoids found in peppers offer potential health benefits, such as antioxidant and anti-inflammatory properties, and are linked to improved cardiovascular health and metabolism (Asad *et al.*, 2024). Capsaicin, in particular, is known for its wide range of effects, including pain relief, anticancer activity, and the ability to reduce inflammation and obesity. It also helps lower postprandial glucose levels and improve insulin resistance (Fattori *et al.*, 2016). These effects are thought to be mediated through the activation of the
TRPV1 receptor in sensory nerve fibers. However, the precise mechanism remains poorly understood, although it may involve the release of neuropeptides like substance P and CGRP (Calcitonin Gene-Related Peptide) (Russell et al., 2014).

However, excessive consumption of capsaicin can cause gastrointestinal irritation, respiratory issues, certain types of cancer, and other adverse effects in some individuals (Popescu et al., 2020).

Studies suggest that capsaicin can influence uterine contraction by altering the concentration of different neuropeptides (Lin et al., 2022). This is because capsaicin is known to interact with specific receptors, such as the TRPV1 (Transient Receptor Potential Vanilloid 1), found in sensory nerve endings. When activated by capsaicin, these receptors can trigger the release of neuropeptides CGRP and substance P (Tingåker et al., 2008).

These neuropeptides have been associated with regulating uterine activity. While substance P is known to promote uterine contraction, CGRP may have opposite effects, causing uterine relaxation under certain circumstances. Therefore, by affecting the concentration of these neuropeptides, capsaicin can negatively influence uterine contraction (Malvasi et al., 2017).

It is known that during pregnancy, physiological changes occur that result in endocrine and metabolic alterations in the maternal body (Soma-Pillay et al., 2016). Neuroendocrine events in the placenta, fetus, and mother are critical for the onset and maintenance of pregnancy, fetal growth and development, as well as childbirth (De Bonis et al., 2012). Among the changes that occur during pregnancy is the development of the placenta, a selective filter for the passage of substances between the mother and the fetus, performing functions such as excretion, gas exchange, maintenance of homeostasis, hormone secretion, and hematopoiesis (Cindrova-Davies; Sferruzzi-Perri, 2022).

Hence, we devised a novel experimental study utilizing Wistar rats, prompted by the scarcity of research investigating the consequences of chronic capsaicin administration. Our objective was to evaluate maternal and fetal harm stemming from the chronic administration of capsaicin extract.
2 MATERIAL AND METHODS

2.1 OBTAINING THE PEPPER AND EXTRACT PREPARATION

The commercial peppers were prepared by removing their stems, washing them, and then subjecting them to drying in an oven at 40°C for 24 hours to achieve the appropriate moisture content for storage. Afterwards, they were dried and ground in a blender, and 70% INPM alcohol was added to obtain a solution with a concentration of 10% (10g/100mL). The solution was left to stand for seven days, covered with PVC film for extraction. In the next step, the solution was filtered and evaporated. The resulting extract was diluted in physiological saline at a concentration of 1:1 (v/v) for administration to animals.

2.2 EXPERIMENTAL DESIGN

The study and experiments received approval from the Committee on Animal Care and Use of Experimental Animals (CEUA/FCMS, Protocol Number 2016/57) at the Sorocaba campus. Healthy male and female Wistar rats were obtained from the FCMS/PUC-SP animal facility at the Sorocaba campus and housed in the same facility according to animal welfare standards (Percie et al., 2020). During the study, the animals were kept in microenvironment isolation cages (Alesco®), under standard temperature (22-24 °C), humidity (55 ± 5%), and lighting (12:12 h light:dark cycle). They were fed standard diet ad libitum and had free access to filtered water.

For mating, one male was housed with three females overnight. Pregnancy was confirmed by the positive identification of spermatozoa in vaginal lavage smears through microscopic observations (Biological Microscope, Model Axio Lab.A1, ZEISS®), and was designated as day one of gestation (Damasceno et al., 2008).

Pregnant rats were housed individually and randomly divided into 4 groups (n = 5 per group):

- group I – Negative Control, receiving water (no gavage);
- group II – Positive Control, receiving saline solution via gavage;
• group III – Cps 1 – 0.25 mL, receiving pepper extract;
• group IV – Cps 2 – 0.5 mL, receiving pepper extract.

Animals treated with capsaicin extract received the extract after the embryo implantation period (3rd day of pregnancy) until term (20th day of pregnancy), with gavage performed three times per week, specifically on Mondays, Wednesdays, and Fridays.

Animals were weighed and observed throughout gestation for any morbidity daily. On the 20th day of gestation, the animals were anesthetized with Halothane, with an overdose compared to the doses considered anesthetic. The inhalation anesthetic was soaked in cotton and placed with the animal in a closed environment, as determined by CONCEA (Concea, 2015).

The cesarean section procedure was performed with a longitudinal incision in the linea alba to expose the uterus and ovaries. After euthanasia, samples of fetus and placental tissue were collected from each animal.

2.3 EVALUATION OF REPRODUCTIVE CAPACITY

The number of fetuses, implantations, and visible resorptions in the uterine cavities were assessed. Additionally, the corpora lutea were manually removed with a scalpel and counted. The fetuses were then removed from the gestational sacs, counted, weighed, and euthanized with halothane. Reproductive capacity was evaluated based on the percentage of pre-implantation loss, post-implantation loss, offspring vitality, and fetal weight.

The rate of preimplantation (Prem) loss was calculated using the formula:

\[ \text{Prem loss} = \left( \frac{C_l - Imp}{C_l} \right) \times 100 \]  \hspace{1cm} (1)

The rate of postimplantation (Postm) loss was calculated using the formula:

\[ \text{Postm loss} = \left( \frac{Imp - L_f}{Imp} \right) \times 100 \]  \hspace{1cm} (2)

Offspring's survival was determined according to the formula:
\[ \text{Survival} = \left( \frac{L_f}{F_t} \right) \times 100 \quad (3) \]

where

- \( C_l \) is the number of corpus luteum,
- \( \text{Imp} \) is the number of implantations,
- \( L_f \) refers to live fetuses, and
- \( F_t \) is the total number of fetuses.

### 2.4 MORPHOLOGICAL ANALYSIS OF THE PLACENTAS

After opening the embryonic sac, the placentas were removed, weighed on a scale (Ohaus®-AS200S), and used for morphometric evaluation. Histological analysis of the placentas was performed after fixation and processing. Fixation was carried out in buffered formalin for 48 hours, followed by overnight rinsing in running water. The placentas were then stored in 70% alcohol. Subsequently, they were processed using the PT12 Automatic Tissue Processor. The tissues were embedded in histological paraffin blocks. Histological sections were made using a rotary-manual microtome with a thickness of four micrometers, stained with hematoxylin and eosin, and mounted with histological resin (Tolosa \textit{et al.}, 2003). Quantitative analysis consisted of measurements in micrometers of placental width and thickness, performed using a microscope equipped with a digital camera (Biological Microscope, Model Axio Lab.A1, ZEISS®).

### 2.5 FETAL MORPHOLOGICAL ASSESSMENT

Fetuses were separated from the respective placentas and weighed. The fetuses were euthanized with halothane (saturated vat). Body measurements were taken for dimensions including anterior-posterior and lateral-lateral skull, anterior-posterior and lateral-lateral thorax, and craniocaudal length, measured in millimeters.
2.6 STATISTICAL ANALYSIS

Data are presented as mean ± standard deviation. Normality was assessed, and since the data were non-parametric, the Kruskal-Wallis test was applied, followed by the Duncan test to determine differences among treatment protocols. Additionally, a non-parametric Two-Way Analysis of Variance was conducted to verify the influence of time and treatments on weight gain. A significance level of $p < 0.05$ was used, with results considered significant at $p < 0.05$. The litter was used as the comparison unit. Statistical analysis was performed using the Statistica® 8.0 and Graph Pad Prism® 8 programs.

3 RESULTS

3.1 MATERNAL PARAMETERS

Figure 1 illustrates the variation in weight gain among pregnant rats distributed across various experimental groups. The analyzed data, expressed as mean ± standard, reveal that there were no statistically significant differences in weight gain among the groups throughout the gestation period. Such uniformity indicates that the administration of capsaicin extract, at the dosages used, did not impact the weight gain of the pregnant rats compared to the control groups.

Figure 1 – Weight gain of pregnant rats in in the negative control (CN), positive control (CP), 0.25 mL (CP 1), and 0.5 mL (CP 2) groups. Data are presented as the mean ± SD or percentage (%), n=5 rats/group.

Source: Author
A Table 1 presents the reproductive capacity data. A notable increase in pre-implantation losses was observed in the treatment groups compared to the positive and negative control groups. There was no difference in post-implantation losses and fetal vitality, indicating that exposure to pepper extract at any concentration primarily affects pre-fetal implantation. Regarding fetal weight, we observed a reduction in fetal weight in all groups compared to the negative control, as well as in the treatment groups compared to the positive control. Additionally, we noted variations among the treatment groups.

Table 1 – The reproductive capacity in the negative control (CN), positive control (CP), 0.25 mL (CP 1), and 0.5 mL (CP 2) groups. Data are presented as the mean ± SD or percentage (%), n=5 rats/group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CN</td>
</tr>
<tr>
<td>Offspring vitality (%)</td>
<td>100 ± 0.0</td>
</tr>
<tr>
<td>Pre-Implantation loss (%)</td>
<td>8.5± 6.0</td>
</tr>
<tr>
<td>Post-Implantation loss (%)</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Fetus weight (g)</td>
<td>3.9 ± 0.86</td>
</tr>
<tr>
<td>Number of litters</td>
<td>5</td>
</tr>
<tr>
<td>Number of fetuses</td>
<td>37</td>
</tr>
</tbody>
</table>

Note: *p< 0.05 compared to CN; #p< 0.05 compared to CP; a*p< 0.05 compared to CP 1, using Kruskal-Wallis followed by Duncan test.

Source: Author

Figure 2 presents histological examinations of placental sections from different experimental groups of pregnant rats, showing variations in tissue composition and anomalies. In the Negative Control Group, well-preserved placental structures including basalis decidua (d), spongiotrophoblast (E), and trophoblastic labyrinth (L) with intact maternal-fetal villi indicate normal placental morphology without experimental intervention. In the Positive Control Group, the presence of placental glycogen deposition in the spongiotrophoblast region highlights potential responses to a standard treatment or experimental condition. In the Treatment Group 0.25 mL, similar to the negative control, this panel reveals well-preserved basalis decidua (d), spongiotrophoblast (E), and trophoblastic labyrinth (L), suggesting minimal impact at this concentration of capsaicin extract on placental architecture. However, in the Treatment Group 0.50 mL, notable pathological changes are evident, including deposition of hyaline material (*) and blood precipitation (arrow) between maternal-fetal villi, indicating significant alterations potentially due to higher concentrations of capsaicin extract.
Figure 1 – Histological analysis of placental tissue. HE 100x

Note: (A) Negative control group showing presence of basalis decidua (d), spongiotrophoblast (E), and labyrinth trophoblast (L) well preserved with maternal-fetal villi; (B) Positive control group: showing presence of placental glycogen deposition in the region of spongiotrophoblast; (C) Treatment group 0.25 mL: Presence of basalis decidua (d), spongiotrophoblast (E), and labyrinth trophoblast (L) well preserved; (D) Treatment group 0.50 mL: (*) Deposition of hyaline material; (arrow), precipitation of blood between maternal and fetal villi.

Source: Author

Table 2 displays fetal morphometric measurements. Regarding external abnormalities, a significant decrease was observed in all measurements in the treatment groups compared to the control groups.

Table 2 – Mean ± SD length (mm) of sections of the head and body of fetuses of maternal in the negative control (CN), positive control (CP), 0.25 mL (CP 1), and 0.5 mL (CP 2) groups. Data are presented as the mean ± SD or percentage (%), n=5 rats/group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CN</th>
<th>CP</th>
<th>CP 1</th>
<th>CP 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of litters</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Number of fetuses</td>
<td>37</td>
<td>29</td>
<td>33</td>
<td>44</td>
</tr>
<tr>
<td>Cranium anteroposterior</td>
<td>10.56 ± 0.57</td>
<td>11.01 ± 1.88</td>
<td>8.17 ± 0.67**</td>
<td>7.82 ± 1.27**</td>
</tr>
<tr>
<td>Cranium latero-lateral</td>
<td>14.15 ± 0.47</td>
<td>9.14 ± 0.80*</td>
<td>5.62 ± 0.63**</td>
<td>6.58 ± 0.87**</td>
</tr>
<tr>
<td>Thorax latero-lateral</td>
<td>11.14 ± 1.01</td>
<td>10.86 ± 1.49</td>
<td>6.04 ± 1.14**</td>
<td>6.97 ± 1.54**</td>
</tr>
<tr>
<td>Thorax anteroposterior</td>
<td>10.94 ± 0.67</td>
<td>11.16 ± 1.39*</td>
<td>7.62 ± 1.21**</td>
<td>8.78 ± 1.06**</td>
</tr>
<tr>
<td>Cranium-caudal</td>
<td>33.47 ± 1.31</td>
<td>33.42 ± 4.49</td>
<td>27.91 ± 3.15**</td>
<td>30.75 ± 1.25**</td>
</tr>
</tbody>
</table>

Note: *p < 0.05 compared to CN; **p < 0.05 compared to CP; ***p < 0.05 compared to CP 1, using Kruskal-Wallis followed by Duncan test.

Source: Author
4 DISCUSSION

A capsaicin, also known as the main component responsible for the spicy taste found in peppers of the Capsicum genus, is widely used in cooking due to its flavor and color properties (Rezazadeh et al., 2023). Additionally, it has been employed in traditional medicine to treat a variety of conditions across different parts of the world. In contemporary medicine, formulations containing capsaicin are often topically applied to treat various issues related to neurogenic pain, inflammation, and certain types of cancer (Yuan et al., 2016). Moreover, studies have shown that dietary capsaicin may help reduce oxidative stress, as evidenced by the restoration of antioxidant molecules and enzymes in red blood cells and the liver, as well as the reduction of elevated levels of lipid peroxides in rats. However, excessive use of capsaicin can turn this ingredient into a pro-oxidant (Szallasi, 2022).

Maternal weight plays a crucial role during pregnancy, directly influencing the course of gestation and fetal development. Studies have demonstrated a significant association between pre-pregnancy maternal weight and various obstetric and perinatal complications, including preeclampsia, gestational diabetes, fetal growth restriction, and premature birth (Marshall et al., 2022). Additionally, maternal weight is also closely linked to the baby’s birth weight, which can affect their long-term health.

Capsaicin has been of interest in studies due to its potential effects on body weight and metabolism. Some studies suggest that capsaicin may have thermogenic properties and stimulate metabolism, which could lead to a reduction in body weight in humans (Elmas; Gezer, 2022). However, the effects of capsaicin during pregnancy are not yet fully understood. In our study, we did not find any changes in the weight of the animals.

Excessive consumption of antioxidant-rich products during pregnancy can have adverse effects and potentially contribute to pre-implantation losses. Although antioxidants are generally beneficial to health, in excess, they can disrupt the oxidative balance in the body, causing oxidative stress (Sebastiani et al., 2022).

Pre-implantation losses, also known as early embryonic losses, occur when there is a failure in embryo implantation in the uterus, resulting in early pregnancy termination. In our study, we observed an increase in pre-implantation losses in
the groups treated with capsaicin, possibly related to the oxidative stress that occurs during pregnancy (Jarvis, 2016). This oxidative stress is necessary to regulate physiological processes such as embryo implantation, placental angiogenesis, and fetal development. However, when this oxidative stress is excessive, it can result in cellular damage and dysregulation of vital processes, contributing to pregnancy complications, including pre-implantation losses (Hussain et al., 2021).

Proper fetal development depends on ideal conditions to maintain high rates of proliferation, growth, and cellular differentiation, characteristic of the complex process it involves (Forhead; Fowden, 2014). Thus, disturbances in the supply of macro and micronutrients, oxygen, and hormones during pregnancy, or improper exposure to potentially toxic agents, can compromise fetal development and result in consequences in adulthood (Mousa; Naqash; Lim, 2019). The deposition of hyaline material in tissues can be interpreted as an indicator of tissue damage or an inflammatory response. Capsaicin is known for its pro-inflammatory properties and its ability to cause tissue irritation (Augustine et al., 2021). Therefore, the presence of hyaline material may suggest a localized inflammatory response resulting from exposure to capsaicin.

Additionally, the presence of blood precipitate between maternal and fetal villi is a significant concern, as it may indicate damage to the maternal-fetal interface. This interface is essential for the proper exchange of nutrients, oxygen, and waste between the mother and the fetus. The presence of blood in this region can interfere with this exchange and negatively affect fetal development (Burton; Fowden, 2015).

These findings indicate that capsaicin may have adverse effects on the uterine environment and fetal health. Localized inflammation and interference with the maternal-fetal interface can result in gestational complications such as fetal growth restriction, preeclampsia, or spontaneous abortion (Megli; Coyne, 2022). This is consistent with the results of our study, which identified reduced fetal weight in the treated groups, suggesting that possible excessive consumption of "Dedo de moça" pepper may be detrimental to the fetus.

Fetal morphological assessment is essential for understanding fetal development and identifying any anomalies that may be present (Springhall et al.,
In the context of the study in question, the reduction of all fetal parameters in the capsaicin-treated groups is a significant finding. The reduction in these measures in the treated groups suggests a negative impact on fetal development. This can be attributed to the oxidative stress induced by capsaicin, which may interfere with cell proliferation processes and the proper morphological development of the fetus (Peng et al., 2023).

As mentioned earlier, a proper balance between oxidants and antioxidants is essential for a healthy pregnancy, and excessive consumption of capsaicin may disrupt this balance.

5 CONCLUSION

In summary, our study demonstrated that the administration of "Dedo de moça" pepper extract during gestation in Wistar rats resulted in significant alterations in fetal development, including reduced weight and increased pre-implantation losses. These effects were likely due to interference with placental exchange, leading to intrauterine growth restriction. These findings suggest that consuming this extract during pregnancy may have adverse effects on the uterine environment and fetal development.

Our results can inform both society and the academic community about the potential risks associated with the excessive consumption of capsaicin-rich foods during pregnancy. This information is crucial for developing dietary guidelines and public health recommendations to ensure maternal-fetal health.

However, our study has limitations, such as the specific animal model used and the dosage regimen, which may not directly translate to humans. Future research should focus on different dosages, other animal models, and eventually clinical trials to fully understand the implications of capsaicin consumption during pregnancy. Additionally, studies should explore the underlying mechanisms by which capsaicin affects placental function and fetal development to provide a more comprehensive understanding of its effects.
REFERENCES


