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ELIANA JARDIM FERNANDES

EFEITO DA EXPOSIÇÃO DE NANOPARTÍCULAS DE LUTEÍNA SOBRE O MODELO EXPERIMENTAL TIPO DOENÇA DE PARKINSON EM *Drosophila melanogaster*

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Dissertação apresentada ao programa de Pós-graduação Stricto Sensu em Bioquímica da Universidade Federal do Pampa (UNIPAMPA), como requisito parcial para obtenção do grau de Mestra em Bioquímica.

Orientador: Prof. Dr. Gustavo Petri Guerra

Co-orientadora: Profa. Dra. Marina Prigol

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DEDICATÓRIA

A minha mãe Sônia Margarete,
por estar sempre ao meu lado,
me apoiando e incentivando.

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A universidade Federal do Pampa, e Programa de Pós-graduação em Bioquímica.

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RESUMO

A doença de Parkinson (DP) é a segunda doença neurodegenerativa mais comum em pessoas com idade acima dos 60 anos. Sabe-se que o estresse oxidativo, inibição na atividade do complexo I da cadeia transportadora de elétrons e perda de neurônios na substância negra desempenham um papel crucial na DP. A rotenona (ROT) inibe o complexo I da cadeia respiratória, causa toxicidade e ativa a NADPH oxidase, causa estresse oxidativo, degeneração seletiva do sistema dopaminérgico com distúrbios comportamentais subsequentes, característicos da patogênese da DP. Os tratamentos para a DP promovem apenas o alívio sintomático nos estágios iniciais, não protegem contra a progressão da doença, além disso provocam efeitos colaterais como, sonolência, confusão e piora da função motora. Assim, o desenvolvimento de novas opções terapêuticas, sendo mais eficazes, seguras e que diminuam os efeitos colaterais, consiste em um dos objetivos do cenário científico internacional. A luteína, principal carotenoide encontrado no cérebro humano, possui maior propriedade antioxidante comparada a outros carotenoides. Assim, o objetivo deste estudo foi avaliar o efeito de nanopartículas de luteína sobre um modelo experimental de DP em *Drosophila melanogaster*. Para verificar o efeito da luteína sobre os danos induzido pela ROT, as moscas (de ambos os sexos) com idades entre 1 a 4 dias, foram divididas em 4 grupos: controle, ROT (500 µM), nanopartículas de luteína (6µM), e ROT (500 µM) + nanopartículas de luteína (6µM), durante 7 dias. A porcentagem de sobrevivência foi avaliada durante os 7 dias. Após as moscas foram avaliadas nas tarefas comportamentais e posteriormente utilizadas para o preparo de amostras para a realização análises bioquímicas. Verificou-se que a exposição às nanopartículas de luteína (6 µM durante 7 dias), protegeu contra os danos induzidos pela ROT nos testes locomotores e exploratórios e aumentou a taxa de sobrevivência das moscas. Ainda restaurou os níveis de dopamina (DA), regulou atividade da Acetilcolinesterase (AChE), dos indicadores de estresse oxidativo e viabilidade celular. Estes resultados sugerem o envolvimento do estresse oxidativo, sistema dopaminérgico e colinérgico na DP, fornecendo evidências de que as nanopartículas de luteína são uma possível alternativa no tratamento de DP.

Palavras-chave: Carotenoide; antioxidantes; rotenona; estresse oxidativo; *Drosophila melanogaster.*

ABSTRACT

Parkinson´s disease (PD) is the second most common neurodegenerative disease in people over 60 years old. It is known that oxidative stress, inhibition in the activity of the complex I of the electron transport chain and neurons loss in the substantia nigra play a crucial role in PD. Rotenone (ROT) inhibits the respiratory chain complex I, causes toxicity, activates NADPH oxidase, causes oxidative stress and selective degeneration of the dopaminergic system, with subsequent behavioral disturbs, which are characteristic of PD. Treatments for PD only promote symptomatic relief in the early stages, do not protect against disease progression, also provoke collateral effects as drowsines, confusion and worsening of motor function. Thus, the development of new therapeutic options, which are more effective and safer and also reduce the collateral effects, consists in one of the objectives of the international scientific scenario. Lutein, the main carotenoid found in the human brain, possesses greater antioxidant property compared to other carotenoids. Therefore, the objective of the present study is to evaluate the effect of lutein nanoparticles on an experimental DP model on *Drosophila melanogaster*. In order to verify lutein´s protection effect on the damage induced by ROT, the flies (both sexes) with ages between 1 and 4 days, were divided into 4 groups: control group, ROT (500 µM), lutein nanoparticles (6µM) and ROT (500 µM) + nanoencapsulated (6µM), during 7 days. The survival percentage was evaluated during the 7 days. Afterward, the flies were evaluated in the behavioral tasks and posteriorly used to prepare the samples for the biochemical analysis. It was verified that the exposure to lutein nanoparticles (6 µM during 7 days) protected against the damages induced by ROT in test locomotor and exploratory damages and increased the survival rate of the flies as well. Still, it restored the dopamine (DA) levels, regulated the acetylcholinesterase (AChE) activity, the oxidative stress indicators and the cell viability. These results suggest the involvement of the oxidative stress and the dopaminergic and cholinergic system in PD. They also provide evidence that lutein nanoparticles are a possible alternative for the treatment of PD.

Keywords: Carotenoid; antioxidants; rotenone; oxidative stress; *Drosophila melanogaster.*

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LISTA DE ABREVIATURAS E SIGLAS

AChE - Acetilcolinesterase

ACh – Acetilcolina

AKT - Serina-treonina quinase (AKT)

DA – Dopamina

ERO - Espécies reativas de oxigênio

FADH2 – Dinucleótido de flavina e adenina

NADH - Nicotinamida adenina

Nrf2 - Fator de transcrição nuclear 2

PAL - Protocerebral anterior lateral;

PVP - Polivinilpirrolidona

PPM - Protocerebral posterior medial

PPL - Protocerebral posterior lateral

DP - Doença de Parkinson

ROT - Rotenona

SNC - Sistema nervoso central

TH - Tirosina-hidroxilase

VUM - Ventral não pareado medial (Do inglês Ventral unpaired medial)

SUMÁRIO

1 INTRODUÇÃO

As doenças neurodegenerativas levam ao dano progressivo e morte de células neuronais (MIZUNO, 2014). A Doença de Parkinson (DP) é uma desordem neurodegenerativa que afeta de 2-3% da população acima dos 60 anos, caracterizada pela perda seletiva dos neurônios dopaminérgicos, na substância negra do sistema nervoso central, e pelo acúmulo de corpos de Lewy, desencadeando déficit cognitivo e motor, associado a tremor, rigidez, bradicinesia e instabilidade postural (MORGANTE et al., 2003). Há evidências consideráveis de que o estresse oxidativo danifica seletivamente o sistema de dopamina (DA) e elucida características clínicas e patológicas da DP, assim muitos estudos usam a rotenona (ROT), que é um inibidor do complexo I da cadeia respiratória, para replicar as características da DP (MONTEIRO et al., 2017).

Pacientes que fazem o uso de levodopa (GOETZ, 2011), e antiparkinsonismo anticolinérgicos (AAs) (KAKKAR; DAHIYA, 2015) no tratamento para a DP, relatam efeitos colaterais como boca e olhos secos, constipação e retenção urinária, sonolência, confusão e piora da função motora (FEINBERG, 1993). Assim, se faz necessário o desenvolvimento de novos tratamentos que diminuam os efeitos indesejáveis e que apresentem elevado potencial terapêutico.

Diante desse cenário, estudos tem apontado a ação dos compostos bioativos, constituintes extranutricionais presentes, principalmente, nos alimentos de origem vegetal, que atuam na manutenção da saúde e na redução do risco de doenças, sem o surgimento de efeitos colaterais como no caso de algumas drogas que apresentam elevado potencial terapêutico e promovem bem estar aos pacientes (BANJARI et al., 2018; KUSSMANN et al., 2007; MALAIWONG et al., 2019).

Uma vez que existem evidências demonstrando que o dano oxidativo e inflamatório contribui para a evolução da DP (BEAL, 2006; GAKI; PAPAVASSILIOU, 2014), devido seus efeitos antioxidantes, os carotenoides vem sendo estudados, apresentam efeito neuroprotetor na DP, a ingestão de carotenoides na dieta está associada a um baixo risco de DP (YANG et al., 2017), combate o estresse oxidativo, anormalidades neurocomportamentais e cognitivas (KAUR; CHAUHAN; SANDHIR, 2011). Nesse sentido, estudos mostram que a luteína o principal

carotenoide encontrado no cérebro humano, e está envolvida na proteção da DP (JOHNSON et al., 2011), e é um potente antioxidante que protege os neurônios dopaminérgicos (NATARAJ et al., 2016).

A biodisponibilidade é outro fator importante que deve ser considerado em relação aos efeitos da luteína. A solubilização da luteína em óleo do gérmen de trigo melhora sua biodisponibilidade em camundongos (GORUSUPUDI; BASKARAN, 2013). Nesse sentido, nanopartículas poliméricas têm sido utilizadas para melhorar a ação terapêutica, principalmente em relação à liberação controlada do fármaco, evitando efeitos colaterais indesejáveis (CARO-LEÓN et al., 2018). Assim, a nanoemcapsulação da luteína pode representar um aumento na sua biodisponibilidade e absorção, sendo uma alternativa para o tratamento de doenças.

Drosophila melanogaster é um excelente modelo de organismo utilizado em estudos que avaliam doenças neurodegenerativas, como a DP, possuindo 75% dos genes homólogos à doença humana (KAUR et al., 2015). Este modelo também apresenta anormalidades semelhantes ao DP em humanos, como degeneração dopaminérgica e déficit locomotor (SONG et al., 2017; WHITWORTH; WES; PALLANCK, 2006).

Apesar de todos os estudos já existentes sobre carotenoides e doenças neurodegenerativas, o efeito de nanopartículas de luteína sobre a prevenção ou reversão da DP, assim como, questões quanto a biodisponibilidade e possíveis mecanismos de ação envolvendo estresse oxidativo, sistema colinérgico e dopaminérgico, ainda não foram investigados. Assim, a realização do presente estudo é importante para determinar os efeitos das nanopartículas luteína, um possível candidato no tratamento da DP e seus mecanismos de ação.

2 REVISÃO BIBLIOGRÁFICA

2.1 Doença de Parkinson

Doenças neurodegenerativas causam a destruição progressiva e irreversível das células do sistema nervoso central (SNC), provocando ao paciente gradativa perda das funções motoras, fisiológicas e capacidade cognitiva (PRZEDBORSKI; VILA; JACKSON-LEWIS, 2003).

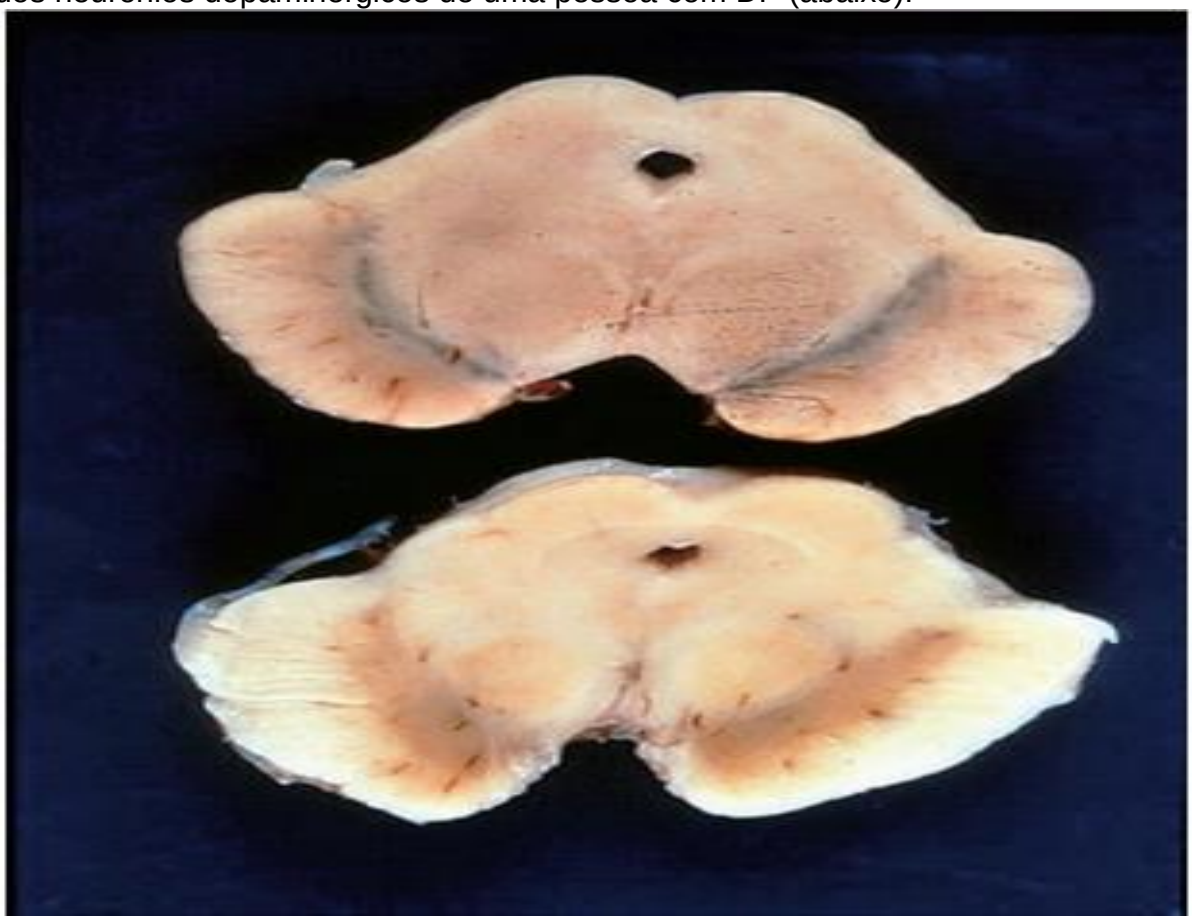
A DP, segunda doença neurodegenerativa mais comum em pessoas com idade acima dos 60 anos, apresenta uma alta incidência nessa faixa etária (CHEN et al., 2014; RAO et al., 2016; STEFANO et al., 2018).

Segundo a OMS, aproximadamente 200 mil pessoas eram acometidas pela DP no Brasil no ano de 2005, e a projeção é de que esse número praticamente dobre nos próximos 20 anos, atingindo, em 2030, mais de 9 milhões de pessoas (DORSEY, 2007). Além do efeito devastador nos pacientes e seus familiares, o Parkinson, representa um sério problema financeiro para a economia mundial. Nos Estados Unidos, o custo anual por pessoa é estimado em cerca de 23000 dólares, com custos totais da doença ultrapassando 14 bilhões de dólares, sendo, que as despesas com medicamentos correspondem a 35 % destes gastos (BOHINGAMU MUDIYANSELAGE et al., 2017; KOWAL et al., 2013).

A característica patológica da DP é a degeneração seletiva de neurônios dopaminérgicos na região compacta da substância negra, diminuição de DA no corpo estriado, e pelo acúmulo de corpos de Lewy. (Figura 1) (BONNET; HOUETO, 1999; GAO; WU, 2016; HIRSCH et al., 2016; SINGLETON; FARRER; BONIFATI, 2013; STEFANIS, 2012) .

Ainda, a fisiopatologia dessa doença aponta a ocorrência das conformações oligoméricas solúveis da alfa-sinucleína, conhecidas como protofibrilas, que são altamente tóxicas e provocam o rompimento da homeostase celular, estando associada a morte celular, indicando a contribuição dos aglomerados de alfa-sinucleína para a patogênese da DP (STEFANIS, 2012).

Figura 1. Imagem da pigmentação dos neurônios dopaminérgicos na substância negra. Neurônios dopaminérgicos de pessoas saudáveis (acima), e despigmentação dos neurônios dopaminérgicos de uma pessoa com DP (abaixo).



Fonte: Adaptado de (LITTLE et al., 2008)

A DP manifesta-se devido à perda relativamente seletiva de neurônios dopaminérgicos importantes para a regulação da função motora, e essas deficiências motoras aparecem quando 80% da DA ou 60/70% dos neurônios dopaminérgicos da substância negra já estão destruídos (DAUER; PRZEDBORSKI, 2003; SONG et al., 2017).

Essas disfunções no sistema dopaminérgico culminam com os sintomas progressivos e permanentes da DP. Nesse sentido a literatura aponta que a DP pode ser dividida em 4 estágios, sendo estágio 1: envolvimento unilateral, pouco comprometimento funcional. Estágio 2: Envolvimento bilateral, sem comprometimento funcional. Estágio 3: Deficiência leve ou moderada, com diminuição dos reflexos, instabilidade postural, pouca restrição de atividades, porém esses pacientes são fisicamente independentes. Estágio 4: Deficiência severa, alterações físicas e incapacidade de trabalhar, perda das funções motoras, incluindo

bradicinesia, tremor, rigidez muscular e instabilidade postural (CHEN et al., 2014; HOEHN; YAHR, 1967; LEWIS et al., 2011; STEFANO et al., 2018).

Ainda, a DP pode vir acompanhada de outros sintomas não motores incluindo disfunção olfatória, problema cognitivo e psicológicos (DE VIRGILIO et al., 2016).

2.2 Alterações neuroquímicas da Doença de Parkinson

2.2.1 Estresse oxidativo

As causas da DP vêm sendo estudadas e têm sido relacionadas a fatores genéticos, ambientais e imunológicos (DE VIRGILIO et al., 2016). Apenas 10% dos casos de DP tem uma causa genética identificada, mesmo tendo um papel importante no desenvolvimento da doença (LITTLE et al., 2008; MARTIN; DAWSON; DAWSON, 2011). Já os fatores ambientais são mais estabelecidos, como a exposição a herbicidas e pesticidas (TANNER et al., 2011), fatores que levam ao estresse oxidativo, inibição na atividade do complexo I da cadeia transportadora de elétrons, perda de neurônios na substância negra e disfunção mitocondrial, formando espécies reativas de oxigênio (ERO), levando a efeitos deletérios para as células (PARK et al., 2014).

Ainda, devido ao elevado consumo de oxigênio do cérebro e por possuir grande quantidade de ácidos graxos poli-insaturados, se torna vulnerável a danos oxidativos, e acrescido a esse fato, o aumento da idade também eleva o estresse oxidativo, e passa a ser considerado mais um fator para o desenvolvimento das doenças neurodegenerativas (JIMENEZ et al., 2017).

Além disso, o metabolismo celular produz radicais livres, que em excesso são extremamente tóxicos para as células, desencadeado pela alta produção de radicais livres e/ou inadequado mecanismo de defesa (THAKUR; NEHRU, 2014). O sistema de defesa das células é responsável por eliminar os radicais livres, evitando danos celulares, incluindo a enzima superóxido dismutase (SOD), catalase (CAT) e glutationa peroxidase (GPx), que fazem parte desse sistema e protegem contra o estresse oxidativo (BONNET; HOUETO, 1999).

Na DP, a morte celular está relacionada além do estresse oxidativo, com a disfunção no complexo mitocondrial e inflamação, e são considerados os prováveis

causadores dessa patologia, apesar de que suas causas ainda não são bem descritas (BEAL, 2006). No entanto sabe-se que a DP ocorre principalmente em pessoas idosas, estando extremamente relacionada com o aumento do estresse oxidativo e agregação de proteínas que ocorre com o processo do envelhecimento (KARUPPAIAH, 2018; TAMILSELVAM et al., 2013).

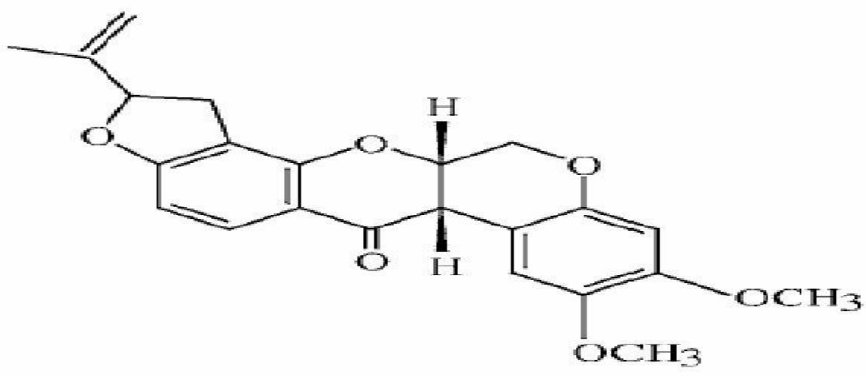
Com aproximadamente 40 subunidades, o complexo I é a maior de todas as proteínas, está localizado na membrana interna da mitocôndria, sendo um dos componentes da cadeia respiratória (CIMDINS et al., 2019; SCHAPIRA, 2010).

O complexo I contribui para fornecimento de energia pelo metabolismo aeróbico, responsável pela oxidação de NADH, utilizando a ubiquinona (Q) como aceitador de elétrons. Tem como função a oxidação e transporte de nicotinamida-adenina-dinucleótido reduzida (NADH) e dinucleótido de flavina e adenina (FADH2). Os complexos I, III e IV, atuam como uma bomba de prótons (FERREIRA; AGUIAR; VILARINHO, 2008).

De acordo com estudos, o desenvolvimento de doenças neurodegenerativas está relacionado com déficit no complexo I, por problemas genéticos e/ou por ser atingido por algumas toxinas que alteram sua função (SCHAPIRA, 2010).

O uso de inseticidas na agricultura existe há dois milênios na China, Egito, Grécia e Índia. A ROT é um inseticida do tipo organofosforado utilizados há mais de 150 anos, é extraído de raízes de *Derris, Lonchocarpus e Tephrosia* (Figura 2), o mais usado é oriundo da *Lonchocarpus*, cultivado na Venezuela e Peru (ISMAN, 2006).

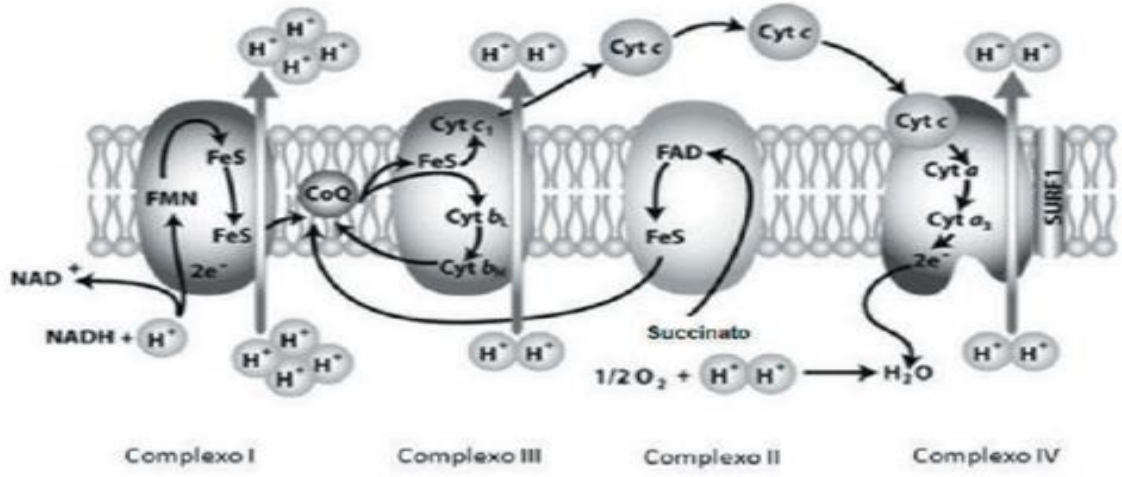
Figura 2. Estrutura química da rotenona.



Fonte: (ISMAN, 2006).

ROT é um pesticida tóxico que inibe o complexo I da cadeia respiratória (Figura 3), e que frequentemente penetra na barreira hematoencefálica devido à sua propriedade lipofílica, não necessitando de transportadores, provocando morte, estresse oxidativo e a perda de neurônios dopaminérgicos, e assim, disfunção da cadeia respiratória, aumento na geração de ROS, que culminam com as alterações comportamentais semelhantes à DP (KARUPPAIAH, 2018).

Figura 3. Representação esquemática dos complexos da cadeia respiratória mitocondrial. Complexo I (nadh-ubiquinona oxidoredutase ec 1.6.5.3); Complexo II (succinato-ubiquinona oxiredutase ec 1.3.5.1); Complexo III (ubiquinol-citocromo c oxiredutase ec 1.10.2.2) e complexo IV (citocromo c oxidase ec 1.9.3.1).



Fonte: (FERREIRA; AGUIAR; VILARINHO, 2008).

Nesse sentido, diante da sua toxicidade, a ROT vem sendo usada através da exposição crônica em *Drosophila* para elucidar um modelo da DP, sendo apontada em muito estudos devido à sua capacidade de provocar a perda dos neurônios dopaminérgicos, afetando os níveis de DA, que vão refletir em alterações na atividade locomotora (SUDATI et al., 2013; TANNER et al., 2011).

Estudos anteriores mostram que a exposição de Drosophila melanogaster à ROT também diminuiu a atividade das enzimas antioxidantes SOD e CAT (LAKKAPPA et al., 2019; ARAUJO et al., 2015), causa danos nos níveis de TBARS (LAKKAPPA et al 2019; COUTO et al 2018), ainda aumenta a atividade da GST (GIRISH et al., 2012) e diminui os níveis de NPSH (Manjunathn et al 2015) e tiol total (RAO et al., 2016).

2.2.2 Metabolismo da Dopamina

A DA é da família das catecolaminas, sintetizada a partir da tirosina, nos terminais dos neurônios dopaminérgicos, nessa síntese a tirosina é hidroxilada pela tirosina-hidroxilase (TH), transportada através da barreira hematoencefálica, (GILMAN AG., HARDMAN JG., 2003).

A DA é responsável pela transmissão de mensagens no cérebro, possui várias funções motoras, fisiológicas e cognitivas, portanto o déficit de DA leva à alterações e incapacidade dessas funções (JEAN-MARTIN BEAULIEU; RAUL R. GAINETDINOV, 2011). A perda exorbitante de neurônios dopaminérgicos, levando à deficiência de DA, é uma das principais características da DP (ZHENG et al., 2018).

Os neurônios dopaminérgicos liberam radicais livres, e estão predispostos ao estresse oxidativo devido ao seu catabolismo da degradação oxidativa, gerando peróxido de hidrogênio, e por possui altas concentrações de ferro, que em concentrações aumentadas é tóxico para os neurônios dopaminérgicos (FOLLMER; NETTO, 2013; NATARAJ et al., 2016; SPINA MB, 1989).

2.2.3 Atividade da Acetilcolinesterase (AChE) e a Doença de Parkinson

Enzima AChE já é bem descrita e utilizada para pesquisar a eficácia do tratamento na DP e outras doenças neurodegenerativas, e a expressão da AChE é proposta para induzir a morte dos neurônios dopaminérgicos, afetando a função motora (DALPIAZ et al., 2007). Ainda, pesticidas dos tipos organofosforados são capazes de alterar a enzima AChE, pois promovem toxicidade (CAVALCANTI et al., 2016).

AChE é uma enzima que tem a função de hidrolisar a acetilcolina (ACh) que é um neurotransmissor encontrado no cérebro, nas sinapses colinérgicas, responsável pelos impulsos nervosos entre outras funções, faz a transmissão de mensagens de um neurônio a outro (ARAÚJO; SANTOS; GONSALVES, 2016). Diante disso, evidencia-se a importância dessa enzima no entendimento das questões relacionadas as alterações motoras. Assim, são de suma importância estudos que avaliem a ação de compostos bioativo capazes de inibir ou ativar a AChE (ZHU et al., 2008).

2.3 Tratamentos da Doença de Parkinson

As estratégias atuais de tratamento e as drogas para DP promovem apenas o alívio sintomático isoladamente nos estágios iniciais da doença. Levodopa e outros medicamentos melhoram os sintomas no início do tratamento, após um tempo os pacientes apresentam sintomas resistentes a esses medicamentos (KARUPPAIAH, 2018). No entanto, nenhum tratamento para a DP possui ação de impedir a progressão da DP.

Pacientes que fazem uso dos tratamentos apresentam problemas na fala, postura, comprometimento do humor, sono e cognitivo, e também efeitos colaterais como psicose, alucinações, demência e depressão (FERRAZ, 1999; GERSZT et al., 2014). Assim, se faz necessário a descoberta de estratégias de tratamentos a partir de recursos naturais, com agentes neuroprotetores para prevenir o surgimento ou deter a progressão da DP (LAKKAPPA et al., 2019).

Estudos mostram que o carotenoide licopeno, protege contra estresse oxidativo, anormalidades neurocomportamentais e cognitivas induzidas pela ROT, um modelo de DP (LIU et al., 2013; KAUR; CHAUHAN; SANDHIR, 2011).

2.4 Compostos Bioativos

As descobertas de novas opções terapêuticas para a DP, através de recursos naturais, podem ser amplamente benéficos e resultar em efeito neuroprotetor, promovendo prevenção ou reversão da DP (KARUPPAIAH, 2018; REIN et al., 2013).

Estudos mostram que o consumo de alimentos naturais com propriedades antioxidantes, diminuem o risco do desenvolvimento de doenças cardiovasculares, câncer e doenças neurodegenerativas (DUTHIE; DUTHIE; KYLE, 2000), sem o surgimento de efeitos colaterais como no caso de algumas drogas farmacêuticas (ESPÍN; GARCÍA-CONESA; TOMÁS-BARBERÁN, 2007; KUSSMANN et al., 2007; REIN et al., 2013).

Os carotenoides são moléculas isoprenóides de 40 carbonos, que produzem a pigmentação vermelha, amarela e alaranjada em plantas, microalgas, bactérias e fungos, além de precursores da vitamina A, apresentam outras funções, tais como

prevenção de câncer, ação inibidora nas mucosas contra úlceras gástricas e aumento da resposta imunológica (BAKÓ; DELI; TÓTH, 2002).

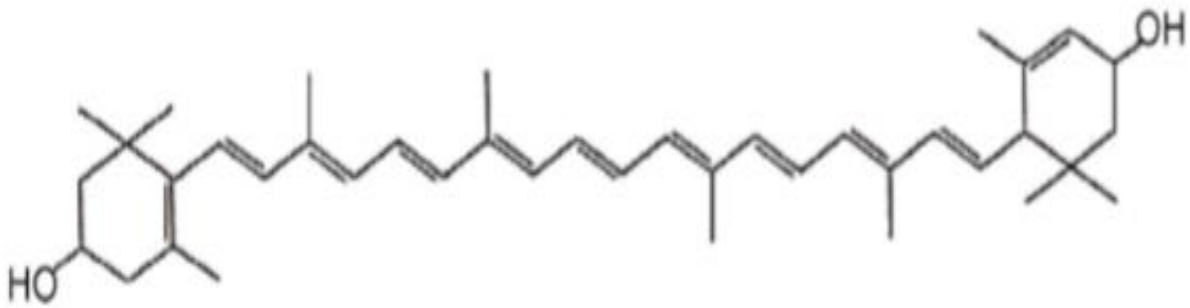
Os carotenoides possuem propriedades antioxidantes, por isso são tão importantes na proteção de doenças neurodegenerativas (ABAYOMI et al., 2019; FIEDOR J, 2014). Alguns estudos mostram que um elevado consumo de carotenoides pode estar relacionado com baixo risco de progredir para a DP (JOHNSON et al., 1999; MIYAKE et al., 2011). Os carotenoides são encontrados em tecidos cerebrais (CRAFT et al., 2004; TAKEDA et al., 2013), estão envolvidos na via de sinalização do sistema nervoso central (SNC), controlam a diferenciação neuronal e o padronização dos tubos neurais (TAFTI; GHYSELINCK, 2007).

2.4.1 Luteína

A luteína é um carotenoide de cor amarela que pertence à classe da xantofila (SASAKI et al., 2010). Não pode ser sintetizada pelos animais, é naturalmente encontrada no reino vegetal, sintetizado por plantas, bactérias e algas, portanto deve ser obtida através da dieta, está presente em vegetais verdes escuros como espinafre, couve e brócolis, também em alimentos de coloração amarelo, como gema de ovo e milho (KRINSKY; LANDRUM; BONE, 2003).

A luteína possui grupos terminais β e ε, é dihidroxicarotenóides com grupos hidroxila (Figura 4) (KHACHIK; BEECHER; SMITH, 1995).

Figura 4. Estrutura química da luteína.



Fonte: (RAO; RAO, 2007).

O elevado número de duplas ligações presentes na estrutura da luteína, atribuí suas propriedades antioxidantes, os anéis ciclohexenol permite a absorção de energia das ERO (EL-AGAMEY et al., 2004).

A luteína possui capacidade de atravessar a barreira hematoencefálica, pode desempenhar efeito neuroprotetor (LI; LO, 2010). A luteína, principal carotenoide presente no cérebro humano (JOHNSON, 2012, 2014), constitui 59% dos carotenoides no cérebro de crianças com menos de 1,5 anos de idade (VISHWANATHAN et al., 2014). Em idosos a concentração de luteína é mais baixa do que em outras faixas etárias (JOHNSON et al., 2010).

Devido aos seus efeitos antioxidantes, anti-inflamatório e neuroprotetor, a luteína tem chamado atenção dos pesquisadores (ABUBAKR et al., 2018; KALARIYA; RAMANA; VANKUIJK, 2012). As principais funções da luteína são como filtro de luz ultravioleta (UV), impede a entrada de luz UV no cristalino ocular e também protege a pele, aumenta o pigmento macular e melhora a visão, e inativador de ERO. (ALVES-RODRIGUES; SHAO, 2004).

Através da propriedade antioxidante da luteína, a mesma é capaz de inativar os radicais livres, promover complexação de íons metálicos e/ou redução dos hidroperóxidos, inibir o oxigênio singlete, e proteger a membrana contra a radiação UV impedindo a peroxidação lipídica (SHAMI; MOREIRA, 2004). Ainda, protege os neurônios da retina contra o estresse oxidativo em células (LI; LO, 2010).

Assim, no trabalho de Kim et al. (2012), a alimentação de cobaias com luteína, foi eficiente para reduzir a peroxidação lipídica e a produção de citocinas pró-inflamatórias no fígado e nos olhos de cobaias expostas ao dano causado pela dieta hipercolesterolêmica, sugerindo que a luteína exerce função antioxidante e anti-inflamatória.

Ainda, de acordo com Vijayapadma et al. (2014), a luteína possui efeito protetor contra o estresse oxidativo induzido por benzopireno em eritrócitos humano, aumenta a atividade das enzimas antioxidantes SOD E CAT e regula os níveis de TBARS. Ainda, foi demonstrado anteriormente que a luteína regula a atividade da GST e protegia contra o estresse oxidativo induzido pelo peptídeo β-amilóide em células endoteliais cerebrovasculares (LIU et al., 2016).

A luteína também se mostrou eficiente contra o comprometimento cognitivo em mulheres e homens idosos (HAMMOND et al., 2017). Segundo Johnson et al,

(2013), concentrações séricas de luteína estão relacionadas a melhor cognição nos idosos. Já, baixas concentrações podem levar ao comprometimento cognitivo em pessoas com idade mais avançada (AKBARALY et al., 2007). A luteína promoveu diferenciação neural (XIE et al., 2019).

A alta concentração de luteína nos tecidos cerebrais está relacionada à melhora da visão, memória e audição em adultos (JOHNSON, 2014). A luteína oferecida através da dieta, em quantidades suficientes, é depositada em células dos tecidos neurais, atuando como protetora de acordo com a demanda (STRINGHAM; STRINGHAM, 2015).

Estudos mostram o envolvimento da luteína no mecanismo do estresse oxidativo, tal fato pode estar relacionado com a ativação do fator de transcrição nuclear 2 (Nrf2), que faz a proteção contra o estresse oxidativo, desencadeia a expressão de fatores de crescimento e genes responsáveis pela desintoxicação de drogas, imunomodulação, sinalização intracelular (DINKOVA-KOSTOVA et al., 2015). O Nrf2 faz a ativação das defesas antioxidantes (FUNAKOHI-TAGO et al., 2018). Estudos revelam que a via de sinalização Nrf2 é ativada via ativação de serina-treonina quinase (AKT), e que a ativação do eixo de sinalização AKT / Nrf2 pode contribuir para a inibição da neuroinflamação para a prevenção da DP (HUANG et al., 2018).

2.4.1.1 Nanopartículas poliméricas de luteína

As nanopartículas poliméricas possuem um vasto potencial, pois têm a função de proteger as drogas encapsuladas, oferecendo estabilidade no trato digestivo, melhor absorção e biodisponibilidade (JIANG et al., 2015), maximizando o potencial terapêutico e proporcionam a liberação do fármaco no sítio de ação desejado, ao mesmo tempo minimizando os efeitos colaterais (REIN et al., 2013; XIE et al., 2011).

A luteína é insolúvel em água e sensível a degradação pela luz e calor. Neste sentido nanopartículas são usadas para encapsular a luteína e oferecer maior estabilidade e melhorar as propriedades físico-químicas deste composto (CHITTASUPHO; POSRITONG; ARIYAWONG, 2019).

Segundo Do Prado Silva et al. (2017), a encapsulação foi capaz de aumentar a solubilidade da luteína em água em mais de 43 vezes, e que a administração de luteína livre 100mg · kg $^{-1}$ e nanopartículas de polivinilpirrolidona (PVP), que é um polímero solúvel em água, carregadas com luteína a 10 e 1,5mg · kg $^{-1}$ aumentaram o índice de reconhecimento de objeto de camundongos, o estudo comprova que são necessárias doses bem menores de luteína encapsulada para promover o mesmo efeito oferecido pela luteína livre.

A utilização de nanopartículas vem sendo cada vez mais empregada, pois pode elevar consideravelmente a eficácia dos medicamentos e oferecer uma melhor qualidade de vida aos pacientes (ZHANG et al., 2017).

2.5 Modelo de *Drosophila Melanogaster* e DP

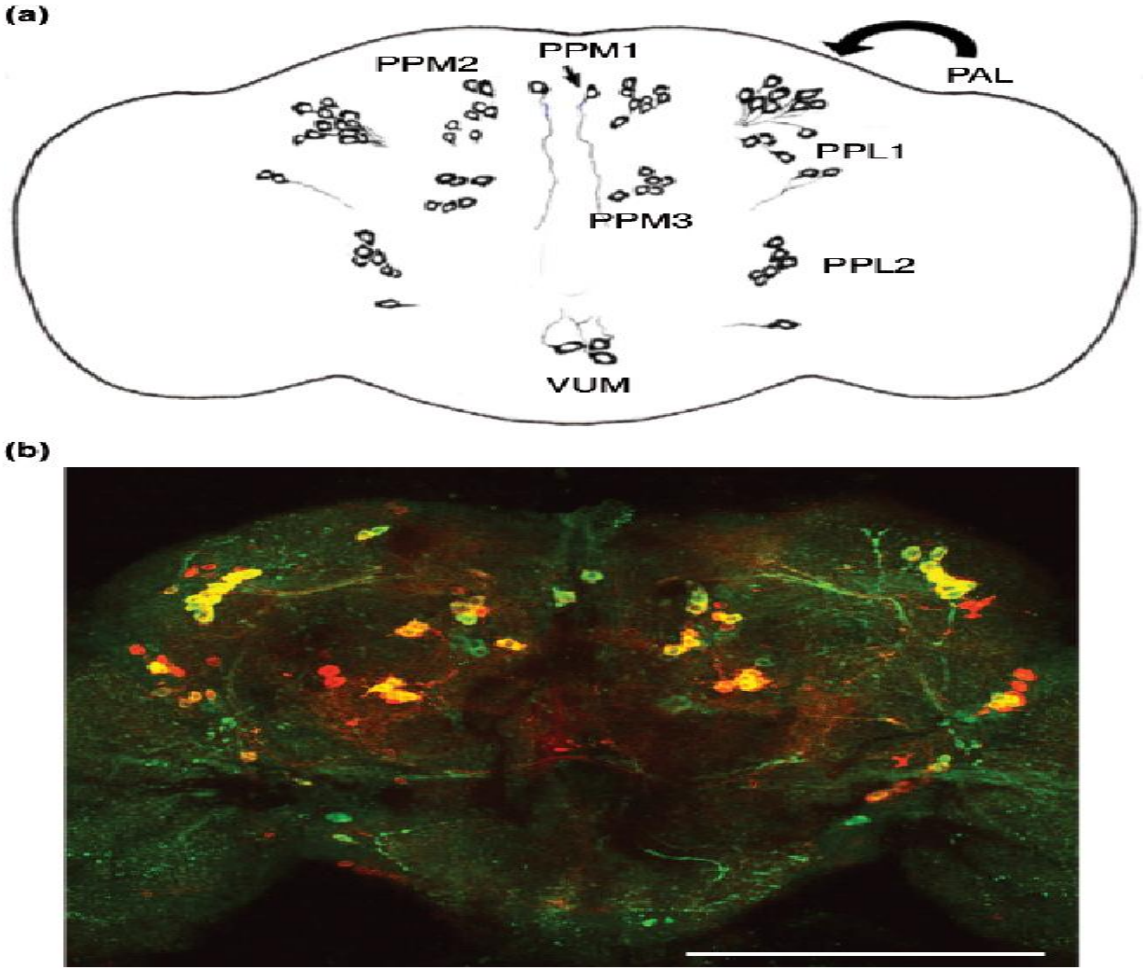
No que se refere ao estudo da DP, o modelo *Drosophila melanogaster* vem sendo amplamente utilizado em estudos de doenças neurodegenerativas como DP, Alzheimer, Doença de Huntington, e síndrome de Angelman (GIRISH; MURALIDHARA, 2012; MACKAY; ANHOLT, 2006).

É um modelo utilizado para estudos de distúrbios neurocomportamentais, pois possui a presença de genes que são semelhantes aos genes humanos, incluindo genes da DP, apresenta homologia de cinco dos seis genes relacionados à DP (BOTELLA et al., 2009; WHITWORTH; WES; PALLANCK, 2006).

De 59 genes neurológicos humano estudados, a *Drosophila* possui 38 genomas ortólogos. Dos 289 genes de doenças humanas, a mesma possui 177 ortólogos, aproximadamente 70% (RUBIN et al., 2000).

O modelo *Drosophila* possui um grande potencial em estudos voltados a DP, possibilita inclusive analisar a integridade dos neurônios dopaminérgicos presentes nesse modelo. A *Drosophila* possui um sistema nervoso complexo com aproximadamente 100.000 neurônios e desses cerca de 200 neurônios que contém o neurotransmissor DA, sendo um excelente modelo para estudos do desenvolvimento e disfunção neural (Figura 5) (WHITWORTH; WES; PALLANCK, 2006).

Figura 5. Sistema nervoso de *Drosophila*. (a) representação esquemática da distribuição dos neurônios da no cérebro adulto de *Drosophila*. (b) neurônios revelados por microscopia confocal. Abreviaturas: PAL - Protocerebral lateral anterior; PPM - Protocerebral posterior medial; PPM - Protocerebral posterior medial; PPL - Protocerebral posterior lateral; VUM - Ventral não pareado medial.



Fonte: (WHITWORTH; WES; PALLANCK, 2006).

A expressão da proteína alfa-sinucleína e DA no modelo de *Drosophila*, desenvolveu déficit de acuidade e discriminação, portanto confere as características da DP, sendo adequado para mimetizar a DP (CHEN et al., 2014).

O modelo *Drosophila* é bem caracterizado, possui comportamentos relevantes aos seres humanos incluindo memória (TULLY et al., 1994), perda dos neurônios e capacidade de escalada (SONG et al., 2017), e exploração em campo aberto (BURNS et al., 2012).

Ainda, esse modelo alternativo se destaca no que se refere a DP pois consegue mimetizar alterações motoras importantes, que são marcas registradas da DP, através do método de Geotaxia negativa (COULOM, 2004). Para estudar os déficits comportamentais semelhantes à DP em *Drosophila*, também é possível

avaliar as alterações na capacidade locomotora, no teste de campo aberto de acordo com Hirth (2010), que avalia a capacidade exploratória da mosca.

Portanto, a *Drosophila melanogaster* já foi utilizada em múltiplos estudos envolvendo o estresse oxidativo, o qual associa-se a patogênese de várias doenças (PARKES et al., 1998). E ainda esse modelo também apresenta-se vantajoso ao estudo de doenças neurodegerativas e buscas de novos compostos pois é de facíl manutenção, curto ciclo de geração, maior numero de progênies, e metabolismo semelhante à humanos, e assim, devido á essas e outras propriedades tem sido cada vez mais utilizado em novos estudos (CHINTAPALLI; WANG; DOW, 2007).

3 OBJETIVOS

3.1 Objetivo geral

Avaliar o efeito de nanopartículas de luteína no modelo experimental tipo DP em *Drosophila melanogaster,* bem como seus possíveis mecanismos de ação.

3.2 Objetivos específicos

- Avaliar o efeito da luteína na forma livre e na forma de nanopartículas sobre o comportamento motor e taxa de sobrevivências em *Drosophila melanogaster;*
- Avaliar o efeito de nanopartículas de luteína sobre as alterações motoras induzidas pela ROT, em *Drosophila melanogaster;*
- Determinar os níveis de DA em *Drosophila melanogaster* submetidas as nanopartículas de luteína e ao modelo experimental de DP;
- Avaliar os indicadores de estresse oxidativo em *Drosophila melanogaster* expostas as nanopartículas de luteína e ao modelo experimental de DP;
- Avaliar a atividade da AChE em *Drosophila melanogaster* expostas as nanopartículas de luteína e ao modelo experimental de DP;
- Avaliar viabilidade celular em *Drosophila melanogaster* submetidas as nanopartículas de luteína e ao modelo experimental de DP.

4 MANUSCRITO CIENTÍFICO

Os resultados os quais fazem parte desta dissertação apresentam-se sob a forma de manuscrito científico, o qual encontra-se aqui estruturado sob o título: “Exposure to lutein nanoparticles attenuates Parkinson's model -induced damage in *Drosophila melanogaster*: restoration of cholinergic and dopaminergic system and antioxidants enzymes”. Os itens Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas, encontram-se inseridos no próprio manuscrito. O manuscrito apresenta-se na forma que será submetido a revista “ Neurotoxicology”.

Exposure to lutein nanoparticles attenuates Parkinson's model -induced damage in *Drosophila melanogaster*: restoration of antioxidants, dopaminergic and cholinergic system

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Abstract

Parkinson's is a neurodegenerative disease, characterized by the loss of dopaminergic neurons, cholinergic alterations and oxidative damages. Lutein, the main carotenoid found in the human brain, is widely known by its antioxidants properties. Accordingly, it is believed that lutein is able to prevent neurodegenerative diseases and cognitive deficits. However, lutein bioavailability is limited due to its poor water solubility. In the present study, we investigated whether lutein protects rotenone-induced locomotor and exploratory damage and neurotoxicity in *Drosophila melanogaster*, as well as possible mechanisms of action, involving oxidative stress, cholinergic and dopaminergic system. The fruit flies of 1–4 days old were divided into four groups and exposed to a standard diet (control), a diet containing either rotenone (500 µM), lutein nanoparticles (6 µM) or rotenone (500 µM) and lutein nanoparticles (6 µM) for 7 days. After the exposure to those diets, the flies were submitted to negative geotaxis and open field tasks, to evaluate locomotor and exploratory activity. Furthermore, the survival percentage was assessed during 7 days of exposure. After the behavioral test, the flies were used for the determination of dopamine levels, acetylcholinesterase activity, oxidative stress indicators (superoxide dismutase, catalase, thiobarbituric acid reactive substances, glutathione S-transferase and non-protein thiol) and cell viability (MTT and resazurin). The exposure to rotenone induced a locomotor damage and decreased the survival rate, as well as dopamine levels, acetylcholinesterase activity, altered oxidative stress indicators and it also reduced cell viability. The exposure to lutein nanoparticles (6 µM during 7 days) protected the flies against locomotor damage and the decrease survival rate induced by rotenone, besides, it restored the dopamine levels, acetylcholinesterase activity, oxidative stress indicators and cell viability. These results provide evidence that lutein nanoparticles are an alternative treatment for rotenone-induced damage, and suggest the involvement of oxidative stress, dopaminergic and cholinergic system.

Keywords: carotenoids; xanthophyll; geotaxis; oxidative stress; nanoencapsulation; dopaminergic degeneration.

1. Introduction

Parkinson's disease (PD), the second most common neurodegenerative disease for people over the age of 60, affects about 1-2% of the world's population (Fatima et al., 2019; Sprenger and Poewe, 2013). The projection is that by 2030 more than 9 million people will be affected (Dorsey, 2007).

PD is characterized by the selective dopaminergic neurons degeneration in the compact region of the substantia nigra, and by the Lewy Bodies accumulation (Gao and Wu, 2016; Hirsch et al., 2016). Lewy bodies are intraneuronal inclusions composed mainly of α-synuclein, ubiquitin and synfilin-1 proteic aggregates, synaptic proteins that cause the dopaminergic neurons degeneration, with a consequent decrease in dopamine levels (Jadiya et al., 2011) and increases of oxidative stress (GUO, S.; BENZEROUAL, 2013), important factors in the progression of PD (Staveley, 2014). All these factors characterizes a progressive and permanent motor disturb, signaled by muscular rigidity, bradykinesia, tremor and postural instability (Cutsuridis and Perantonis, 2006; Lewis et al., 2011; van der Burg et al., 2006).

Currently the available treatments involves pharmacological strategies that delay the natural evolution of the disease symptoms through dopaminergic stimulation or cholinergic and glutamatergic inhibition (Hornykiewicz, 2010; Taddei et al., 2017). In fact, it has been shown that these drugs improves some of the symptoms of patients with the disease (D., 2006; Oertel and Schulz, 2016), however, the drugs act by temporarily restoring the dopaminergic function, without demonstrating protective action on the neurodegeneration process. Furthermore, there are some points that limit or impair the use and effectiveness of these drugs. Firstly, the improvement of symptoms is temporary, and may disappear over time, with the disease progression or the treatment discontinuation (Clissold et al., 2006;

McColl et al., 2002). Secondly, the efficacy of these drugs may vary according to the patient (Akram et al., 2017; Lang et al., 2006). Thirdly, in some patients the treatment provokes side effects, including, nausea, vomiting, hypotension, tachycardia, mydriasis, insomnia and depression (Oertel and Schulz, 2016; Olanow, 2008). Fourthly, motor abnormalities are diagnosed when the neurodegenerative process is at an advanced stage, with approximately 70% of degenerated neurons and dopamine levels reduced by 80% in the striatum (Braak et al., 2004).

Therefore, studies that contribute to the development of treatments able to modify the natural evolution of the disease and its symptoms, avoiding dopaminergic degeneration and oxidative stress, should be considered. The evidence to support this is important role of bioactive compounds present in foods (Espín et al., 2007; Rein et al., 2013). In this sense, a study indicates that the neuroprotective effect of bioactive compounds present in green tea occurs due to antioxidant properties, decreased α-synuclein aggregation and modulation of intracellular signaling pathways (Caruana and Vassallo, 2015).

Due to their antioxidant and anti-inflammatory potential, carotenoids plays an important role in studies related to PD (Kim et al., 2017; Lee et al., 2011; Zhang et al., 2002), since there is evidence which shows that oxidative and inflammatory damage contributes to the evolution of DP (BEAL, 2006; Gaki and Papavassiliou, 2014). In line with this view, patients with PD present reduced levels of carotenoids in the serum compared to normal patients (Kim et al., 2017). Oral administration of lycopene reverses rotenone-induced α-synuclein increases in mice (Liu C-B, Wang R, Pan H-B, Ding Q-F, 2013). There are lines of evidence which demonstrate that lutein, xanthophyll carotenoid, is a possible candidate to the treatment of neurodegenerative diseases. Firstly, lutein is the main carotenoid found in the human

brain, forming approximately 30% of the total carotenoids concentration. It displays an ability to cross the blood–brain barrier, with preferential uptake compared to most carotenoids, showing a positive correlation between the serum and the brain lutein levels (Craft et al., 2004; Vishwanathan et al., 2014). Secondly, lutein protects dopaminergic neurons by increasing antioxidant defenses and decreasing mitochondrial dysfunction (Nataraj et al., 2016). Thirdly, there is a positive correlation between intake of carotenoids including lutein and PD risk (SCHEIDER et al., 1997; JOHNSON et al., 1999).

In addition, polymeric nanoparticles have been extensively studied aiming to increase bioavailability, absorption and facilitate the entry of drugs or bioactive compounds through biological barriers, maximizing the therapeutic potential and at the same time minimizing the side effects (Kussmann et al., 2007; Rein et al., 2013). Thus, lutein nanoencapsulation may represent an increase in its bioavailability and absorption, which is an alternative for the treatment of diseases. In accordance with this view, oral administration of free lutein (100 mg/kg) and lutein nanoparticles (1.5 and 10 mg/kg) improves object discrimination index in mice, which means that a similar effect is achieved by lutein nanoparticles in doses 66 times lower than free lutein (do Prado Silva et al., 2017).

Therefore, in the present study, we investigated whether lutein protects rotenone-induced locomotor and exploratory damage and neurotoxicity, as well as possible mechanisms of action, involving oxidative stress, cholinergic and dopaminergic system.

2. Materials and Methods

2.1 Chemicals

Lutein (kindly gifted by Pincredit Bio-tech Co.) was dissolved in olive oil. Lutein in the form of nanoparticles was encapsulated in polyvinylpyrrolidone (PVP) matrix by the dissolution in common solvent method. Nanoparticles were characterized in respect to morphology, water solubility, and interactions between PVP and lutein in according to (do Prado Silva et al., 2017). Rotenone (ROT) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and diluted in ethanol 98%. Reagents for HPLC were obtained from Sigma-Aldrich (ST Louis, MO, USA). All the other reagents were of analytical grade from the campus of the UNIPAMPA.

2.2 *Drosophila melanogaster* stock

The fruit flies (*Drosophila melanogaster* wild-type - Harwich strain) were obtained from the National species Stock Center (Bowling Green, Ohio, USA), kept for about 4 days in an glass bottles in the BOD incubator, under controlled light conditions (12 hour light / dark cycle, temperature (25 ± 1 ° C) and 60% humidity and fed with 10 mL of standard food comprising corn flour (76.59%), wheat germ (8.51%), sugar (7.23%), powdered milk (7.23%), salt (0.43%), an 0.08% v/w methylparaben, and a pinch of dry yeast, until the treatment was done.

2.3 Experimental design

2.3.1 Experiment 1: Effect of free lutein or lutein nanoparticles on locomotor and exploratory activity and survival percentage

A concentration-response curve for lutein was performed to evaluate the effect on locomotor and exploratory activity and to define the concentration for subsequent experiments. The flies of both sexes from 1 to 4 days of age, were divided into seven groups (50 flies each) exposed to a standard diet (control group), a diet containing olive oil at a final concentration of 0.5%, free lutein (2 or 200 μM), dissolved in olive oil or lutein nanoparticles, dissolved in water (2, 6 or 20 μM) for 7 days. After the exposure to the diet, the flies were submitted to negative geotaxis and open field tasks to evaluated locomotor and exploratory activity. Furthermore, the survival percentage was assessed during 7 days of exposure. The treatment schedule is depicted in Figure 1A.

2.3.2 Experiment 2: Effect of lutein nanoparticles on locomotor and exploratory deficit induced by rotenone exposure and oxidative stress indicators, dopamine level and acetylcholinesterase (AChE) activity

Once it had been determined that lutein nanoparticles at the concentration of 6 μM did not alter locomotor activity and increased survival rate per se, we tested whether this concentration of lutein nanoparticles protects the damage induced by ROT. The flies were divided into four groups (50 flies each) exposed to a standard diet (control), a diet containing either ROT (500 μM), lutein nanoparticles (6 μM) or ROT (500 μM) and lutein nanoparticles (6 μM) for 7 days. After exposure to diet, the

flies were submitted to negative geotaxis and open field tasks to evaluated locomotor and exploratory activity. Furthermore, the survival percentage was assessed during 7 days of exposure. The concentration of ROT (500 μM) was selected based on a previous study which show it cause 50% death of the flies during the experimental period (Araujo et al., 2015; Sudati et al., 2013). The diet treatment consisted of 1% w/v yeast beer, 2% w/v sucrose, 1% w/v milk powder, 1% agar w/v, and 0.08% w/v metilparabeno. The total food medium in all groups contained a volume of 0.5% ethanol. After the behavioral test, the flies were used for the determination of oxidative stress indicators Superoxide dismutase (SOD), Catalase (CAT), Thiobarbituric acid reactive substance (TBARS), Glutathione-S-transferase activity (GST), non-protein (NPSH) thiol, dopamine levels, AChE activity and cell viability (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and resazurin). The treatment schedule is depicted in Figure 3A.

2.4 In vivo assays

2.4.1 Survival percentage

The survival rate was evaluated by counting once daily the number of living flies until the end of the experimental period (7 days). Around 90 flies per group were included in the survival data and the total number of flies represents the sum of six independent experiments (15 flies/each treatment repetition). The data were expressed as percentage of surviving flies.

2.4.2 Negative geotaxis assay

The locomotor activity of the flies was evaluated with the task of negative geotaxis as described by (Feany and Bender, 2000), with some modifications. Assay which evaluates the locomotor and climbing capacity of flies. In short, after 7 days of treatment, 15 flies from each group were briefly anesthetized with ice and placed individually in a vertical glass test tube with a diameter of 1.5 cm. After 10 min of recovery from the ice exposure, the flies were gently tapped to the bottom of the tube. We measured the time spent by each fly to reach the top of the column (6 cm) and flies that remained at the bottom were counted separately, in a maximum evaluation time of 120 seconds. The test was repeated five times for each fly, with a 1 min interval, and the data was analyzed according to the meantime. Four to six independent experiments were performed.

2.4.3 Open field test

To evaluate the exploratory activity, the open field task was performed with 15 flies from each group, according to the method described by (Hirth, 2010), with some modifications. Each fly was maintained on a petri dish divided by squares measuring 1x1 cm each, kept there for a 10 min recovery period before beginning the test and the number of crossings was determined during a 60-second-period. The data represent the mean of 4-6 independent experiments.

2.5 Ex vivo assays

2.5.1 Sample preparation

Immediately after the behavioral evaluations the flies were euthanized on ice, homogenized in HEPES buffer (20 mM, pH 7.0), 10:1 (flies/volume µL) for 2 min, with or without head according to each analysis to be performed. The resulting homogenate was then centrifuged at 10,000 rpm x 10 min at 4 ºC and the supernatant fraction (S1) was used for the determination of enzymatic and non-enzymatic indicators of oxidative stress, dopamine levels, AChE activity and cell viability. The protein content was measured colorimetrically using the Bradford method (Bradford, 1976) and bovine serum albumin (1 mg/mL) was used as the standard.

2.5.2 Superoxide dismutase (SOD) activity

The activity of SOD was determined by monitoring the inhibition of quercetin auto-oxidation, according to (Kostyuk and Potapovich, 1989), and modifications as made by (Franco et al., 2009). Briefly, the homogenates containing 20 heads and 20 bodies of flies from each group were centrifuged (at 14,000 rpm for 30 min at 4°C). The reaction mixture of sodium phosphate buffer (0.025 M/EDTA 0,1 mM, pH 10), N,N,N,Ntetramethylethylenediamine (TEMED), 10 µL of the head or body tissue sample, and 0.15% quercetin dissolved in dimethyl formamide was monitored at 406 nm for 2 min to evaluate its autooxidation. The results represent the mean of four independent experiments in each group. The ability of the test sample to inhibit

quercetin oxidation by 50% at 26 °C is defined as one unit of the enzyme and the activity was expressed as U/mg protein.

2.5.3 Catalase (CAT) activity

The CAT activity was assayed according to (AEBI, 1984), with modifications as per (Paula et al., 2012). Briefly, the homogenates containing 20 heads and 20 bodies of flies from each group were centrifuged (at 14,000 rpm for 30 min at 4°C). The CAT activity was estimated by adding 30 µL head or body sample to a reaction mixture contained phosphate buffer (0.25 M/EDTA 2.5 mM, pH 7.0), hydrogen peroxide (10 mM) (H_2O_2) and Triton X-100 (0.012%). The decomposition of H_2O_2 was monitored at 240 nm for 2 min, and the activity was expressed as U/mg protein (1 U decomposes 1 µmol H_2O_2/min at pH 7 and 25°C) and it was determined from four independent experiments.

2.5.4 Thiobarbituric acid reactive substance (TBARS) levels.

Lipid peroxidation was estimated by measuring TBARS and it was expressed in terms of the malondialdehyde (MDA) content, according to the method described by (Ohkawa et al., 1979). In this method, MDA, an end product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a colored complex. Briefly, the heads of 20 flies from each group were homogenized in a 120 µL HEPES buffer and the bodies were homogenized in a 400 µL HEPES buffer (pH 7.0) and centrifuged at 1,000 rpm for 10 min (4°C). The content of TBARS was measured in a

medium containing 50 µL of tissue homogenate of either the head or body of the flies, 50 µL of 1.2% sodium dodecyl sulfate (SDS), 125 µL of acetic acid buffer (0.45 M, pH 3.4), and 125 µL of 0.28% TBA. The mixture was then heated at 95 °C for 120 min in a water bath. After it cooled to room temperature, the absorbance was measured in the supernatant at 532 nm. The results were calculated as µmol MDA/mg of protein.

2.5.5 Glutathione-S-transferase activity (GST)

The GST activity was quantified as described by (Habig and Jakoby, 1981) using 1-chloro-2–4-dinitrobenzene (CDNB) as a substrate. Twenty heads and twenty fly's bodies from each group were homogenized and centrifuged at 14,000 rpm for 30 min at 4 °C. The assay reaction mixture consisted of 30 µL of head or 60µL body sample, with 1,000 µL a solution (0.25M Kpi buffer / EDTA (2.5 mM, pH 7.0), distilled water and 100 mM GSH) and 20 µL of 50 mM CDNB. The reaction was monitored for 2 min at 340 nm using a spectrophotometer. The GST activity is expressed as mmol/mg protein.

2.5.6 Determination of thiol content

The determination of non-protein (NPSH) thiol was estimated as described by (Ellman, 1959). Briefly, the heads of 35 flies were homogenized in 350 µL Tris buffer (pH 8.0) and bodies of 35 flies were homogenized in 1,400 µL Tris buffer (pH 8.0). For the NPSH assay, the 300 µL homogenate of either the head or body was

precipitated with the 100 µL 0.5M PCA, followed by centrifugation at 10,000 rpm at 4°C for 5 min. It was used 50µL of supernatant, and added 190 µL of 0.5 M Tris/HCl buffer (pH 8.0). For both assays, after a 15 min incubation with 10 µL 5,5' dithiobis-2-nitrobenzoic acid (DTNB) 5 mM at room temperature (25 °C), the absorbance was measured by the spectrophotometry at 412 nm. Results is expressed as a percentage of the control group as µmol thiols/min/mg protein.

2.5.7 Determination of dopamine levels by HPLC-DAD

To determine DA using high performance liquid chromatography (HPLC), 30 head of flies per treatment group were homogenized in 0.9% NaCl (288 µL) and 0.5 M HCl (12 µL), the homogenates were centrifuged at 10,000 rpm x 10 min at 4 °C. In 0.22-µm PTFE filters the supernatant was filtered and then stored at 80°C until it was used. The supernatant of the samples (20 µL) was injected into the HPLC system by an auto sampler device. The YL9100 HPLC system consisted of a vacuum degasser and a quaternary pump connected to a reverse phase column (Synergi 4 µm Fusion-RP 80 Å 4.6 x 250 mm; Phenomenex) coupled to a diode array detector (DAD). The mobile phase consisted of methanol and water (12:88 v/v) adjusted to pH 3 with phosphoric acid and the flow rate was maintained at 0.8 mL min^{-1} (Bianchini et al., 2019). The detection was performed at 198 nm, and the results of the dopamine levels were expressed as µg DA/mg protein.

2.5.8 Determination of AChE activity.

The AChE activity was measured by the method described by (Ellman et al., 1961), using acetylthiocholine iodide as a substrate in homogenates of the head or body of flies. Each sample was assayed in triplicate. The rate of hydrolysis of acetylthiocholine iodide was measured at 412 nm through the release of thiol compounds, which reacted with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), producing the colored product thionitrobenzoic acid. The data were expressed as µmol/h/mg protein.

2.5.9 MTT reduction assay

Cellular viability was measured by MTT reduction as described by (Hosamani and Muralidhara, 2013). The head and body samples of the flies were incubated in MTT at 37 °C for 60 min, after the removal of MTT the sample was incubated in DMSO at 37 °C for 30 min. MTT reduction was measured at 540 nm. The results were expressed as percentage of the control.

2.5.10 Resazurin Reduction Test

This method evaluates the ability of viable cells to reduce resazurin to resorufin, a fluorescent molecule as described by (Franco et al., 2009). The homogenate of the heads and bodies of the flies were centrifuged at 3,570 rpm for 10 min at 4 °C. The 20µL of supernatant was then incubated with 180 µL of 20 mM Tris buffer (pH 7.0) and 10 µL resazurin for 2 hours. The fluorescence was measured by

the EnsPireR multi-mode plate reader (Perkin 214 Elmer, USA) from 579 nm to 588 nm.

2.6 Statistical analysis

The GraphPad Prism 7 software was used for statistical analysis and plotting graphs. Statistical analyses were performed by one-way or two-way analysis of variance (ANOVA), followed by Bonferroni's post hoc test, depending on the experiment. Lifespan measurement was determined by comparing the survival curves with a log-rank (Mantel–Cox) test. Values of $p < 0.05$ were considered statistically significant. All data are expressed as the mean and S.E.M.

3. Results

3.1 Experiment 1

Figures 1B, 1C and 2 show the effect of the exposure to free lutein (2 or 200 μM) or lutein nanoparticles (2, 6 or 20 μM), for 7 days, on climbing time and crossing number in the geotaxis and open-field task respectively and the survival percentage of *Drosophila melanogaster*. Statistical analysis (one-way ANOVA) revealed that exposure to lutein nanoparticles, significantly decreased the climbing time [$F_{(6,29)} = 7.89$; $P < 0.05$] and increased the crossing number [$F_{(6,29)} = 4.89$; $P < 0.05$]. Post hoc comparisons demonstrated that lutein nanoparticles, at the concentration of 2 and 20 μM, improved locomotor and exploratory activity in the geotaxis (Fig. 1B) and open-field task (Fig. 1C), compared to the control group and free lutein (2 and 200 μM).

Statistical analysis revealed that exposure to lutein nanoparticles, significantly increased the survival percentage. The comparisons demonstrated that lutein nanoparticles, at the concentration of 2 and 6 μM, improved the lifetime of flies (Fig. 2) compared to the control group and free lutein (2 and 200 μM). Based on the results from this assay, in the subsequent experiments only of lutein nanoparticles at the concentration 6 μM (concentration which had no effect per se) was used.

3.2 Experiment 2

Figures 3B, 3C and 4 show the effect of the exposure to ROT (500 μM), lutein nanoparticles (6 μM) and the co-exposure to ROT and lutein nanoparticles, for 7 days, on climbing time and crossing number in the geotaxis and open-field task respectively and the survival percentage of *Drosophila melanogaster*. Statistical analysis (two-way ANOVA) revealed a significant effect for the interaction factor (lutein nanoparticles versus ROT) [$F_{(1,20)} = 45.78$; $P < 0.05$] on the climbing time. Post hoc comparisons demonstrated that lutein nanoparticles co-exposure protected the flies against the ROT-induced locomotor deficit in the geotaxis task (Fig. 3B). Statistical analysis (two-way ANOVA) also revealed a significant effect for the interaction factor (lutein nanoparticles versus ROT) [$F_{(1,20)} = 18.28$; $P < 0.05$] on the crossing number. Post hoc comparisons demonstrated that lutein nanoparticles co-exposure protected the ROT-induced locomotor and exploratory deficit in the open-field task (Fig. 3C). Statistical analysis revealed that exposure to ROT, significantly increased the mortality of flies over experimental period compared to the control group. However, lutein nanoparticles co-exposure protected the flies against the ROT-induced mortality (Fig. 4).

3.3 Determination of oxidative stress indicators

Figures 7-11 show the effect of the exposure to ROT (500 μM), lutein nanoparticles (6 μM) and the co-exposure to ROT and lutein nanoparticles for 7 days, on oxidative stress indicators (SOD, CAT, TBARS, GST and NPSH) in the head and body of *Drosophila melanogaster*. Statistical analysis (two-way ANOVA) revealed a significant effect for the interaction factor (lutein nanoparticles versus ROT) on oxidative stress indicators: SOD [$F_{(1,12)} = 27.01$; $P < 0.05$], CAT [$F_{(1,16)} = 8.34$; $P < 0.05$], TBARS [$F_{(1.14)} = 4.94$; $P < 0.05$] and GST [$F_{(1,15)} = 23.64$; $P < 0.05$)] in the head and SOD [$F_{(1,16)} = 12.79$; $P < 0.05$], CAT [$F_{(1,12)} = 8.67$; $P < 0.05$], TBARS [$F_{(1,16)} = 10.17$; $P < 0.05$], GST [$F_{(1,16)} = 23.89$; $P < 0.05$)] and NPSH [$F_{(1,12)} = 9.04$; $P < 0.05$)] in the body. Post hoc comparisons demonstrated that lutein nanoparticles co-exposure protected the flies against the oxidative stress indicators alteration induced by ROT in the head (SOD, CAT, TBARS and GST; Fig. 5A-8A) and in the body (SOD, CAT, TBARS, GST and NPSH; Fig. 5B-9B). ROT did not induce any alterations for NPSH in the head (Fig. 9A).

3.4 Determination of Dopamine levels

Figure 10 shows the effect of the exposure to ROT (500 μM), lutein nanoparticles (6 μM) and the co-exposure to ROT and lutein nanoparticles, for 7 days, on dopamine levels in the head of *Drosophila melanogaster*. Statistical analysis (two-way ANOVA) revealed a significant effect for the interaction factor (lutein nanoparticles versus ROT) [$F_{(1,36)} = 8.69$; $P < 0.05$] on dopamine levels. Post hoc

comparisons demonstrated that lutein nanoparticles co-exposure protected the flies against the dopamine levels decrease induced by ROT (Fig. 10).

3.5 Determination of AChE activity

Figure 11 shows the effect of the exposure to ROT (500 µM), lutein nanoparticles (6 µM) and the co-exposure to ROT and lutein nanoparticles, for 7 days, on AChE activity in the head and body of *Drosophila melanogaster*. Statistical analysis (two-way ANOVA) revealed a significant effect for the interaction factor (lutein nanoparticles versus ROT) on AChE activity in the head [$F_{(1,12)} = 6.17$; $P < 0.05$] and body [$F_{(1,12)} = 11.57$; $P < 0.05$]. Post hoc comparisons demonstrated that the exposure to ROT decreased AChE activity in the head (Fig. 11A) and body (Fig. 11B). The co-exposure with lutein nanoparticles protected the flies against the AChE activity decrease induced by ROT in the head, however, it did not protect them against this decrease in the body.

3.6 Determination of cell viability by reduction of MTT and resazurin

Figures 12 and 13 show the effect of the exposure to ROT (500 µM), lutein nanoparticles (6 µM) and the co-exposure to ROT and lutein nanoparticles, for 7 days, on cell viability by the reduction of MTT and resazurin in the head and body of *Drosophila melanogaster*. Statistical analysis (two-way ANOVA) revealed a significant effect for the interaction factor (lutein nanoparticles versus ROT) on the reduction of MTT in the body [$F_{(1,12)} = 6.89$; $P < 0.05$], however, it did not show a

significant difference in the head. Post hoc comparisons demonstrated that lutein nanoparticles co-exposure protected the flies against the MTT decrease induced by ROT in the body (Fig. 12B). Statistical analysis also revealed a significant effect for the interaction factor (lutein nanoparticles versus ROT) on resazurin in the head [$F_{(1,12)} = 15.11$; $P < 0.05$], however, it did not show significant a difference in the body. Post hoc comparisons demonstrated that lutein nanoparticles co-exposure protected the flies against the resazurin decrease induced by ROT in the body (Fig. 13A), which suggests that lutein nanoparticles protected the flies against cell damage.

4. Discussion

In the present study, we evaluated the effect of lutein nanoparticles on PD model induced by rotenone in *Drosophila melanogaster* and the possible mechanism involved. Lutein nanoparticles (2 and 20 μM) showed to improve locomotor and exploratory activity per se in *Drosophila melanogaster* when compared to the control group (Fig. 1). In addition, lutein nanoparticles (2 and 6 μM) increased the survival rate per se in flies (Fig. 2), which suggests that it does not present neurotoxic effect. Lutein bioavailability is limited due to its poor water solubility (Arunkumar et al., 2015). Thus, nanoencapsulation appears as an alternative to increase the solubility of lutein and consequently to increase its bioavailability and absorption, which improves its biological effects. Interestingly, lutein nanoparticles did not only improve locomotor activity when compared to the control group, but also when compared to lutein itself in its unencapsulated form, with hydrophobic characteristics at concentrations 10 to 100 times lower. These results corroborate with previous reports which state that the encapsulation of bioactive compounds increases their biological

effects (ARUNKUMAR et al., 2015; OKONOGI; RIANGJANAPATEE, 2015; DO PRADO SILVA et al., 2017).

As expected, our results also showed that exposure to rotenone caused locomotor damage and neurotoxicity, decreased the survival rate (Fig. 3 and 4), as well as altered oxidative stress indicators, such as SOD, CAT, TBARS, GST and NPSH (Fig. 5-9), and dopamine levels (Fig. 10), AChE activity (Fig. 11) and reduced cell viability (Fig. 12-13) in the head and the body of the flies.

However, the most important finding is that this study provides evidence for the protective potential of exposure to lutein nanoparticles (6 μM during 7 days) on rotenone-induced locomotor damage in the geotaxis and open-field task (Fig. 3) and survival rate (Fig. 4). This is the first report to describe the ability of lutein nanoparticles in promoting survival and avoiding locomotor impairments in the PD experimental model in *Drosophila melanogaster*. Corroborating with our findings regarding lutein neuroprotection, it has been described that lutein improves the cognitive and memory deficits caused by Huntington's disease (Binawade and Jagtap, 2013), ethanol (Geiss et al., 2019) and age-related diseases (JOHNSON, 2012; KESSE-GUYOT et al., 2014; RENZI et al., 2014; NOLAN et al., 2015). Moreover, there is a positive correlation between intake of lutein and PD risk (SCHEIDER et al., 1997; JOHNSON et al., 1999).

Interestingly, our results showed that lutein nanoparticles restored the oxidative stress indicators (Fig. 5-9), dopamine levels (Fig. 10) and AChE activity (Fig. 11) in *Drosophila* exposed to rotenone and that this recovery in the neurochemical parameters seems to reflect protection against locomotor damage and the improvement in the survival rate of the flies.

Dopamine is a neurotransmitter synthetized by the enzyme tyrosine hydroxylase of the dopaminergic neurons and widely distributed in the substantia nigra pars compacta (SNpc) which projects to the striatum (Cichewicz et al., 2017; Figueira et al., 2017). Dopamine plays an important role in the control of multiple brain functions, including voluntary movement (Jean-Martin Beaulieu; Raul R. Gainetdinov, 2011). Dopaminergic neurons degeneration causes the reduction of dopamine levels in the striatum, which results in specific neurological symptoms, features of PD (Hirth, 2010; Zheng et al., 2018). The degenerative effect induced by xenobiotics such as paraquat and Rotenone occurs in a similar manner in the dopaminergic neurons of humans and *Drosophila* (Chaudhuri et al., 2007; Coulom, 2004). Regarding this point, administration or nutritional supplement of lutein attenuates the dopaminergic neurodegeneration induced by rotenone or MPTP in rodents, respectively (Makhija et al., 2014; Nataraj et al., 2016). Our results corroborate with this evidence, showing that the exposure to lutein nanoparticles increased dopamine levels, which suggests protection aganist rotenone-induced damage. Although the pathological hallmark of PD is the loss of dopaminergic neurons, changes in the cholinergic system have also been observed in the disease.

AChE is the enzyme that catalyzes the neurotransmitter acetylcholine (ACh) hydrolysis in cholinergic synapses, which regulates motor function and locomotion (Kim and Lee, 2013; Kostelnik et al., 2017). The degradation of ACh is required to disrupt the interaction between neurotransmitter and receptor, and to allow the cholinergic neuron to return to its resting state upon activation (Araújo et al., 2016). Evidence has shown reduced activity of AChE resulting in increases in the ACh availability in the synaptic cleft in neurodegenerative disorders such as PD (Zhu et al., 2008). In this sense, the dopaminergic neurons degradation associated to a

decrease in the AChE activity causes an imbalance between dopamine/ACh (Scarduzio et al., 2017). Since dopamine acts as an inhibitory neurotransmitter and ACh as an excitatory in the striatum, the dopamine/ACh imbalance provokes cholinergic neurons hyperactivity which difficult movement control (Calabresi et al., 2000; Rizzi and Tan, 2017; Zhu et al., 2008; Ztaou and Amalric, 2019). Our result showed that lutein nanoparticles protected against the decrease in AChE activity in *Drosophila melanogaster* exposed to Rotenone. Furthermore, lutein also attenuates ethanol-induced memory deficit by restoring AChE activity in rats (Geiss et al., 2019).

Rotenone has often been considered an effective chemical model to PD in *Drosophila melanogaster* and rodents, since it produces anatomical, neurochemical and neuropathological alterations similar to those observed in human disease (Coulom, 2004; de Freitas Couto et al., 2019; Hirth, 2010; Sudati et al., 2013; Valdez et al., 2019). Rotenone causes inhibition of the mitochondrial complex I, which increases the production of reactive oxygen species and, consequently, generates oxidative stress (Betarbet et al., 2000; Karuppaiah, 2018; Wang et al., 2017). In the present study, we found significant oxidative stress indicators alterations in flies exposed to rotenone, as evidenced by the decrease of SOD and CAT antioxidant enzyme activity, increases of TBARS levels and GST activity, the decrease of NPSH and cell viability. Oxidative stress is considered to be responsible for the dopaminergic neurons degeneration and decreases in AChE activity, causing an imbalance between dopamine and ACh, being those the main factors attributed to the locomotor dysfunction present in PD (Aosaki et al., 2010; Araujo et al., 2015; Cannon and Greenamyre, 2010; Lehmann and Langer, 1983; Sanders and Greenamyren, 2013).

Moreover, we showed that lutein restored the rotenone-induced oxidative stress indicators alterations, this effect may be associated to antioxidant and free radical scavenging activity, due to its high number of conjugated double bonds and hydroxyl group attached to each end of the molecule (Conna, 1991; El-Agamey et al., 2004). In this sense, previous studies have shown reduction in malondialdehyde (MDA) levels and up-regulation of gene expression of SOD and CAT in flies fed with lutein (Zhang et al., 2014). Furthermore, lutein administration prevents the MPTP-induced increased TBARS, SOD and CAT in mice (Nataraj et al., 2016), as well as the increase of MDA levels and decrease of GSH concentration and GPx activity in diabetic mice (Muriach et al., 2006).

In summary, our results support that lutein nanoparticles protected the flies against rotenone-induced locomotor damage, and that effect appears to involve a set of factors, such as the protection of oxidative stress and the increase of antioxidant defenses, which is associated to dopaminergic and cholinergic regulation in *Drosophila melanogaster*. However, the protective effect of lutein seems not to be limited only to the events shown here. In addition, cortical carotenoids seem to play other synaptic functions, like the increase of communication among neurons and gap junction (Conner et al., 1997; Takahashi et al., 2012). Although we do not have experimental evidence, it is interesting to consider that the protective effect of lutein nanoparticles on locomotor damage may involve complementary signaling cascades, beyond those investigated in this study.

In this view, lutein can directly combat oxidative stress due to its structure and ability to neutralize free radicals (Roberts et al., 2009; Shami and Moreira, 2004), as well as trigger signaling cascades such as PI3K/Akt (Lee et al., 2006; Li et al., 2012). PI3K/Akt is an intracellular signaling pathway that promotes growth,

proliferation, cell survival, transcription and protein synthesis in response to extracellular signals (Astous et al., 2006; Humbert et al., 2002; Wei et al., 2002). PI3K activation phosphorylates and activates Akt, which has a downstream target including nuclear factor erythroid 2–related factor 2 (Nrf2) (Astous et al., 2006; Li et al., 2014). The Nrf2 is a transcription factor that regulates the expression of genes that, similarly to mammals, in *Drosophilas*, encode antioxidant enzymes (Jing et al., 2015; Ma, 2013). Evidence including in vivo, postmortem and genetic studies, shows that deregulation of the PI3K/Akt pathway, associated to significant reduction in Nrf2, has been implicated in the induction of PD (Gao and Wu, 2016; Gunjima et al., 2014; C. Li et al., 2018). Regarding this point, lutein significantly activates Nrf2 in mice microglial BV-2 cells (Wu et al., 2015), mice liver (Li et al., 2015), human retinal pigment epithelial cells (ARPE-19 cells) (Frede et al., 2017) and osteoporosis rat model (H. Li et al., 2018). Thus, a possible additional mechanism of action for the effects of lutein on protection against rotenone-induced damage may involve the activation of PI3K/Akt/Nrf2 signaling pathway, beyond the effects on the dopaminergic and cholinergic system balance in *Drosophila melanogaster*, demonstrated in study.

In summary, our results support the fact that lutein nanoparticles protected against rotenone-induced damage, suggesting that this neuroprotective effect on locomotor activity and survival involves oxidative stress and dopaminergic and cholinergic neurotransmission in *Drosophila melanogaster*. These currently reported results support lutein nanoparticles as a therapeutic target for the development of drugs able to protect PD, thus allowing the identification of effective pharmacological strategies for treatments. However, further investigation is needed to clarify the

precise mechanisms involved in the neuroprotective effects of lutein nanoparticles on PD symptoms.

Conflict of interest: The authors declare that there are no conflicts of interest.

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Figure Legends

Figure 1. (A) Schedule of the experimental protocol for the concentration–response curve of lutein. Effect of free lutein (2 or 200 μM) or lutein nanoparticles, (2, 6 or 20 μM) for 7 days, on locomotor and exploratory activity in the *Drosophila melanogaster*. (B) Negative geotaxis assay; (C) Open-field. Data are mean ± SEM, for n = 4-6 in each group. * Indicates a significant difference ($p < 0.05$) compared to the control group. # Indicates a significant difference ($p < 0.05$) of free lutein compared to the lutein nanoparticles groups (2 or 200 μM).

Figure 2. Effect of free lutein (2 or 200 μM) or lutein nanoparticles, (2, 6 or 20 μM) for 7 days, on survival percentage in *Drosophila melanogaster.* Data were collected every 24 h for each group over 7 days. Data are mean ± SEM, for n = 9-13 in each group. * Indicates a significant difference ($p < 0.05$) compared to the control group. # Indicates a significant difference ($p < 0.05$) of lutein nanoparticles compared to the free lutein group (2 or 200 μM).

Figure 3. (A) Schedule of the experimental protocol. Exposure to ROT (500 μM), lutein nanoparticles (6 μM) and the co-exposure to ROT and lutein nanoparticles for 7 days, on locomotor and exploratory activity in *Drosophila melanogaster.* (B) Negative geotaxis assay; (C) Open-field. Data are mean ± SEM, for n = 6 in each group. * Indicates a significant difference ($p < 0.05$) compared to the control group. # Indicates a significant difference ($p < 0.05$) compared to the ROT group.

Figure 4. Exposure to ROT (500 μM), lutein nanoparticles (6 μM) and the co-exposure to ROT and lutein nanoparticles for 7 days, on survival percentage in *Drosophila melanogaster*. Data were collected every 24 h for each group over 7

days. Data are mean ± SEM, for n = 6 in each group. * Indicates a significant difference ($p < 0.05$) compared to the control group. # Indicates a significant difference ($p < 0.05$) compared to the ROT group.

Figure 5. Exposure to ROT (500 μM), lutein nanoparticles (6 μM) and the co-exposure to ROT and lutein nanoparticles for 7 days, on SOD activity in the (A) head and (B) body of *Drosophila melanogaster*. Data are mean ± SEM, for n = 4-6 in each group. * Indicates a significant difference ($p < 0.05$) compared to the control group. # Indicates a significant difference ($p < 0.05$) compared to the ROT group.

Figure 6. Exposure to ROT (500 μM), lutein nanoparticles (6 μM) and the co-exposure to ROT and lutein nanoparticles for 7 days, on CAT activity in the (A) head and (B) body of *Drosophila melanogaster*. Data are mean ± SEM, for n = 5 in each group. * Indicates a significant difference ($p < 0.05$) compared to the control group. # Indicates a significant difference ($p < 0.05$) compared to the ROT group.

Figure 7. Exposure to ROT (500 μM), lutein nanoparticles (6 μM) and the co-exposure to ROT and lutein nanoparticles for 7 days, on TBARS levels in the (A) head and (B) body of *Drosophila melanogaster*. Data are mean ± SEM, for n = 4-5 in each group. * Indicates a significant difference ($p < 0.05$) compared to the control group. # Indicates a significant difference ($p < 0.05$) compared to the ROT group.

Figure 8. Exposure to ROT (500 μM), lutein nanoparticles (6 μM) and the co-exposure to ROT and lutein nanoparticles for 7 days, on GST activity in the (A) head and (B) body of *Drosophila melanogaster*. Data are mean ± SEM, for n = 4-5 in each group. * Indicates a significant difference ($p < 0.05$) compared to the control group# Indicates a significant difference ($p < 0.05$) compared to the ROT group.

Figure 9. Exposure to ROT (500 μM), lutein nanoparticles (6 μM) and the co-exposure to ROT and lutein nanoparticles for 7 days, on NPSH assay in the (A) head and (B) body of *Drosophila melanogaster*. Data are mean ± SEM, for n = 4 in each group. * Indicates a significant difference ($p < 0.05$) compared to the control group. # Indicates a significant difference ($p < 0.05$) compared to the ROT group.

Figure 10. Exposure to ROT (500 μM), lutein nanoparticles (6 μM) and the co-exposure to ROT and lutein nanoparticles for 7 days, on dopamine levels in the head of *Drosophila melanogaster*. Data are mean ± SEM, for n = 9-11 in each group. * Indicates a significant difference ($p < 0.05$) compared to the control group. # Indicates a significant difference ($p < 0.05$) compared to the ROT group.

Figure 11. Exposure to ROT (500 μM), lutein nanoparticles (6 μM) and the co-exposure to ROT and lutein nanoparticles for 7 days, on AChE activity in the (A) head and (B) body of *Drosophila melanogaster*. Data are mean ± SEM, for n = 4 in each group. * Indicates a significant difference ($p < 0.05$) compared to the control group. # Indicates a significant difference ($p < 0.05$) compared to the ROT group.

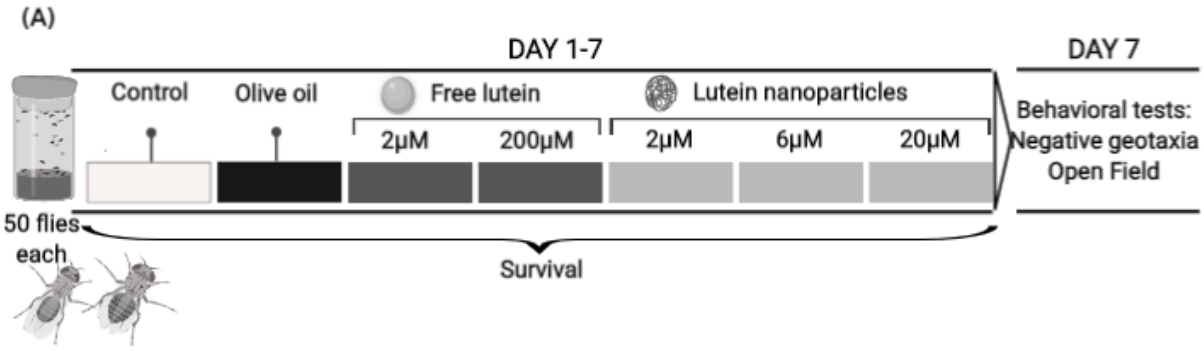
Figure 12. Exposure to ROT (500 μM), lutein nanoparticles (6 μM) and the co-exposure to ROT and lutein nanoparticles for 7 days, on cell viability by the reduction of MTT in the (A) head and (B) body of *Drosophila melanogaster*. Data are mean ± SEM, for n = 4-5 in each group. * Indicates a significant difference ($p < 0.05$) compared to the control group. # Indicates a significant difference ($p < 0.05$) compared to the ROT group.

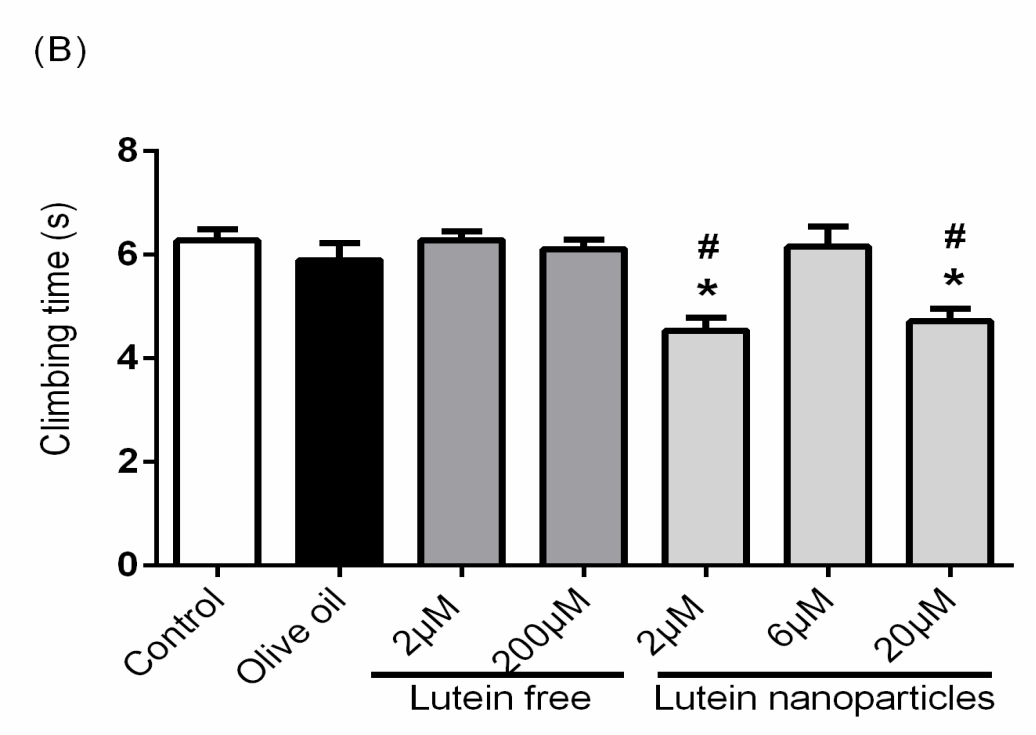
Figure 13. Exposure to ROT (500 μM), lutein nanoparticles (6 μM) and the co-exposure to ROT and lutein nanoparticles for 7 days, on cell viability by reduction of

resazurin in the (A) head and (B) body of *Drosophila melanogaster*. Data are mean ± SEM, for n = 4 in each group. * Indicates a significant difference ($p < 0.05$) compared to the control group. # Indicates a significant difference ($p < 0.05$) compared to the ROT group.

Figures:

Figure 1





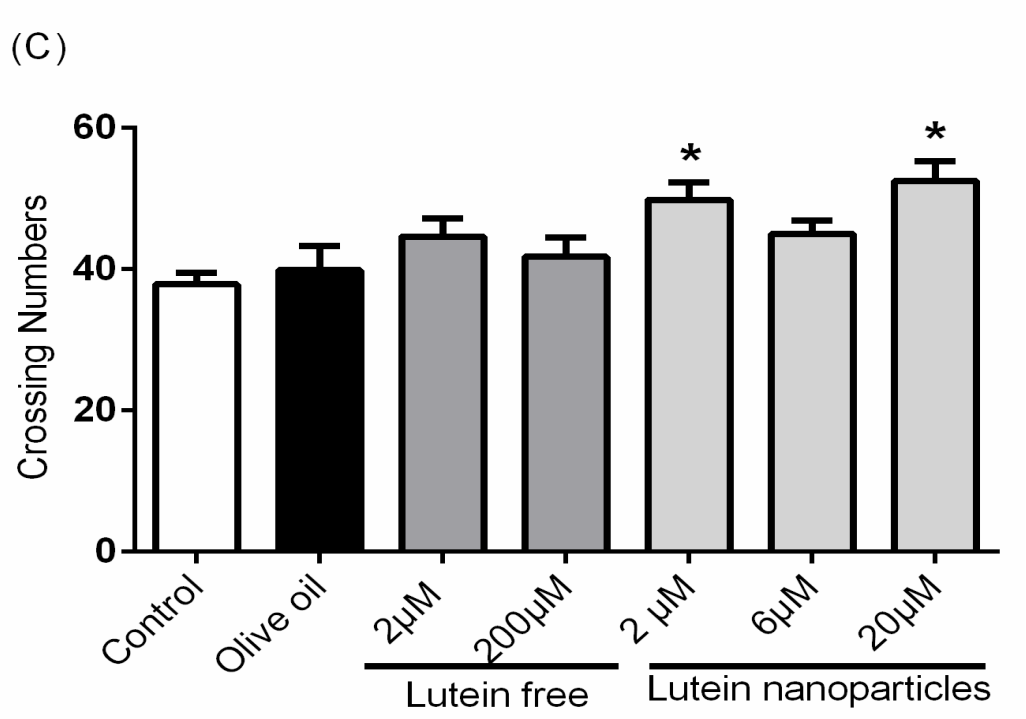

(C)
Crossing Numbers
60
40
20
0
*
*
Control
Olive oil
2μM
200μM
2 μM
6μM
20μM
Lutein free
Lutein nanoparticles

Figure 2

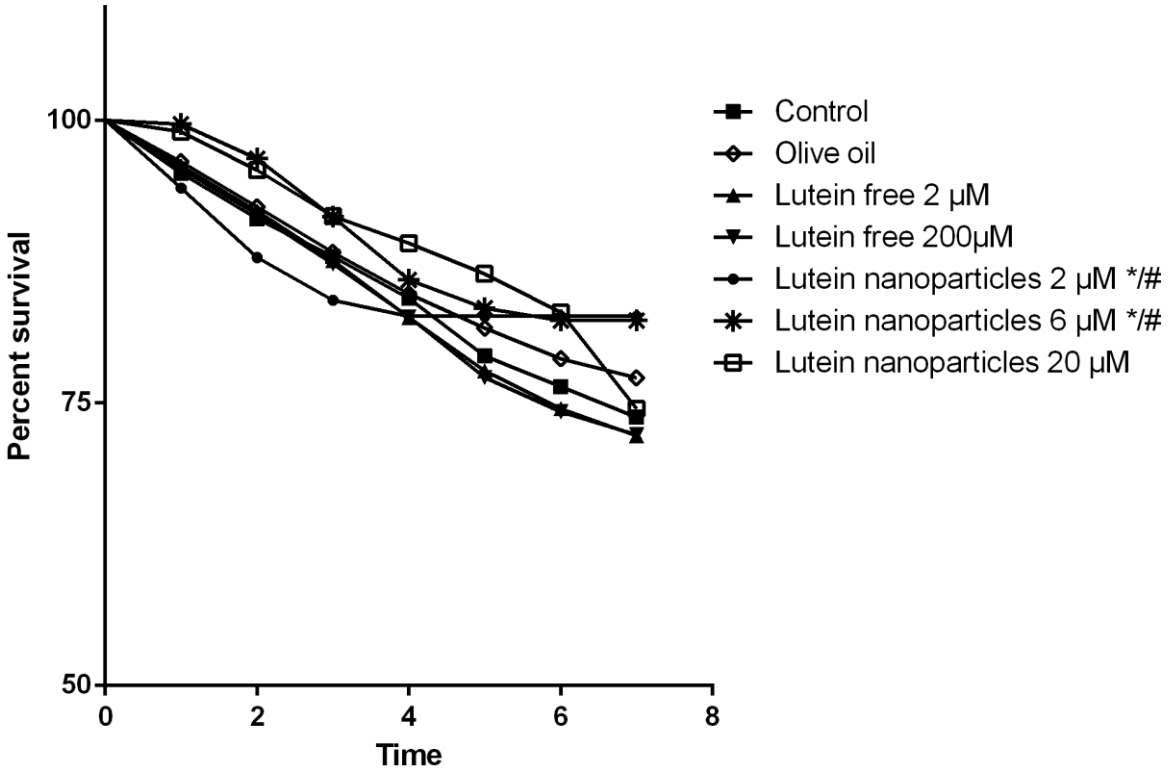
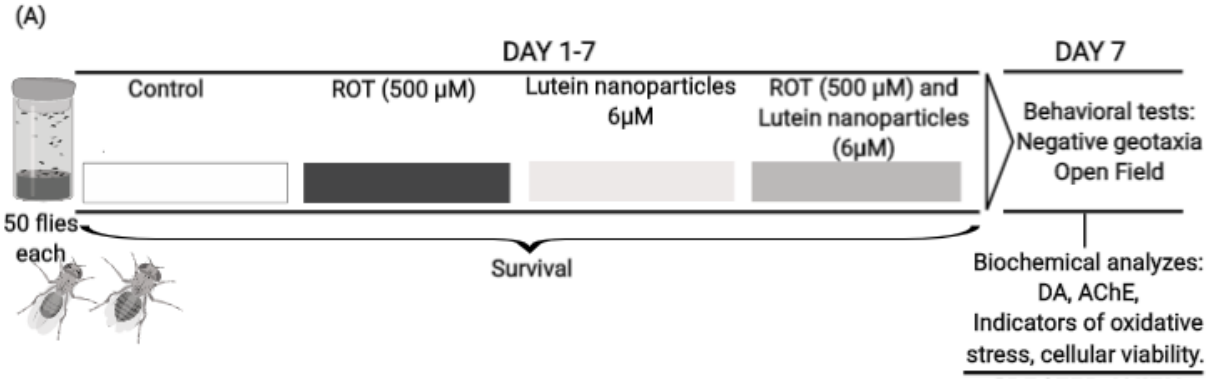
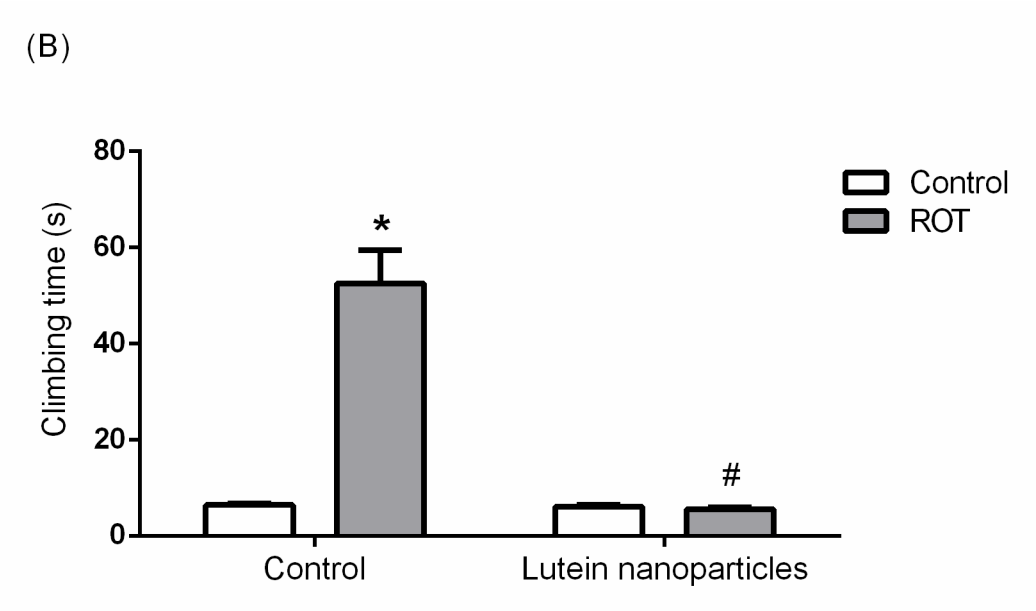

Percent survival
100
75
50
0
2
4
6
8
Time
Control
Olive oil
Lutein free 2 μM
Lutein free 200μM
Lutein nanoparticles 2 μM */#
Lutein nanoparticles 6 μM */#
Lutein nanoparticles 20 μM

Figure 3


(A)
DAY 1-7
DAY 7
Control
ROT (500 µM)
Lutein nanoparticles 6µM
ROT (500 µM) and Lutein nanoparticles (6µM)
Behavioral tests:
Negative geotaxia
Open Field
50 flies each
Survival
Biochemical analyzes:
DA, AChE,
Indicators of oxidative stress, cellular viability.


(B)
Climbing time (s)
80
60
40
20
0
*
#
Control
ROT
Control
Lutein nanoparticles

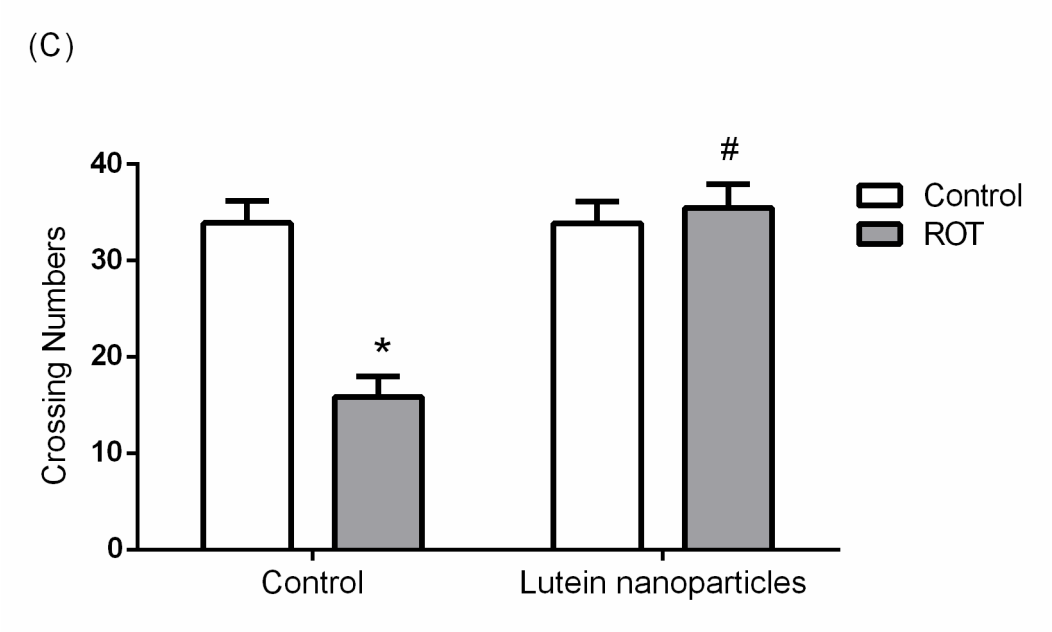

(C)
Crossing Numbers
40
30
20
10
0
*
#
Control
ROT
Control
Lutein nanoparticles

Figure 4

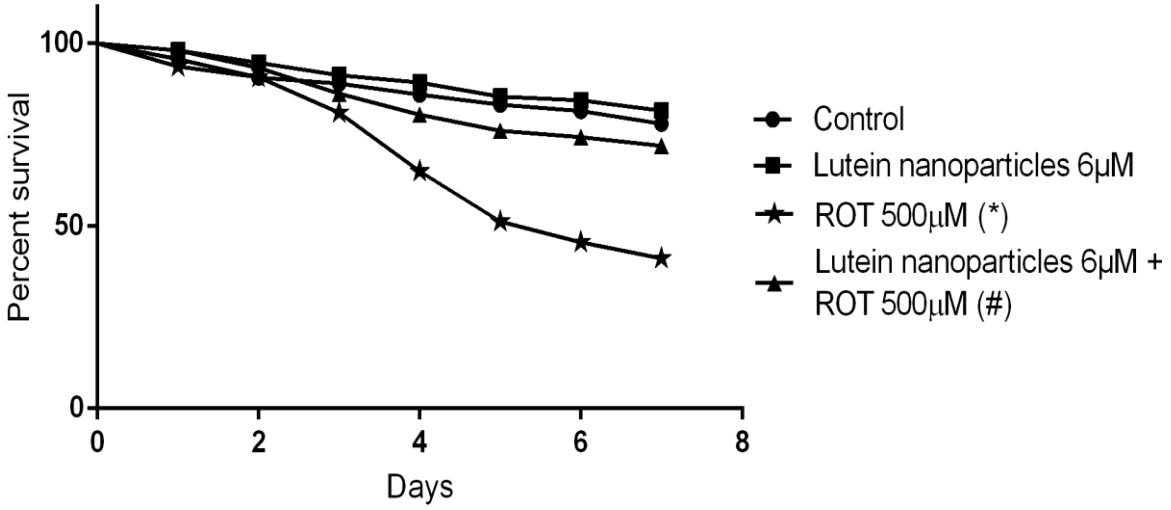

Percent survival
100
50
0
0
2
4
6
8
Days
Control
Lutein nanoparticles 6μM
ROT 500μM (*)
Lutein nanoparticles 6μM +
ROT 500μM (#)

Figure 5

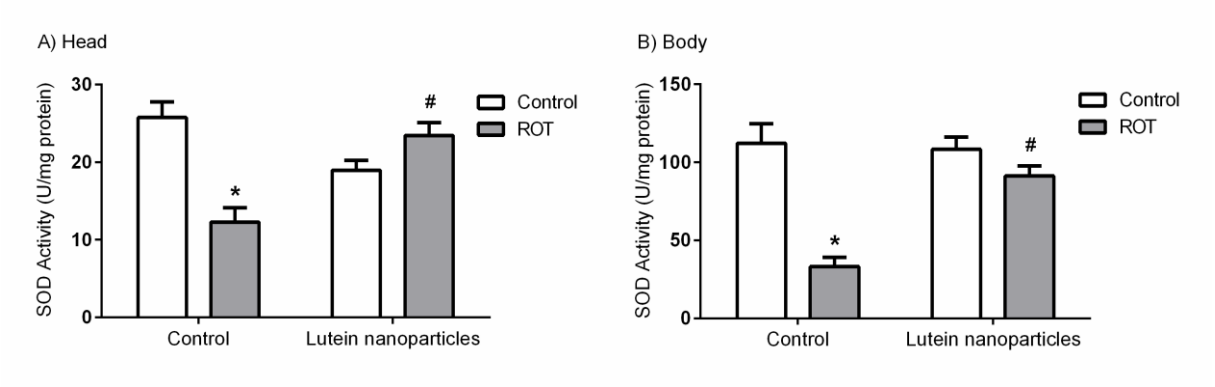


Figure 6

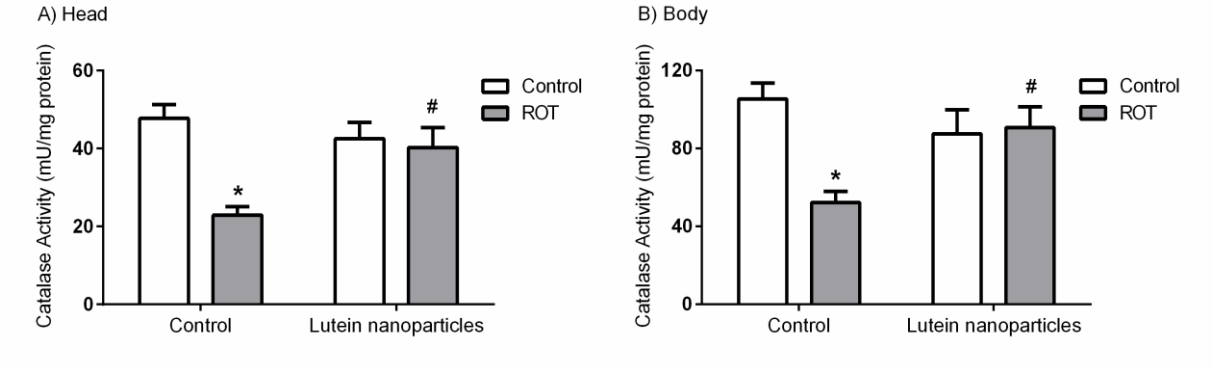


Figure 7

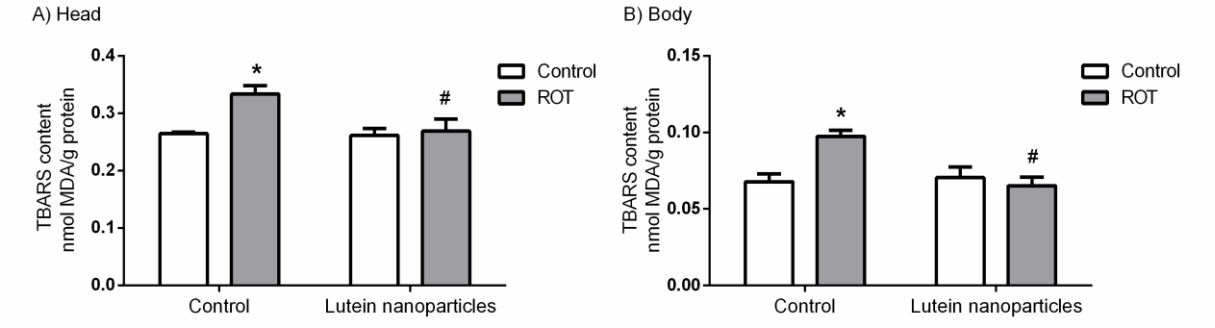


Figure 8

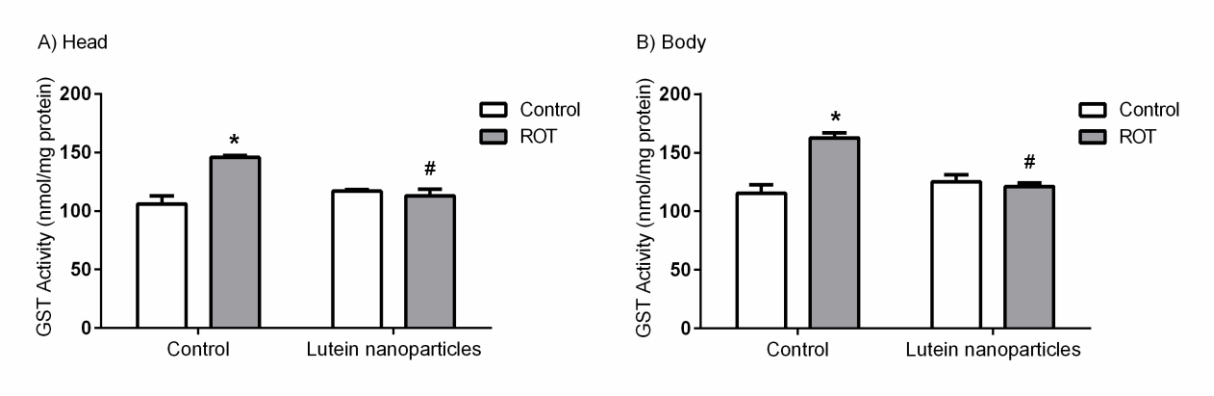


Figure 9

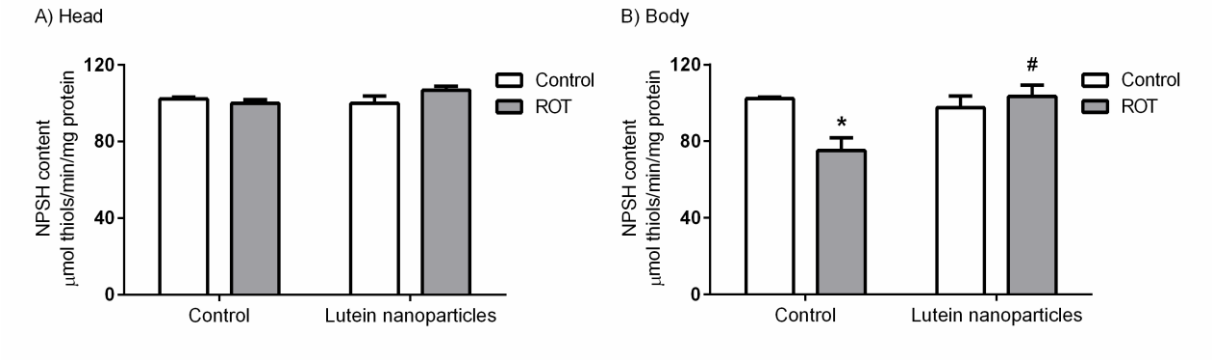


Figure 10

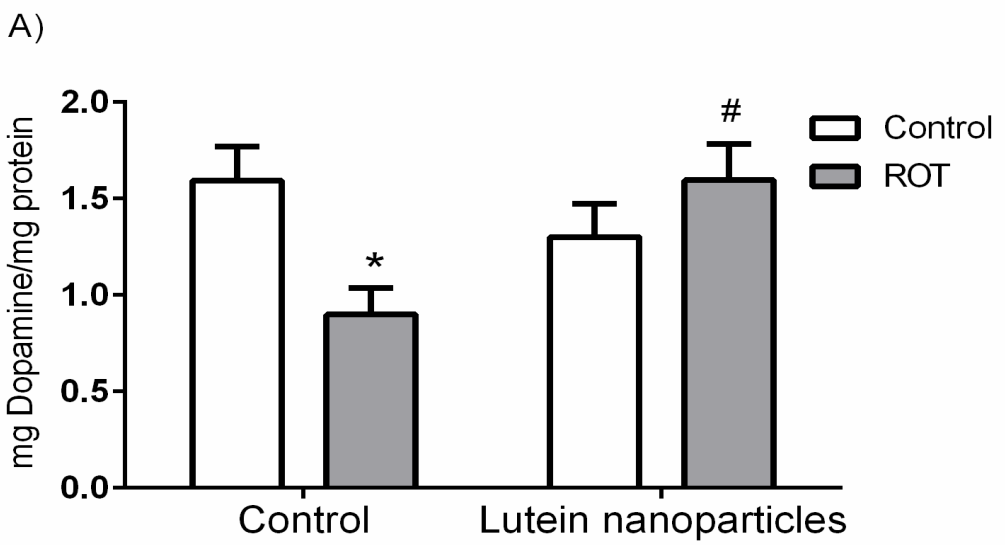


Figure 11

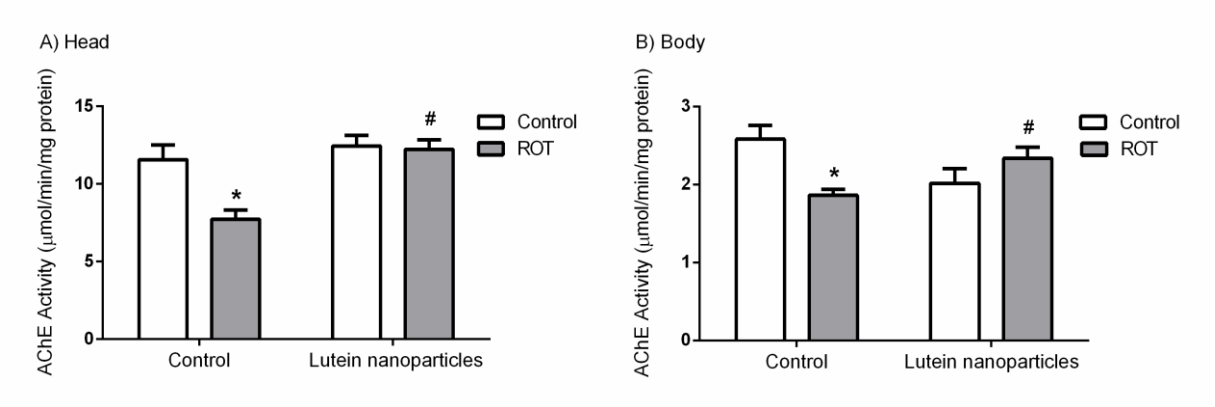


Figure 12

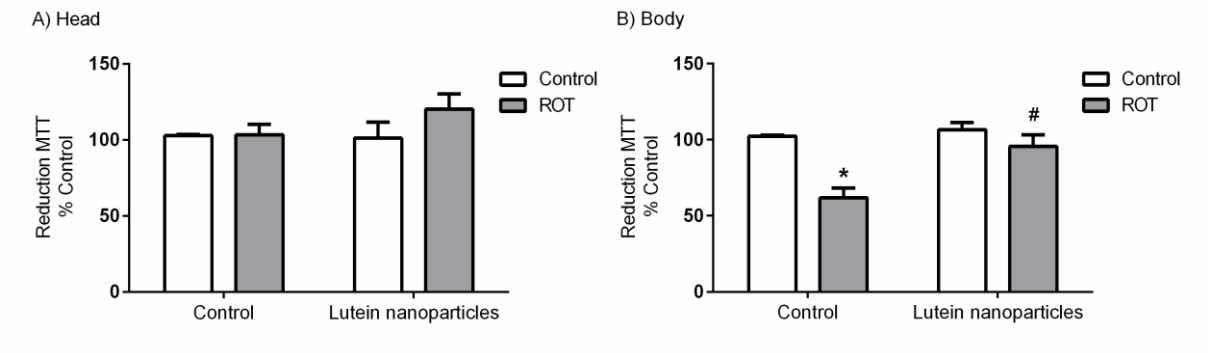
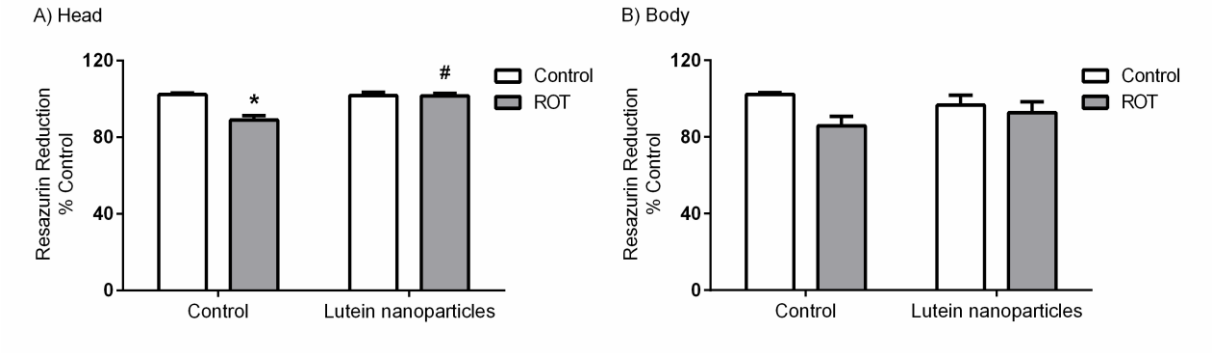


Figure 13



5 CONCLUSÕES

Nossos resultados indicam que as nanopartículas de luteína aumentam a sobrevivência, previnem danos locomotores e exploratórios. Além disso, regulam os indicadores de estresse oxidativo, aumentam os níveis de DA, regulam a atividade da AChE e viabilidade celular em *Drosophila melanogaster* exposta à ROT. Esses achados sugerem que nanopartículas de luteína podem ser um possível candidato no tratamento da DP. Além disso, esses resultados confirmam que a Drosoplila melanogaster é um excelente modelo para investigar alterações nos parâmetros bioquímicos e comportamentais em doenças neurodegenerativas.

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