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Amelioration of Acrolein Effects on the Small Intestine of Rats by Curcumin and Compound 20

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DISSERTATION

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By

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Texas Southern University, 2022

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Abstract

Acrolein is an unsaturated aldehyde which produce chemicals used as an intermediate reactive aldehyde in the chemical industry. The primary component of acrolein, is derived from the incomplete combustion of wood, plastic, fossil fuels, the main component of cigarette smoke, the burning of fats and overheating of oil. The purpose of this research is to investigate whether curcumin and compound 20 prevents the induction of inflammatory and oxidative response caused by acrolein in the small intestine. Curcumin is the yellow pigment, lipophilic polyphenol substance that gives turmeric the medicinal properties. It can also define as the primary bioactive substance in turmeric with anti-inflammatory, and antioxidant properties.

Objectives

- Evaluate acrolein effects in the small intestine; Method - Measuring lipid peroxidation and antioxidant activities.

- Compare curcumin and compound20 effects in reversing/inhibiting the effects of acrolein in the small intestine. Method - Measuring lipid peroxidation and antioxidative activities.
- Determine the mechanism that compound 20 reverse/inhibit the changes caused by acrolein in the small intestine. Method - Cytokines activities and Nrf2 activity and expression.

Methodology/Results

In vivo, male Sprague–Dawley rats received curcumin or compound 20 mixed in peanut butter via voluntary oral consumption followed by intraperitoneal (IP) administration of acrolein. The levels of glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) were detected by a GSH assay kit, SOD kit and MDA kit respectively. In addition, levels of the anti-inflammatory cytokine IL-10 in ileum was detected and showed increased whereas pro-inflammatory cytokine expression (TNF-α) was found to be significantly lower in the ileum of treated animals as compared to the control except for significantly increase with the acrolein treated group. GSH was found to be significantly increased in the treated animals as compared to the control. SOD did not show significant increase except animals treated with compound20 compared to control and significantly in group treated with acrolein compared to control. The MDA lipid peroxidation showed significant increase in animals treated with acrolein compared to the control. In this study, curcumin is shown to be capable of targeting the Nrf2 signaling pathway in protecting the cells against inflammatory and oxidative damage.

Conclusion

These results suggest that curcumin and compound20 might be a useful agent against small intestine dysfunction caused by acrolein-induced inflammatory and oxidative response.

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A/ACR	Acrolein
A+Cu	Mixture of acrolein and curcumin
A+Cmpd20	Mixture of acrolein and compound20
Cu	Curcumin
Cmpd20	Compound20
GSH	Glutathione
SOD	Superoxide Dismutase
GST	Glutathione-S-Transferase
IL-10	Interleukin 10
TNF	Tumor necrosis factor
w/w	Weight ratio
Nrf2	Nuclear factor-2

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CHAPTER 1

INTRODUCTION

The purpose of this research was to investigate whether curcumin and compound 20 prevents the induction of inflammatory response caused by acrolein in the small intestine. Chapter one provided an overview of the research project in terms of problem, purpose, and methods that we would use to achieve the study's aim. This chapter presents a thorough review of past literature related to the subjects of curcumin, acrolein toxicity, and inflammatory effects in the small intestine. First, we review background literature on curcumin, acrolein, and compound 20. Second, we review existing literature on the mechanism of action of curcumin and acrolein toxicity. Lastly, we will review the literature on the impact of curcumin on acrolein toxicity in rats and mice.

Acrolein is a simple unsaturated aldehyde. It is a colorless liquid with a pungent, choking odor. It's very toxic by inhalation and can course strong irritants to eyes, skin and nasal passage. Acrolein is present in the incomplete combustion of trees, plastics. Found in tobacco, gasoline, overheating oil and other plants. Acrolein is the byproduct of lipid peroxidation and lipid peroxidation contribute to the generation of more acrolein. Its primary metabolism occurs through glutathione alkylation.

Curcumin is an extract from a rhizome called *Curcuma langa* which is commonly known as Turmeric (from the ginger family). It is the yellow pigment, lipophilic polyphenol substance that gives the plant turmeric its medicinal properties. It is hydrophobic meaning it is insoluble in water but soluble in oil, ethanol and acetone. Curcumin has been around for thousands of years. It has been used in many countries in Asia and Africa

as spice know as curry. It also has been used in food coloring, dye and in Chinese medicine. Many recent studies show curcumin as an antioxidant, anti-inflammatory and anticarcinogenic agent. Large oral consumption is excreted due to its poor absorption from the small intestine and high metabolism in the liver.

Compound20 is a novel synthetic of curcumin, that was synthesized here at TSU in Dr. Chelliah's lab. It was modified to improve the absorption in the body compared to curcumin.

CHAPTER 2

HISTORY and/or LITERARY REVIEW

Acrolein

Acrolein is a clear or yellow liquid with a pungent odor when burned. Acrolein reacts quickly when exposed to other substances, in addition to burning and evaporating quickly (Xiong et al., 2018). In other definitions, acrolein is an alpha-beta electrophilic unsaturated aldehyde that covalently binds to cell thiols. Acrolein is formed from burning organic matter such as tobacco or fuels such as oil and gasoline. It can also be formed from the breakdown of pollutants in the open air (Xiong et al., 2018). After the burning of fuels and fossils, acrolein forms when vegetable fats and animal oils are heated. Acrolein is used as an intermediate in the chemical manufacturing of acrylic acid and as a biocide in controlling algae and plant growth in agricultural sectors. In addition to being formed from burning animal and vegetable fats, tobacco, wood, plastics, gasoline, and diesel fuel, acrolein is also naturally occurring in the body in very small amounts (Xiong et al., 2018). In the human body, acrolein is formed as a by-product of lipid oxidation and the metabolism of alpha-hydroxyamino acids. Generally, the general population is exposed to limited amounts of acrolein which is increased by exhaust fumes and smoke from wood and plastic.

The reactivity of acrolein is dependent on its electrophilic and unsaturated nature. For instance, a further examination of acrolein revealed that its electrophilic and unsaturated nature enhanced the speed with which it bound and depleted nucleophiles

such as glutathione (Gupta et al. (2021). Additionally, acrolein reacts with proteins via Schiff base formation and Michael addition. In proteins, acrolein targets the nucleophilic side chain of histidine, lysine, and cysteine residues. Zhang et al. (2018) contended high concentrations of acrolein impacted cell cytotoxic reactions that lead to genotoxicity and cell mutation.

On the other hand, Gupta et al. (2021) established that acrolein initiated lipid peroxidation, induced cell apoptosis, and enhanced the expression of glutathione S-transferases. When examining acrolein toxicity, Gupta et al. (2021) observed that acrolein increased oxidative stress in high concentrations by reducing the levels of the antioxidant glutathione. Decreased glutathione levels paved the way for high levels of oxidative stress and free radicals.

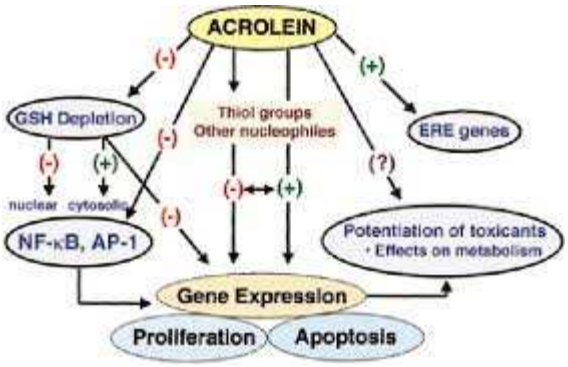

Figure 1: Molecular effects of acrolein

https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.semanticscholar.org%2Fpaper%2FThe-molecular-effects-of-acrolein.-Kehrer-

Acrolein Toxicity

Even though the levels of acrolein are low in human beings, Chen et al. (2018) noted that exposure to high concentrations of heated vegetable and animal fats, tobacco, plastic and wood smoke, exhaust fumes from burned gasoline exposed people to the side effects of acrolein. People may experience the toxic effects of acrolein after inhalation, ingestion, or dermal exposure (Zirak et al., 2019). At high concentrations, acrolein results in skin irritation, irritation of the nose, lungs, throat, lung hemorrhage, pulmonary edema, and death (Chen et al., 2018). Consequently, exposures to high concentrations of oral acrolein resulted in severe stomach ulceration, bleeding, and vomiting. Zirak et al. (2019) presented that oral exposure to more than 2mg/kg or higher of acrolein resulted in more severe experiences of stomach irritation. Examining the dermal effects of acrolein, Chen et al. (2018) reported that skin exposure to acrolein liquid resulted in ulceration, lacrimation, reddening, and stinging of the eyes. In a different study, Xiong et al. (2018) evaluated the mode of action of acrolein toxicity in the human airways tissue model. Xiong et al. (2018) found that continued exposure of the human airways to acrolein for a consecutive period of ten days resulted in significant GSH depletion, protein oxidation, oxidative stress, and inflammation.

Dwivedi et al. (2015) conducted a study to investigate the levels of acrolein exposure at which people experienced acute irritation. Dwivedi et al. (2015) studied a total of nine healthy volunteers who were exposed to 0.05ppm and 0.1ppm of acrolein for two hours. The results showed of the study revealed that exposure at 0.001 of acrolein

resulted in mild eye irritation. The results further showed that there were no significant effects of exposure to pulmonary function and nasal swelling. In a near similar study, Moghe et al. (2015) investigated the molecular mechanisms of acrolein toxicity in human diseases. Moghe et al. (2015) first defined acrolein as a highly reactive unsaturated aldehyde with serious implications to human health. According to Moghe et al. (2015), high acrolein exposure at cellular levels exposes the human body to a range of toxic effects, including oxidative stress, membrane damage, endoplasmic reticulum stress, and immune dysfunction and DNA adduction. On DNA adduction, Moghe et al. (2015) found that acrolein readily reacted with deoxyguanosine to produce two exocyclic DNA adducts from cross-link interchain with double-stranded DNA. The results of both studies showed acrolein interfered with the viability of cellular membranes.

Advancing the study conducted by Moghe et al. (2015), Ou et al. (2020) investigated the interaction of acrolein, acrylamide, and 5-hydroxymethylfurfural with DNA and amino acids. According to Ou et al. (2020), acrolein is electrophilic. The unsaturated carbonyl group easily reacts with the amino, thiol and imino groups of proteins, amino acids, and DNA through nucleophilic reactions. A closer examination of the reaction between acrolein, acrylamide, and hydroxymethylfurfural revealed that both compounds formed adducts with protein, amino acids, and DNA. For instance, Ou et al. (2020) presented that the adducts formed by these compounds were mutagenic and caused significant mutations in the DNA.

Additionally, these compounds compromised the integrity of cell membranes. In a similar study, Zirak et al. (2019) investigated the mechanisms behind the atherothrombotic effects of acrolein. According to Zirak et al. (2019), acrolein interrupted

the permeability of endothelial cells. Degrading the viability of endothelial cells, Zirak et al. (2019) established that oxidative stress and enhancement of inflammatory processes and the activation of metalloproteases initiated and accelerated the formation of atherosclerotic lesions. Further examination revealed that acrolein induced systemic dyslipidemia.

Reversing Acrolein-induced Inflammation

In the existing literature, several studies have been conducted on ways of reversing acrolein-induced inflammation. In one study, Wang et al. (2019) investigated the efficacy of the compound sildenafil in reversing airway inflammation induced by acrolein. According to Wang et al. (2019), sildenafil is a potent inhibitor that actively breaks down guanosine monophosphate, responsible for hormonal signaling to initiate inflammation in endothelial cells. Wang et al. (2019) used a sample of 67 rats initially treated with sildenafil before acrolein exposure. To measure the efficacy of sildenafil, Wang et al. (2019) extracted bronchoalveolar lavage fluid from the nasal tract and measured the expression levels of pro-inflammatory cytokines. Apart from pro-inflammatory cytokine levels, Wang et al. (2019) also measured histological scores, mucin production, and levels of nitric acid metabolites. In their findings, Wang et al. (2019) reported that sildenafil significantly reversed the expression of pro-inflammatory cytokine levels in the bronchoalveolar lavage fluid, nitric oxide metabolite, and mucin production. As such, sildenafil subsequently reduced inflammation of the airway in the rats. However, Wang et al. (2019) pre-treated the rats with sildenafil before the introduction of acrolein. Pre-treatment is not a common method of administering

medication; hence Wang et al. (2019) suggested that the study be replicated with a comparison of pre-treatment and post-treatment results.

In another study, Gökçe et al. (2020) examined the efficacy of selenium in reversing acrolein-induced liver inflammation and apoptosis in rats. In their study, Gökçe et al. (2020) included a sample of 100 male Winstar albino rats, which were randomly assigned to four groups and administered with acrolein + placebo, acrolein, selenium, and acrolein + selenium. Particularly, the experimental groups were administered with acrolein, selenium, and acrolein + selenium, while the control group was administered with acrolein + placebo. Electronic microscopy and immunohistochemical were performed, and liver apoptotic indexes were calculated. After calculating the liver apoptotic indexes for all the rats, Gökçe et al. (2020) found that the mean apoptotic index for rats in the acrolein and control groups increased significantly over the administration period.

On the contrary, apoptotic indexes for rats in the experimental group significantly reduced compared to rats in the acrolein and control categories. As such, Gökçe et al. (2020) demonstrated that selenium might have liver anti-inflammatory properties and anti-apoptotic properties induced by acrolein. However, Gökçe et al. (2020) did not address any potential side effects of selenium intake. Additionally, Gökçe et al. (2020) did not specify the dosage amounts of selenium, acrolein, and placebo administered to the Winstar rats. Lastly, Gökçe et al. (2020) used a sample of male albino rats without proper documentation of why a specific rat breed and gender were preferred.

In yet another study, da Silva Bittencourt et al. (2020) evaluated the protective effects of guarana extracts on acrolein-induced inflammation of the central nervous

system (CNS). da Silva Bittencourt et al. (2020) used a sample of 60-day old Winstar rats, which were pre-treated with guarana extracts (conc. 350mg/kg) and acrolein toxicity induced (conc. 2.5 mg/kg) per day for 42 days. In their findings, da Silva Bittencourt et al. (2020) reported guarana extracts had a significant protective effect against acrolein-induced inflammation of the liver and toxicity in the CNS.

In general, past research contains evidence on the efficacy of several treatment modalities in reversing the inflammatory effects of acrolein toxicity. However, several gaps need to be addressed. First, while acrolein has been proven to cause inflammation of the endothelial cells of the intestines, there is a scarcity of research on existing treatment modalities for reversing acrolein-induced inflammation in the intestines of rats. Past scholars have focused on reversing acrolein toxicity in the liver (da Silva Bittencourt et al., 2020), airway and nasal tract (Wang et al., 2019), and the central nervous system (da Silva Bittencourt et al., 2020; Gökçe et al., 2020). Second, while curcumin has been shown to possess anti-inflammatory properties, no prior study was found that specifically addresses the question of whether the administration of curcumin can reverse acrolein-induced inflammation of the small intestines in rats.

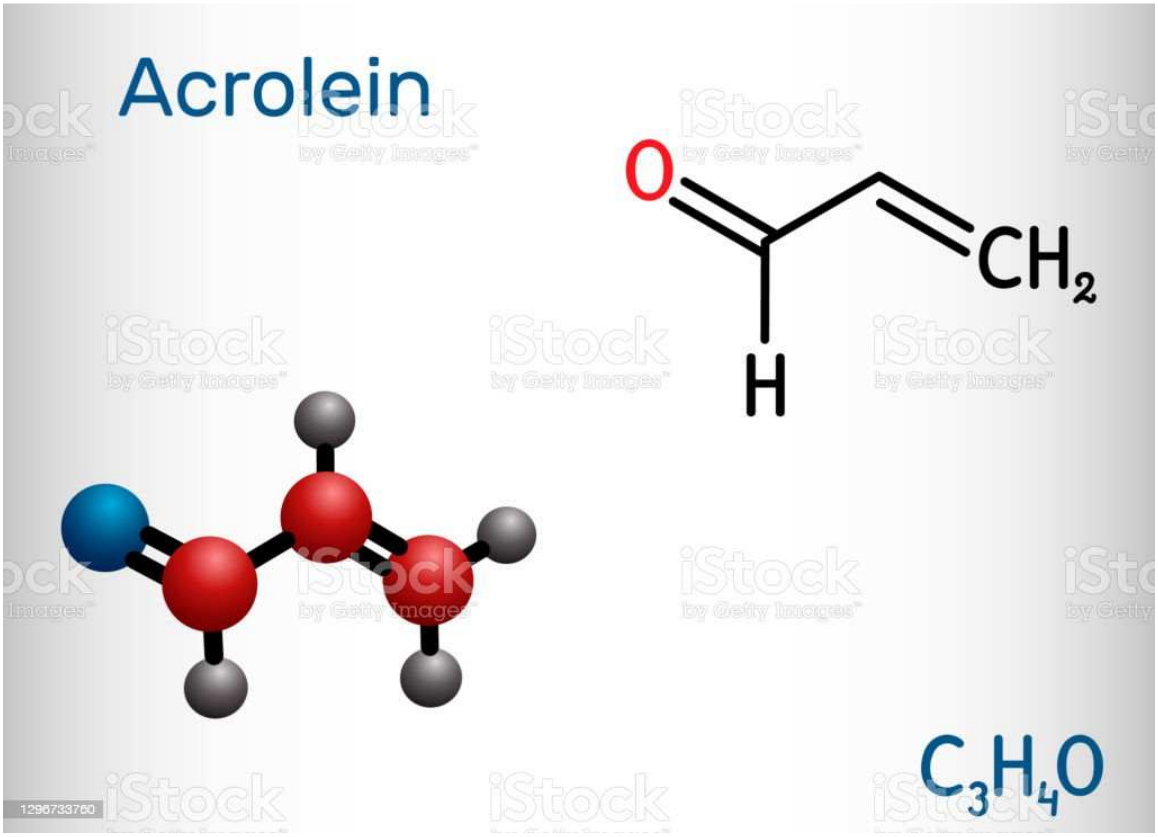

Figure 2: Acrolein chemical structure

https://pubchem.ncbi.nlm.nih.gov/image/imgsrv.fcgi?cid=7847&t=l

Curcumin

Over the years, significant attention has been turned to turmeric following its health benefits and use as a spice. According to Hewlings and Kalman (2017), research on the health benefits of turmeric revealed that it had anti-inflammatory properties. Turmeric (*Curcuma longa)* is a rhizomatous perennial plant of the ginger family that is widely grown in Asia's Southern and South Western regions with a tropical climate (Kocaadam & Sanlier, 2017). As a culinary spice, turmeric is widely used in India, China, Thailand, Iran, and Malesia due to the additional effects on the taste, color, and nature of the prepared food. According to Kocaadam and Sanlier (2017), research on the medicinal benefits of turmeric dates back centuries. Kocaadam and Sanlier (2017) stated that ancient Chinese and Indians used turmeric to alleviate the effects of stress, depression, and dermatologic infections such as skin lesions. However, examining the most current research on the medicinal effects of turmeric revealed turmeric has an active component known as curcumin that gives it its medicinal value.

Curcumin is an orange-yellow, lipophilic polyphenol substance that gives turmeric its medicinal properties. Patel (2021) also defined curcumin as the primary bioactive substance in turmeric with anti-inflammatory, anticancer, and antioxidant properties. According to Hewlings and Kalman (2017), the anticancer, anti-inflammatory, and antioxidant properties of curcumin make the substance ideal for treating various health complications such as cardiovascular diseases, diabetes, auto-immune infections, and neurological diseases. In diet, Ware (2018) showed that curcumin improved digestion and added flavor to food. Ware (2018) reported that turmeric has been incorporated into

Ayurvedic medicine and used as a digestive agent due to curcumin. In addition to the positive health benefits of curcumin, consuming large amounts of turmeric ingredients may lead to short-term stomach complications. Prolonged consumption of curcumin as an ingredient may eventually lead to long-term complications such as peptic and gastric ulcers. While curcumin stimulates the release of gastric acid that may enhance digestive capabilities, it may negatively affect some people by exacerbating the risk of developing gastric ulcers.

According to Hewlings and Kalman (2017), while curcumin has some desirable effects, including reducing inflammations, countering the effects of cancer by modulating cell proliferation, and reducing oxidative stress via its antioxidant properties, it also has several drawbacks. One of the drawbacks of curcumin is that it is poorly soluble in water, a property that significantly limits its bioavailability (Hewlings & Kalman, 2017). Similar findings were reported by Higdon and Delage (2021), who, after evaluating different clinical trials, concluded that the systemic bioavailability of curcumin administered orally was low. Examining curcumin concentration in the plasma or serum, Higdon and Delage (2021) found that curcumin metabolites and derivatives were found to be in high concentration than curcumin itself. The low concentration of curcumin compared to its metabolites and derivatives explains its slow absorption and limited bioavailability. Apart from poor solubility and subsequent slow absorption, Hewlings and Kalman (2017) observed that the low bioavailability of curcumin could also be attributed to its rapid metabolism and elimination from the body.

Given its importance and role in medicine, several clinical trials have been conducted to improve the bioavailability of curcumin. Following several studies

conducted in literature, various substances have been reported to significantly increase the bioavailability of curcumin. For instance, Hewlings and Kalman (2017) and Patel (2021), piperine could increase curcumin's bioavailability by more than 2000%. In addition to piperine, other adjuvants used to increase curcumin's bioavailability include nano curcumin, curcumin phospholipid complexes, liposomal curcumin, and structural analogs of curcumin such as turmeric oil (Lopresti, 2018). While these adjuvants have shown tremendous success in improving curcumin's bioavailability, Lopresti (2018) noted that there were limited human clinical trials and as such more studies were still warranted.

In a summary, curcumin is important not only as a food ingredient but also for medicinal purposes. Overwhelming evidence has been presented in past literature on the medicinal properties of curcumin. Notably, curcumin has anti-inflammatory, antioxidant, and anticancer properties that make it ideal for treating different complications such as diabetes, cancer, and cardiovascular diseases. However, curcumin has poor solubility in water, a property that reduces its bioavailability considerably. More research should be conducted on how to improve the bioavailability of curcumin to improve its usage for medicinal purposes.

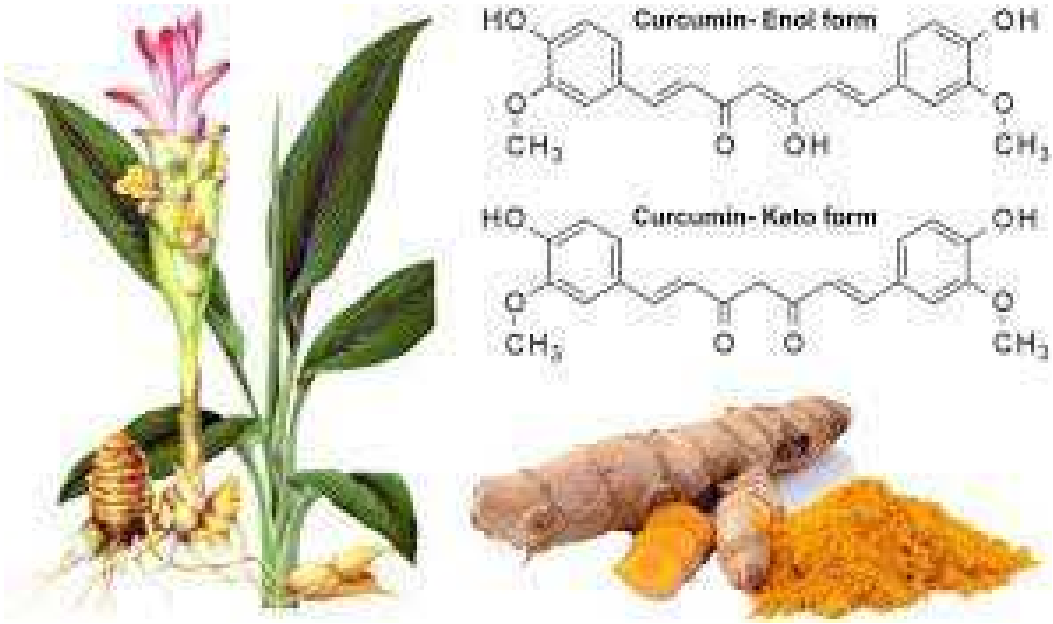

Figure 3: Curcumin

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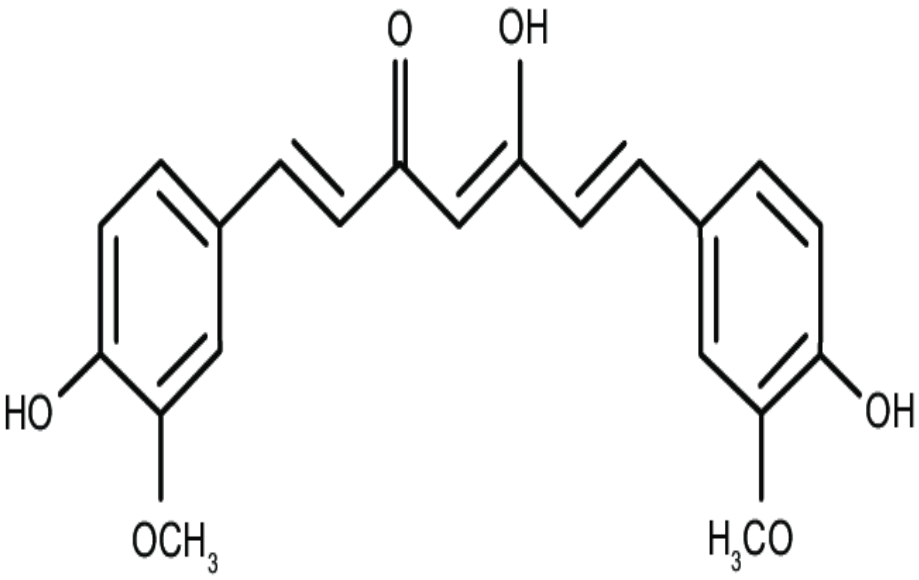

Figure 4: Curcumin chemical structure

https://www.researchgate.net/profile/Pasupathi-Sundharamoorthy/publication/264246601/figure/fig1/AS:601624816926731@1520449942338/chemical-structure-of-curcumin.png

Mechanism of Action

As discussed in the previous section on curcumin, past literature presents overwhelming evidence on the medicinal value of curcumin, particularly in terms of its antioxidant, anticancer, and anti-inflammatory properties (Higdon & Delage, 2021). While in the previous section, we focused on general evidence on the medicinal value of curcumin, this section is dedicated to a description of the chemical properties and mechanism of action of curcumin that brings about its antioxidant, anti-inflammatory, and anticancer properties.

First, on curcumin's antioxidant properties, Hewlings and Kalman (2017) described curcumin as an active and effective scavenger of reactive nitrogen species and reactive oxygen species (ROS). The scavenging capability of curcumin is enhanced by its ability to inhibit the expression of ROS-generating enzymes such as xanthine

hydrogenase/oxidase, cyclooxygenase, and lipoxygenase (Hewlings & Kalman, 2017). Curcumin also regulates the catalase, glutathione, and superoxide dismutase activities that are highly effective in neutralizing free radicals (Hewlings & Kalman, 2017; Razavi & Hosseinzadeh, 2020).

In addition to directly scavenging free radicals, Higdon and Delage (2021) asserted that curcumin activates the release and expression of other antioxidant enzymes such as glutamate-cysteine ligase (GCL). Glutamate-cysteine ligase (GCL) is the first and rate-limiting enzyme in the biosynthesis of glutathione. GCL catalyzes the reaction between cysteine and glutamate to form gamma-glutamylcysteine. According to Higdon and Delage (2021), curcumin elevates the expression of GCL by activating the nuclear factor E2-related factor 2 dependent pathway, which also elevates the levels of other detoxifying agents. Additionally, due to its lipophilic characteristics, curcumin effectively scavenges the peroxyl radicals such as vitamin E (Hewlings & Kalman, 2017). Although the anti-oxidation properties of curcumin out way the drawbacks, Higdon and Delage (2021) asserted, it was yet unclear how curcumin acted as a direct antioxidant in vivo. According to Dei Cas and Ghidoni (2019), curcumin's poor bioavailability in humans limits its concentration in the plasma and serum; however, orally administering curcumin increased its concentration in the gastrointestinal tract, which in turn enhanced its protective capabilities of the mucosal wall in the gut.

In a random collection of studies to further elucidate the antioxidant properties of curcumin, Jagetia and Rajanikant (2015) investigated whether curcumin stimulated antioxidant reactions in mouse skin exposed to fractionated gamma irradiation. According to Jagetia and Rajanikant (2015), fractionated irradiation is an important

radiotherapy treatment for neoplasia. However, continued and prolonged exposure to high levels of radiation impacts negatively on the skin and may have adverse effects on the skins' metabolic and molecular status. Treating the mouse skin with curcumin before exposing it to fractionated radiation activated the skin's anti-oxidation activities, such as increasing the concentration of glutathione, superoxide dismutase, and glutathione peroxidase (Jagetia & Rajanikant, 2015). In another study, Shabeeb et al. (2020) investigated the protective effects of curcumin on the damaged by radiotherapy-induced oxidation. Examining data from four rats, Shabeeb et al. (2020) reported that radiotherapy-induced oxidative damage elevated malondialdehyde levels (MDA) while at the same time depleting the levels of sodium dismutase, catalase, and glutathione peroxidase. However, when curcumin was administered to the rats, Shabeeb et al. (2020) reported that curcumin elevated the levels of SOD, CAT, and DSH-Px, restoring the oxidative capacity of the skin. Adding to the results posted by Shabeeb et al. (2020), Jagetia and Rajanikant (2015) found curcumin to reduce the activities of the enzymes that facilitated lipid peroxidation, ensuring the antioxidant process to continue un-interfered. In general, curcumin interferes with the oxidation mechanism of radiations by elevating the levels of enzymes that neutralize free radicals.

In another study, Goo et al. (2020), when studying the anti-apoptotic, anti-inflammatory, and antioxidant properties of curcumin on acrylamide-induced rats, reported that curcumin increased glutathione levels, superoxide dismutase (SOD), and Glutathione-Px activities in the brain. Consequently, curcumin reduced the concentration and expression of TNF-alpha, beta interleukin-1, and Malondialdehyde (MDA). Jakubczyk et al. (2020), in the meta-analysis of randomized control trials, established that

curcumin reduced the concentration of MDA and increased the antioxidant capacity of curcumin ($p=0.0045$). Reactive oxygen species and reactive nitrogen species, when left unchecked, result in chronic health problems such as inflammatory diseases and hence the need for antioxidants such as curcumin to counter their negative effects, as shown in the above discussion.

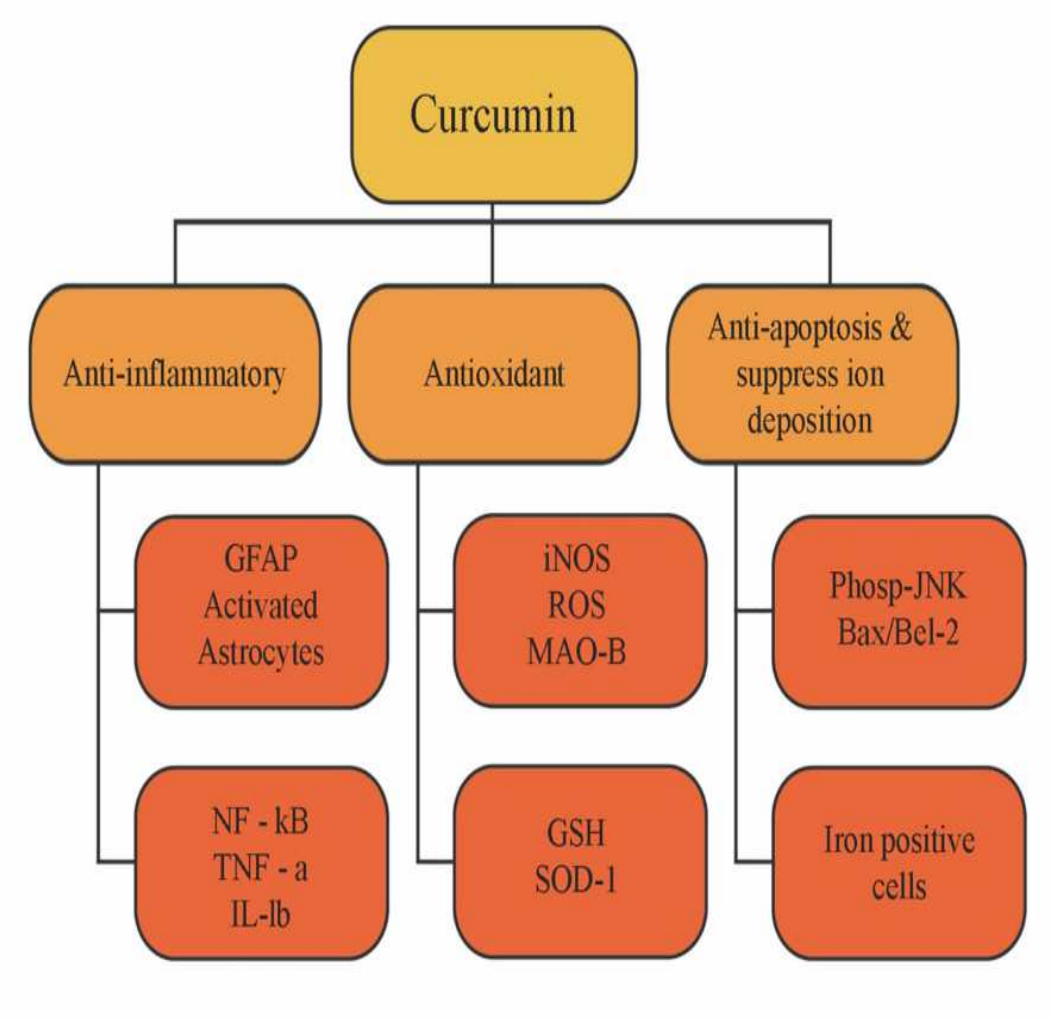

Figure 5: Mechanism of action of curcumin.

https://www.google.com/url

Protective Effects against Induced Toxicity

Previously, the researcher discussed the health benefits of curcumin which were grouped into antioxidant, anticancer, and anti-inflammatory activities; according to Higdon and Delage (2021, and Hewlings and Kalman (2017), curcumin activated signaling pathways and enzymes such as GSH and SOD to scavenge free radicals that increase oxidative stress and inflammatory reaction. In this section, the researcher will examine the protective effects of curcumin on induced toxicity.

Cho et al. (2020) investigated the protective mechanism of curcumin against inflammation and apoptosis induced by lipopolysaccharides in the human neutrophil. In their research, Cho et al. (2020) incubated isolated neutrophil cells with and without lipopolysaccharides (LPS). They used enzyme-linked immunosorbent assays to assess pro-inflammatory cytokines such as tumor necrosis factor-alpha, interleukin-6, and interleukin-8, Western blot analysis to assess the expression of mitogen-activated protein kinase and fluorescence-activated cell sorting to assess the apoptosis of neutrophil cells. At the end of the study, Cho et al. (2020) found that curcumin diminished the expression of interleukin-8, interleukin-6, and tumor necrosis factor-alpha.

Similar findings were reported by Ganji et al. (2021), who investigated the protective effects of curcumin on the toxicity induced by lipopolysaccharides. In an extensive literature review, Ganji et al. (2021) established that curcumin exerted its anti-inflammatory properties that interfered with the inflammatory pathways activated by LPS. Ganji et al. (2021) showed that curcumin inhibited the expression of cytokines, interleukins, and the NF-kB inflammatory pathway. However, Ganji relied on a systematic review of literature findings, which were quite generic.

Shaterpour et al. (2017) investigated the protective effects of curcumin on lithium-induced nephrotoxicity in rats. According to Shaterpour et al. (2017), excessive application of lithium salts results in serious kidney infections such as renal tubular acidosis, natriuresis, and tubulointerstitial nephritis. In the experimental study, Shaterpour et al. (2017) found that while there were limited studies on lithium-induced nephrotoxicity, curcumin increased BUN, creatinine, and Malondialdehyde concentration that countered the effects of lithium in the kidneys. In a similar study, Farkhondeh et al. (2018) studied how curcumin protected renal failure induced by toxic agents. While limited clinical studies are evidencing the protective effects of curcumin against toxin-induced renal failure, Farkhondeh et al. (2018) found that curcumin was effective against toxic nephrotoxic agents such as garlic cisplatin, lead, cadmium, nicotine, and methotrexate. Garlic, a nephrotoxic agent, induces its toxic effects by limiting the activity of antioxidant enzymes. Therefore, to prevent kidney damage due to garlic agents, Farkhondeh et al. (2018) observed that curcumin modulated the oxidant-antioxidant system. Moreover, on methotrexate and Malathion, curcumin protected the kidneys against injury by preventing oxidative stress and increasing antioxidant activities.

Huang et al. (2018) examined whether curcumin was effective against cyclosporine A-induced rat nephrotoxicity. The objective of their study was to establish whether curcumin mitigated renal oxidative stress as a result of CsA. A closer examination of renal cells exposed to CsA recorded a significant increase in reactive oxygen species and Malondialdehyde, decreased levels and activities of sodium dismutase, catalase, and glutathione peroxidase. However, treating the HK-2 cells with curcumin decreased the levels of ROS and MDA by increasing the levels of GSH-Px,

catalase, and SOD. Other effects of curcumin in the HK-2 renal cells were elevated serum creatinine levels and blood urea nitrogen and decreased creatinine clearance rate. In protecting the kidneys against toxic agents mentioned above, curcumin was found to block enzymes and pathways that increased oxidative stress in the kidneys. At the same time, curcumin being an antioxidant agent, served to ensure a balance in the oxidant-antioxidant system to prevent renal injury and damage.

In addition to protecting against renal failure, some scholars have investigated the role of curcumin in protecting against liver toxicity. In one such study, Otuechere et al. (2014) investigated how curcumin protected the liver against propanil-induced toxicity in rats. Male Wistar rats were divided into four groups designated as control, propanil group treated with 20mg /kg, curcumin (50mg/kg) + propanil group (20mg/kg), and curcumin only group (50mg/kg). After 28 days, the Wistar male rats treated with propanil showed a significant increase in the activities of plasma aspartate aminotransferase and alkaline phosphatase. Additionally, there were increased lipid peroxidation and increased levels of serum enzymes and glutathione. In Wistar rats treated with curcumin and propanol, Otuechere et al. (2014) found that curcumin countered the effects of propanil intoxication by decreasing serum enzyme-plasma levels aspartate aminotransferase and alkaline phosphatase. Decreasing the activity of these serum enzymes reduced lipid peroxidation and decreased oxidative stress due to increased glutathione levels.

In a similar study, Youssef et al. (2019) reported that continued oral administration of lithium carbonate for one month decreased the levels of enzymes catalase, glutathione, and sodium dismutase. Additionally, lithium carbonate increased the action of glutathione peroxidase and the thickening of veins found in liver cells.

Equally, Youssef et al. (2019) found that lithium carbonate elevated observed aberrations in the structure of chromosomes in addition to influencing the numerical aberration of chromosomes, including polyploidy, hypoploidy and hyperploid. Treating the liver cells with curcumin improved the hepatic structure by decreasing the vacuolar deposition degeneration on the vacuolar walls and diminished the observed chromosomal aberrations.

Additional evidence on curcumin's effectiveness in protecting against induced toxicity was observed in the study conducted by Sudjarwo et al. (2017). Sudjarwo et al. (2020) studied the protective effects of curcumin on lead acetate-induced testicular toxicity in Wistar rats. The corresponding results showed that lead acetate decreased Sodium superoxide and glutathione peroxidase levels but increased the levels of Malondialdehyde. Lead acetate also decreased sperm count, viability, and motility and altered the histopathology of Wistar rat testis.

Mohammed et al. (2020) investigated the protective effects of curcumin nanoparticles against cardiotoxicity induced by doxorubicin in rats. Mohammed et al. (2020) observed that treating rats exposed to doxorubicin with curcumin prevented oxidative stress and restored the activities of AchE and LDH diminished by doxorubicin. Correspondingly, curcumin nanoparticles prevented the decline of ATPase activity, sodium, and potassium. Mohajeri and Sahebkar (2018) reiterated that curcumin countered the toxic effects of doxorubicin by activating phase II enzymes such as glutathione S-transferase and glutathione peroxidase. Sijadrwo et al. (2017) observed the antioxidant activity of curcumin alleviated the oxidative stress due to decreased SOD, CAT, and GPx levels and increased levels of MDA. Both Mohammed et al. (2020) and Sijadrwo et al.

(2017) showed that curcumin effectively protected against the toxic effects of doxorubicin and lead acetate by reducing oxidative stress through increased levels of CAT, SOD, and GPx.

In a fact, past literature provides overwhelming evidence on the protective effects of curcumin against induced toxicity from different compounds. Past scholars reported that curcumin decreased induced toxicity in the kidney (Cho et al., 2020; Shaterpour et al., 2017), liver (Youssef et al., 2019, reproductive systems (Sudjarwo et al., 2017). Additionally, findings from past literature have illustrated the general antioxidant properties of curcumin in the body (Ganji et al., 2020). However, a literature review is quite generic; it focuses on the general antioxidant effects of curcumin in different parts of the body. In the section that follows, the researcher will review more specific literature on curcumin's anti-inflammatory properties and its effects in reversing inflammation in endothelial cells of intestines in rats.

Anti-inflammatory Effects

The anti-inflammatory effects of curcumin have also been used to describe its mechanisms in biological reactions in the rat model or the human body. According to Hewlings and Kalman (2017), oxidative stress results in unpleasant bodily occurrences, including inflammation. Hewlings and Kalman (2017) and Biswas (2016) discussed that inflammatory cells yielded reactive species that, in turn, resulted in oxidative stress at inflammation sites. To combat the effects of inflammation and inhibit the development of diseases such as Alzheimer, Higdon and Delage (2021) suggested that curcumin blocked inflammatory mediators such as chemokines, cytokines, growth factors, and adhesion molecules. Subsequently, curcumin inhibited the actions of inflammatory enzymes such

as lipoxygenase, cyclooxygenase, and inducible nitric oxide synthase (iNOS). In most inflammatory diseases, Hewlings and Kalman (2017) presented that the tumor necrosis factor (TNF-alpha) was the major inflammatory mediator.

According to Higdon and Delage (2021), the tumor necrosis factor-alpha is an inflammatory cytokine produced by macrophages during inflammation. TNF-alpha activates different types of signaling reactions whose result is cell death. The actions of TNF-alpha are regulated by the activation of nuclear factor (NF-kB). Its inhibition of the NF-kB pathway describes the anti-inflammatory mechanism of curcumin, mitogen-activated protein kinase signaling pathway (MAPK), the Janus Kinase (JAK)/Signal transducer, and activator of transcription (STAT) dependent signaling pathway. In addition to inhibiting the pro-inflammatory pathways mentioned above, Higdon and Delage (2021) showed that the downregulation of p38-MAPK activity, pro-inflammatory cytokine alpha, and myeloperoxidase activities in mice were a result of curcumin inhibition of dextran sulfate sodium. Additionally, curcumin diminished the effects of the immune responses triggered by collagen splenocytes. Correspondingly, curcumin's anti-inflammatory responses were observed in the reduced production of COX-2-induced prostaglandin, TNF-alpha, and beta interleukin-1 (Higdon & Delage, 2021).

In addition to the anti-inflammatory and antioxidant properties, curcumin has also shown some anticancer properties. According to Higdon and Delage (2021), the anticancer activities of curcumin are described based on its effects on the biotransformation enzymes such as cytochrome P450, glutathione synthase, and quinone reductase. However, worth noting, the effects of curcumin on biotransformation enzymes vary depending on the dose of curcumin, type of animal model, and route of

administration. Another important anticancer activity is when curcumin inhibits the proliferation and induction of apoptosis by downregulating the activities of the Nrf2-dependent pathway and inhibiting of NF-kB signaling pathway (Kunnumakkara et al., 2017). Curcumin has also been found to inhibit tumor invasion and angiogenesis by downregulating E-cadherin and Y-cadherin proteins. Additionally, the proliferation, migration, and invasiveness of small cell lung cancer were blocked by curcumin, which inhibited interleukin-6, reducing JAK/STAT signaling pathway activation and the vascular endothelial growth factor (Kunnumakkara et al., 2017).

Anti-inflammatory Effects in Intestinal Endothelial Cells of Rats

In the existing literature, researchers have presented evidence on the inflammatory effect of curcumin, particularly in the endothelial cells of small intestines of rats. In one study, Kumar et al. (2010) examined the efficacy of curcumin in treating gastrointestinal disorders caused by inflammation of the endothelial cells of the intestines in mice. In the study, three chemicals were used: curcumin as the main intervention, vehicular fluid as the control, and barium sulfate as an agent for inducing inflammation. The rats were randomly assigned to two groups – A or B. Rats in group A were given a single dose of vehicular fluid. In contrast, rats in group B were administered a single dose of curcumin with a concentration equivalent to 1gram per kilogram of the average rat's body weight. The rats in both groups were then given a dose of Barium Sulphate, which was to induce inflammation. In their findings, Kumar et al. (2010) reported a significant difference in mean length of intestines traversed by Barium Sulphate between rats in group A and rats in group B. Notably, the mean length of intestines traversed by Barium Sulphate for group A rats was significantly longer than mean length traversed for group B

rats. According to Kumar et al. (2010), these findings indicate curcumin is an important anti-inflammatory compound for treating gastrointestinal disorders as it reduces the mobility of induced toxins such as Barium Sulphate.

In another study, Aldini et al. (2012) aimed to assess curcumin's effectiveness in treating inflammatory bowel disease by measuring its effect on intestinal motility. According to Aldini et al. (2012), inflammatory bowel disease is characterized by reduced gastrointestinal motility. In their study, Aldini et al. (2012) used three reagents; Curcuma extracts, Carbachol, and Dextran Sulphate. Aldini et al. (2012) used a sample of 58 Balb/c mice. Chronic and acute inflammation of the mice' isolated intestines was induced using Sodium solution (2.5% concentration) and Dextran Sulphate (5% concentration), respectively. In one group of the Balb/c mice, Curcuma was administered for seven days, while in the second group, a placebo was administered for 21 days. The results of the experiment indicated Curcuma significantly reduced inflammation in the ileum of the Balb/c mice. As such, Aldini et al. (2012) concluded that Curcuma extract has a myorelaxant effect on the ileum of mice, which reduced the tendency of gastrointestinal inflammation.

Zeng et al. (2013) conducted a study to examine whether curcumin effectively treated inflammation of the colon in rats. Inflammation was induced in rats using 2, 4, 6-trinitrobenzene acid. The rats were then randomly assigned to two groups – group A and group B. Rats in group A were treated with curcumin. In contrast, those in group B were treated with a placebo (sulfasalazine) once a day for a total of seven days. The inflammatory response was measured in terms of the extent of mucosa damage inflammatory activity index and histological score. Zeng et al. (2013) also measured the

expression of enzymes NF-kB and TLR4, responsible for signaling inflammatory response, which was detected using the Western blot. As per the findings obtained, there was a significant reduction in inflammatory activity index, histological score, and extent of mucosa damage in the rats treated with curcumin ($p < 0.05$). On the contrary, there was no significant change in inflammatory activity index, histological score, and extent of mucosa damage in the rats treated with the placebo ($p > 0.05$). However, Zeng et al. (2013) found no significant differences in the expression of TLR4 and NF-kB enzymes responsible for triggering inflammatory response pathways.

Bastaki et al. (2016) used a sample of male Winstar rats to evaluate the efficacy of curcumin in reducing toxicity-related inflammatory bowel disease. Bastaki et al. (2016) induced inflammation in the Wintar rats using acetic acid (1 ml, 4% concentration) via anal administration. Bastaki et al. (2016) then administered Curcuma powder (conc. 100mg/kg) every day for three consecutive days to the intervention group. The control group was administered with a placebo. The researchers then measured glutathione levels, cytokine IL-23, and myeloperoxidase in both intervention and control samples. A significant reduction in acetic acid-induced inflammation in the rats administered with Curcuma longa ($p < 0.05$). Additionally, rats administered with Curcuma longa showed a significant reduction in average colon ulcer score ($p < 0.05$). As such, the findings from the study by Bastaki et al. (2016) showed that treatment of Inflammatory Bowel Disease (IBD) with Curcuma longa might significantly reduce gastrointestinal inflammation and tendency of ulcer development.

Yao et al. (2021) conducted a study to determine whether curcumin effectively reduced inflammation-related diarrhea and constipation. The study's sample included

Sprague-Dawley rats, which were initially treated with intracolonic acetic acid (conc. 4%) to induce gastrointestinal inflammation. Yao et al. (2021) obtained blood samples from the rats, which were used to establish the expression levels of different cytokines necessary for triggering an inflammatory response in the gut; IL-IB and TNF. Notably, Enzyme-linked immunosorbent assay (ELISA) kits were used to measure the serum levels of the two cytokines. In their findings, Yao et al. (2021) reported that induction of curcumin significantly reduced the serum expression levels of the pro-inflammatory trigger cytokines (TNF-alpha and IL-IB). Additionally, the findings indicated that induction of curcumin was negatively associated with myosin light-chain (MLC) hence significantly reducing diarrhea and constipation.

The literature reviewed in this section provides overwhelming evidence that curcumin has anti-inflammatory properties. In essence, evidence indicates that curcumin is capable of reversing inflammation induced by different toxic compounds such as acetic acid (Bastaki et al., 2016; Yao et al., 2021), barium sulfate (Kumar et al., 2010), Carbachol (Aldini et al., 2012), and trinitrobenzene acid (Zeng et al., 2013). While acrolein also induces inflammation in different parts of the body, there is limited research on the impact of curcumin on acrolein-induced inflammation. In the section that follows, a review of literature on the effectiveness of curcumin in reversing acrolein-induced inflammation.

Curcumin and Acrolein Toxicity

Under the subsection acrolein toxicity, the researcher showed the negative health effects of exposure to acrolein; according to Bein and Leikauf (2020), exposure to high levels of acrolein results in severe skin irritations stinging eyes, which can lead to eye damage. Further, exposure to acrolein through breathing irritates the nose, throat, and lungs. To the lungs, Snow et al. (2017) contended, exposure to acrolein irritated the lungs causing shortness of breath and coughing. Snow et al. (2017) further posited, heavy exposure to acrolein led to pulmonary edema (building up of fluid in the lungs) that explains the experiences of shortness of breath. In addition to irritation and pulmonary edema, Snow et al. (2017) found that acrolein exposure may result in mutation. Yeager et al. (2016) examined the mechanism of action of acrolein toxicity to describe the occurrence of the above effects. Yeager et al. (2016) proposed different ways through which acrolein toxicity occurred using the respiratory system. First, Yeager et al. (2016) suggested that acrolein chemically reacted with the macromolecules and proteins lining the respiratory tract cells. The second mechanism of action is through cellular oxidative stress, including the effect on the oxidant-antioxidant system; third, it induces chronic inflammation and initiates necrotic cell death. According to Yeager et al. (2016), other mechanisms occur through tissue remodeling and loss of membrane credibility through the release of reactive oxygen and nitrogen species.

Among the key effects of acrolein was that it might induce cell mutation. Gupta et al. (2021) discussed that acrolein was electrophilic and unsaturated. It easily reacted with the thiol, imino, and hydroxy groups of DNA, amino acids, and proteins, altering their normal structure exposing the proteins and DNA to mutations. Tsai et al. (2021)

established that being an oncogenic agent, acrolein induced rapid cell proliferation and cell cycling and cell migration in NIH/3T3 cells. Tsai et al. (2021) further presented that acrolein activated the RAS/MAPK pathway that contributed significantly to the development of colon cancer.

Furthermore, acrolein was found to form adducts with DNA, with the adduct Acr-dG being high in CRC tumor cells. Building on the findings of Yeager et al. (2016), Wang et al. (2017) found acrolein to be responsible for the damages observed in mitochondrial DNA. According to Wang et al. (2017), being a strong electrophile, acrolein reacted with the nucleophilic reactive groups of guanosines to yield the mutagen cyclic propano-deoxyguanosine (PdG) adduct. Similarly, acrolein reaction with histidine, cysteine, and lysine residues of proteins has inactivated the proteins. Additionally, acrolein reacts with DNA repair proteins, predisposing damaged DNA to mutations. Overall, inhibition of mitochondrial bioenergetics resulting in mitochondrial fission followed by mitophagy and acrolein-induced DNA damages that might trigger mitochondrion apoptosis.

Following the above effects of acrolein toxicity, the antioxidant, anticancer, and anti-inflammatory effects of curcumin have been investigated in various experimental studies to reiterate its effectiveness in alleviating the toxic effects of acrolein. In one such study, Shafie et al. (2019) compared the effects of nanocurcumin and curcumin on acrolein-induced mitochondrial toxicity from the liver of a rat. Similar to Tsai et al. (2017), Shafie et al. (2019) asserted that acrolein reduced the concentration of intracellular glutathione and its antioxidant capacity, crippling mitochondrial function. Shafie et al. (2019) used male Wistar rats weighing between 180-200mg to accomplish

their study. The liver obtained from the male Wistar rats was treated with different concentrations of curcumin and nano curcumin, and then the liver was exposed to very toxic levels of acrolein. Mitochondrial viability was evaluated and data analyzed with a one-way ANOVA test. Analyzing the obtained results, Shafie et al. (2019) found that even though curcumin and Nanocurcumin protected the mitochondria from the adverse effects of acrolein, the protection was not solid. According to Shafie et al. (2019), more clinical studies were still needed to examine curcumin's protective effects in vivo effectively.

Jo et al. (2019) described acrolein as a global pollutant with adverse effects when inhaled through automobile dust or cigarette smoke. According to Jo et al. (2019), inhalation of acrolein is associated with chronic obstructive pulmonary disease development. Exposure and inhalation of acrolein activate enzymes and metabolic reactions that generate reactive oxygen species that react with pulmonary cells, resulting in their untimely breakdown if uncontrolled. In their study, Jo et al. (2019) sought to examine the amelioration of acrolein-induced pulmonary injury by investigating how curcumin inhibited both intrinsic and extrinsic apoptosis signaling pathways. With its strong antioxidant properties, Jo et al. (2019) reported that curcumin suppressed intrinsic and extrinsic apoptosis signaling pathways by modulating p53 and MAPK signaling cascade activities. Jiang et al.(2021) presented that in addition to modulating the p53 and MAPK signaling cascades in pulmonary injury caused by acrolein, curcumin formed an adduct with acrolein inactivating its ability to generate reactive oxygen species.

Doggui et al. (2013) conducted a study to investigate how curcumin protected the neurons from the toxic effects of acrolein and reported curcumin's mechanism of action.

Doggui et al. (2013) treated human neuroblastoma cells SK-N-<SH with acrolein and five micromolar of curcumin. Treating the human neuroblastoma cells with acrolein induced the secretion and activities of NF-kB, Sirt1, and Nrf2 (Doggui et al., 2013). Elevated NF-kB, Srt1, and Nrf2 resulted in oxidative stress in the neuroblastoma cells with subsequent production of reactive oxygen/nitrogen species. Uncontrolled ROS and RN's reactions expose the cells to adverse conditions resulting from their disintegration or death. To counter the effects of acrolein-induced cell apoptosis due to ROS activity, Doggui et al. (2013) presented that treating the neuroblastoma cells with curcumin downregulated the production and activity of NF-kB, Nrf2, and Srt1. Curcumin further activated the phosphorylation AKt which enhanced its antioxidant activity. Although curcumin has been shown to counter the effects of acrolein, Doggui et al. (2013) argued that its poor bioavailability hinders its effectiveness when orally delivered.

Shi et al. (2018) investigated how acrolein-induced neurotoxicity in HT22 mouse hippocampal cells was attenuated by curcumin. In the study, Shi et al. (2018) treated the HT22 murine hippocampal neural cells with 25 micromolar acrolein. Some of the HT22 cells were pre-treated with a 5micromolar of curcumin, while others were not treated with curcumin. For data analysis, the scholars measured the levels of glutathione, SOD, and MDA by GSH assay kit, while cell apoptosis and viability were measured using the CCK8 assay and cytometric analysis. Analyzing the obtained data, Shi et al. (2018) found that curcumin attenuated acrolein-induced oxidative stress following the downregulation of SOD, MDA, and CAT. Saghiri et al. (2020) investigated the effects of cyclophosphamide-induced lung injury in rats found that cyclophosphamide resulted in

severe inflammatory edema, significant increases of MDA, and protein carbonyl (PCO) and caspase-3.

Additionally, exposed rats to CP were also reported to have enlarged blood vessels and obstructed alveolar. However, treating the CP-toxicated rats with curcumin reversed the effects of inflammatory edema, increased SOD and GPx, and decreased the levels of caspase-3 and MDA and PCO. Increased levels of SOD and GPx mitigated the oxidative stress protecting the cell's oxidative damage and subsequent apoptosis due to high levels of ROS.

Curcumin and Acrolein-induced Inflammation

Curcumin is a strong anti-inflammatory agent. As an anti-inflammatory agent, curcumin targeted and reduced elevated levels of inflammatory factors and enzymes in addition to inhibiting inflammatory signaling pathways (Boarescu et al., 2019). The reactive oxygen species generated due to exposure of rat or human cells to acrolein (Jo et al., 2019) have some inflammatory activities on the cell countered by treating the cells with curcumin. Lee et al. (2020) investigated whether curcumin attenuates acrolein-induced COX-2 expression and production of prostaglandin in endothelial cells of the human umbilical vein. Acrolein activates and rapidly facilitates inflammatory responses by increasing the production and concentration of inflammatory enzymes such as cyclooxygenase -2 (COX-2). The aim of the study conducted by Lee et al. (2020) was to investigate the anti-inflammatory effects of curcumin on the inflammation due to acrolein on HUVECs. Analyzing the results on reactive oxygen species, induction proteins, mRNA, and prostaglandins, Lee et al. (2020) established that curcumin countered the inflammatory responses of COX-2 and inhibited the production of prostaglandin due to

acrolein. In this particular study, Lee et al. (2020) described that curcumin attenuated acrolein-induced inflammation by inhibiting the synthesis of adhesion molecules that inactivated the phosphorylation of p38 mitogen-activated protein kinase, protein kinase C and cAMP.

Lewis (2021) carried out a study similar to that of Lee et al. (2020); however, in their study, Lewis (2021) examined the effects of curcumin on inflammation induced by inhalation of woodsmoke. Woodsmoke from the burning of wood and plastics form some of the sources of acrolein. According to Lewis (2021), inflammation from woodsmoke affects many people, given that more than 3billion people rely on biomass as their main source of heating. Long-term exposure to wood smoke in an enclosed environment has been associated with developing chronic upper respiratory infections, asthma, lung cancer, and an increased risk of developing heart disease. As an initiator of inflammation, woodsmoke activates the nuclear factor kappa beta pathway (NFkB) and toll-like receptor (TLR) pathways. Activation of NFkB and TRL pathways results in the buildup of oxidative stress, which enhances lipid peroxidation, expression of pro-inflammatory genes, and damages to the DNA and the mitochondrial. Acrolein-induced inflammation is associated with the activation of apoptosis enzymes and the development of chronic health conditions such as emphysema. To counter the effects of woodsmoke-induced inflammation, Lewis (2021) described curcumin down-regulated the action of NFkB and TRL4 signaling pathways by blocking the action of the AKT signaling pathway.

Furthermore, curcumin signaled the release of the peroxisome proliferator-activated receptor-gamma pathway that induced the production of anti-inflammatory cytokines (Shimizu et al., 2019). Lewis (2021) further described that curcumin decreased

the genetic expression of COX-2 and MIP-2. However, the point of contention in the study conducted by Lewis (2018) was that neither woodsmoke nor curcumin affected the release of TNF-alpha and MIP-2 cytokines. As a result, more empirical studies are needed to explore that point of deviation further.

In another study, Zhou et al. (2020) examined the efficacy of curcumin in protecting neuronal cells against acrolein-induced inflammation and toxicity. In their study, Zhou et al. (2020) used a sample of human neuroblastoma cells. To induce toxicity and subsequent inflammation, the researchers treated the human cells with acrolein. Zhou et al. (2020) the measured acrolein-induced activity of Sirt1, Nrf2, and NF-kB cytokines before introducing curcumin to the neuroblastoma cells. The findings revealed that the introduction of curcumin significantly reduced the activity of the three cytokines known to induce inflammation. Apart from reversing the activity of Nrf2, NF-κB, and Sirt1, the introduction of curcumin in the cells also increased the expression of pAkt, which had been decreased by acrolein to initiate an inflammatory response. However, Zhou et al. (2020) acknowledged that curcumin has low solubility in water, reducing biodegradability. To increase solubility and subsequently biodegradability, Zhou et al. (2020) converted Curcuma longa powder into a capsule where the coating was a biodegradable substance (nanoparticles of lactide-co-glycolide). Zhou et al. (2020) further clarified that curcumin could only reverse the inflammatory response induced by acrolein when used as a capsule. Free curcumin did not induce any neuroprotection against the effects of acrolein.

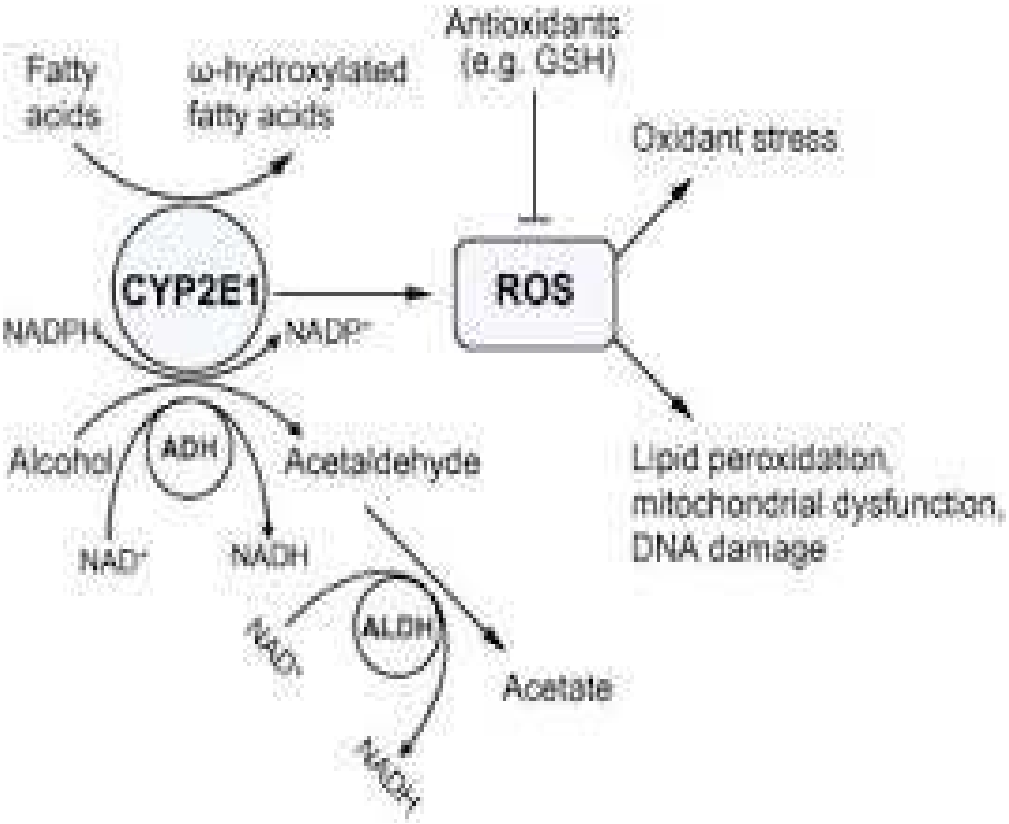

Figure 6: Synthesis of Glutathione

CYP2E1 and oxidant stress in alcoholic and non-alcoholic fatty liver disease, 2013
Nitric Oxide-Mediated Protection of Endothelial Cells From Hydrogen Peroxide Is Mediated by Intracellular Zinc and Glutathione, 2019

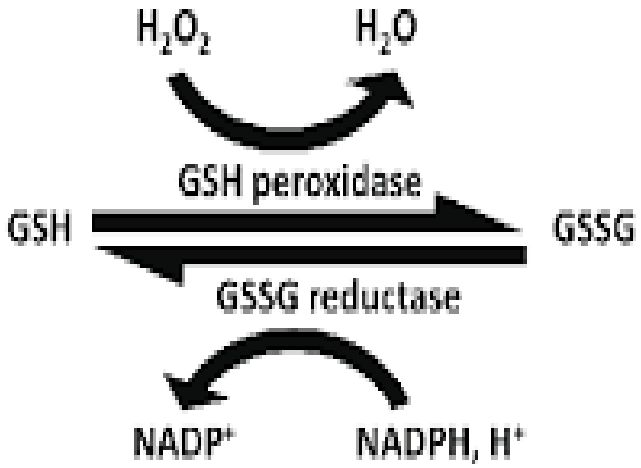

Figure :7 Oxidation/reduction pathway of reduced glutathione GSH

https://www.researchgate.net/publication/341060232/figure/fig1/AS:886193176731649@1588296326869/Oxidation-reduction-pathway-of-reduced-glutathione-GSH-and-oxidized-glutathione-GSSG.png

Research Study Objectives

- Evaluate acrolein effects in the small intestine; Method - Measuring lipid peroxidation and antioxidant activities.
- Compare curcumin and compound20 effects in reversing/inhibiting the effects of acrolein in the small intestine. Method - Measuring lipid peroxidation and antioxidative activities.
- Determine the mechanism that compound 20 reverse/inhibit the changes caused by acrolein in the small intestine. Method - Cytokines activities and Nrf2 activity and expression.

CHAPTER 3

METHOD and MATERIALS

- Animal Treatment

Male Sprague-Dawley rats, (180-280 grams) from Harlan Sprague-Dawley, Houston, TX were used for this research study. The animals were randomly divided into groups(n=5) of control and treatment. The treatment group received acrolein dose of 2mg/kg/day via intraperitoneal for 10 days and others were treated as follows: Curcumin(100mg/kg/day), Compound20(10mg/kg/day), Acrolein(2mg/kg/day) + Curcumin(100mg/kg/day) and Acrolein(2mg/kg/day) + Compound 20(10mg/kg/day), via voluntary consumption. The curcumin and compound 20 were mixed in 8g/kg/day of peanut butter. The control group received an equal volume of PBS and consumed peanut butter voluntary for the same duration of 10 days. The animals were housed in cages individually, under standard conditions (room temperature: 24–26°C; 12/12-h light/dark cycle). The animals had free access to food and water except during treatment periods daily. The rats were acclimatized for 7 days prior to the study. The Texas Southern University Animal Care and Usage Committee approved this research work and NIH guidelines were followed for the Care and Use of Laboratory Animals (NIH publication No. 93-23, revised 1985) and the Animal Welfare Act. The animals were anesthetized with carbon dioxide. At the end of the treatment period, and the animals were disserted, and the small intestines were removed, and cleaned with PBS to remove surrounding fat gently. The cleaned small

intestines were either stored at -80°C for biochemical analysis or -20°C before homogenization. Also, blood samples were collected via cardiac puncture on the day of animal sacrifice and plasma was separated and stored at -80°C until analysis.

Group 1 (Control): The control group(n=5) received the 1% PBS as the vehicle, and voluntary consumed once daily, for 10 days.

Group 2 (Acrolein -Treated): In this group(n=5), rats were treated with Acrolein 2mg/kg per day in PBS after 3-4 hours of fasting by intraperitoneal once daily, for 10 days.

Groups 3 (Curcumin – Treated): In this group(n=5), Cu was mixed in peanut butter and administered by voluntary consumption after 3-4 hours of fasting once daily, for 10 days.

Groups 4 (Compound 20 – Treated): In this group(n=5), Compound20 was mixed in peanut butter and administered by voluntary consumption after 3-4 hours of fasting once daily, for 10 days.

Groups 5 (Curcumin + Acrolein – Treated): In this group(n=5), after 3-4 hours of fasting, Cu was mixed in peanut butter and administered by voluntary consumption once daily while, Acrolein 2mg/kg per day in PBS by intraperitoneal once daily, for 10 days.

Groups 6 (Compound 20 + Acrolein – Treated): In this group(n=5), after 3-4 hours of fasting, Compound 20 was mixed in peanut butter and administered by voluntary consumption once daily while, Acrolein 2mg/kg per day in PBS by intraperitoneal once daily, for 10 days.

	ANIMAL WEIGHTS									
GROUPS	Prior treatment weight(g)					End treatment weight(g)				
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5
Control(C)	221	225	215	225	204	278	263	264	284	273
Acrolein(A)	211	233	211	221	238	182	251	236	209	240
Curcumin (Cu)	216	217	206	225	207	271	270	223	254	267
Compound20(C20)	194	225	223	204	205	264	242	259	240	242
A + Cu	214	183	223	203	204	238	248	265	243	238
A + C20	186	183	215	208	209	227	211	234	234	219

Table 1; Animal weights

- Chemicals

Acrolein was obtained from Sigma-Aldrich Chemical Company. Curcumin was obtained from Alfa Aesar, a Johnson Malthey Company. Rat IL-10 ELISA kit, TNF alpha Rat ELISA, GST Kit, GSH+GSSG Kit, Lipid Peroxidation Kit and SOD Kit were obtained from Abcam. The complete protease inhibitor cocktail was provided from OSVE Pharmaceutical Co. (Tehran, Iran). Sodium dodecyl sulphate (SDS), MDA, thiobarbituric acid (TBA), Tetramethylethylenediamine (TEMED), and β-Mercaptoethanol (β-ME) were provided from Merck. Goat pAb to Ms IgG, Glutathione, IL-10, and TNF were obtained from Abcam.

- Protein content concentration

Prepare the standard solution as follows.

Vial	Vol. of Diluent - dH2O (ul)	Vol. of BSA (ul)	Final BSA concentration
A	0	300 of stock	2000
B	125	375 of stock	1500
C	325	325 of stock	1000
D	175	175 from vial B	750
E	325	325 from vial C	500
F	325	325 from vial E	250
G	325	325 from vial F	125
H	400	100 from vial G	25
I	400	0	0

Table 2: BSA standard for protein concentration

Reagent preparation

10ml of Reagent A was added to 200ul Reagent B for total of 10.2ml.

Procedure

25ul of samples was added to each well in duplicate. Also, 25ul of standard solution was added into their wells in duplicate same as the samples. 200ul of reagent mixture was added to all well plates. Incubate for 30mins at room temperature 37°C. Plate was read on a plate reader at OD 562nm.

- Lipid Peroxidation

For measurement of MDA the marker of lipid peroxidation, the small intestine was homogenized in MDA kit dilution buffer. The homogenate was centrifuged at 3000×g for 10 min, and the obtained supernatant was used as total small intestine homogenized sample. The protein levels and MDA were both measured from the supernatants. The protein content was evaluated using Bradford Protein Assay kit (Bio-Rad Laboratories) and Bovine serum albumin (BSA) as standard. According to the instruction of manufacture, 25 μL of sample supernatants or BSA standards were pipetted into separated microtiter plate wells on a 96 well plate, and then 200 μL diluted dye reagent (containing 1 part Dye Reagent Concentrate with 4 parts deionized water) was added to each well and mixed thoroughly on a plate shaker. After 30 minutes incubation at room temperature, the absorbance of reactions was measured at 562nm. The protein

concentration was calculated by the simultaneously prepared calibration curves using BSA standards. Lipid peroxidation products were assessed by measuring the MDA level according to the method of outlined in the Lipid Peroxidation Assay kit protocol (Abcam). The level of MDA in the supernatant was spectrophotometrically measured thiobarbituric acid-reactive substances with a maximum absorbance at 700nm.

- Reduced GSH Content

GSH content (reduced) was determined according to the method outlined in the GSH+GSSG/GSH Assay kit protocol. The small intestine was homogenized in 0.4ml Glutathione buffer, add 100ul of 5%SSA and mix well. The homogenate was centrifuged at 8000×g for 10 min, and then collected supernatant considered as total small intestine homogenized sample. The protein concentration and GSH were assessed in the supernatants. The protein concentration was determined using Bradford Protein Assay kit (Bio-Rad Laboratories) and BSA as standard. Reduced GSH content was measured using the protocol found in the GSH+GSSG/GSH Assay kit. Then, the development of yellow color was measured using the spectrophotometer at 412nm. The μg/mg was used to express the tissue GSH content.

- Superoxide Dismutase activity

The activity of SOD in the small intestine tissue was determined by available kit provided from Abcam. The small intestine was homogenized in ice-cold 0.1 MTris/HCl, pH 7.4 containing 0.5% Triton X-100, 5 mM β-ME,0.1 mg/mL PMSF and centrifuged at 14,000×g for 5minutes at 4◦C to obtain supernatant. The supernatant was collected and transferred into clean tube. The contents of protein and SOD activity were assessed in the

supernatants. The protein content of obtained supernatants was determined using BCA Protein Assay kit (Pierce) and BSA as standard. According to the instruction of manufacture, 25 µL of sample supernatants or BSA standards was pipetted into separated microtiter plate wells, and then 200µL BCA Working Reagent was added to each well and carefully mixed on a plate shaker for 30 seconds. After 20 minutes incubation at 37◦C.The absorbance of reactions was measured at 450nm. The protein concentration was determined by the simultaneously prepared calibration curves using BSA standards. In this assay, xanthine and xanthine oxidase generate superoxide anion that reacts with tetrazolium chloride to produce a yellow color formazan dye. SOD activity was measured at 450 nm absorbance. The activity of SOD in small intestine tissue was expressed as (inhibition rate%)/mg protein.

- TNF alpha Rat ELISA

All materials were kept at equilibrium and all reagents were prepared at room temperature before use. All standards, controls and samples were performed in duplicate.

All reagents were mixed thoroughly but not create any foam within the vials. Determine the number of microplate strips required and remove from the pouch. 100 µL of each standard and sample, including blank controls were added to the appropriate wells. 50 µL of 1X Biotinylated anti-TNF alpha were added to all wells, covered, and incubate for 3 hours at room temperature (18-25°C). Remove the cover and wash the plate 3x as by aspirating the liquid from each well and add 300 µL of 1X Wash Buffer into each well. 100 µL of 1X Streptavidin-HRP solution was added into all wells, including the blank wells, re-cover and incubate at room temperature for 30 minutes. Wash as described

above and add 100 µL of Chromogen TMB substrate solution into each well. Incubate in the dark for 10-20 minutes at room temperature. 100 µL of Stop Reagent was added into each well and read absorbance on a spectrophotometer using 450 nm as the primary wavelength.

- GST Assay

  All materials and reagents were prepared and equilibrated to correct temperature prior to use. All reagents and samples are prepared using Ab65326 protocol. Sample wells were setup with 50 µL samples. 10 µL of positive control reagent were added into ed Positive control well (adjust volume to 50 µL/well with GST Assay Buffer). 5 µL of Glutathione was added to each sample and control well. 50 µL of Reaction Mix was added into each sample and control sample well. Measure output at OD340 nm on a microplate reader.

- Rat IL-10 Simple Step ELISA

Equilibrate all materials to room temperature before use. Excess microplate strips were removed from the plate frame. 50 µL of all sample or standard were added to appropriate the wells. 50 µL of the Antibody Cocktail was added to each well, seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm. The wells were washed with 350 µL 1X Wash Buffer PT 3x. 100 µL of TMB Development Solution was added to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm. 100µL of Stop Solution was added which changed the color from blue to yellow. Shake plate for 1 minute to mix and record the OD at 450 nm.

- Rat Heme Oxygenase 1

All the reagents were equilibrated at room temperature before use. The small intestine(100mg) was homogenized in 500 µL of chilled 1X Cell Extraction Buffer. The homogenized tissue was Incubated on ice for 20 minutes and centrifuged at 10,000 x g for 20 minutes at 4°C. After centrifuge, transferred the supernatants into clean tubes and discard the pellets. The samples were assay immediately. The sample protein concentration was quantified using BCA. Eight tubes were labelled with Standards 1– 8 and 360µL of 1X Cell Extraction Buffer was added into tube number 1 and 150 µL of 1X Cell Extraction Buffer PTR into numbers 2-8. The Stock Standard were prepared. 50 µL of all samples or standard were added to the appropriate wells in duplicate. 50 µL of the Antibody Cocktail were added to each well, seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm. Perform a 3x 1X Wash Buffer PT and add 100 µL of TMB Development Solution to each well and incubate for 20 minutes in the dark on a plate shaker set to 400 rpm. Add 100 µL of Stop Solution and observe change in color from blue to yellow and shake plate on a plate shaker for 1 minute to mix. Record at OD at 450 nm.

- TNF alpha Rat

  All reagents were brought to room temperature (18-25°C) before to use. The standards were prepared as such; 1,000 pg/mL Standard #1 by reconstituting the standard with 1X Standard Diluent Buffer. Tubes were labelled #2-6 and 100µL of 1X Standard Diluent Buffer added, then Standard #2 was prepared by adding

100 µL of Standard #1 to tube #2 and mix thoroughly. Also, Standard #3 was prepared by adding 100 µL of Standard #2 to tube #3 and mix thoroughly. Final standard conc. (pg/mL) 1-1,000 1, 2- 500, 3-250, 4 -125, 5- 62.5, 6- 31.25. Blood was collected using heparin and centrifuged at 1,000 x g for 30 minutes. Plasma was collected used for assay. All reagents were mixed thoroughly before use. Do not create any foam in the vials. Get the desired number of microplate strips needed to test. Test was performed in duplicate for controls, standards, and samples. 100 µL of each standard and sample, including blank controls were added to the appropriate wells and add 50 µL of 1X Biotinylated anti-TNF alpha to all wells, cover and incubate for 3 hours at room temperature (18-25°C). 3x wash was perform by aspirating the liquid from each well, add 300 µL of 1X Wash Buffer into each well and aspirate the liquid from each well. 100 µL of 1X Streptavidin-HRP solution was added into all wells, including the blank wells and re-cover/incubate at room temperature for 30 minutes. Perform wash again(3x) 100 µL of Chromogen TMB substrate solution was added into each well and incubated in the dark for 20 minutes at room temperature. Finally, 100 µL of Stop Reagent was added into each well and read absorbance of 450nm.

- Nrf2 Transcription Factor

  Prepare reagents per manufacture's instruction found in protocol included in kit (Ab207223). Equilibrate reagents and materials to the correct temperature. Homogenize tissues, centrifuge, and collect the supernatant. Prepare sample and control wells in duplicate. Follow kit protocol Ab207223 strictly and measure the absorbance on spectrophotometer at OD 450.

- Western Blot

  The tissues were homogenized in Radio immune precipitation assay (RIPA) buffer containing protease inhibitor cocktail (Sigma, St. Louis, MO) and centrifuged at 14,000 g for 10 min. The supernatants were collected and equivalent protein in the lysates were determined using the bicinchoninic acid (BCA) method (Pierce, Rockford, IL). Samples containing 55µg of total protein were loaded onto a 12% SDS-PAGE gel. The proteins from the gel were then transferred onto the PVDF membrane at 50 V for 1hour and increase to 150V for 1hour using the Transblot Turbo transfer system from Bio-Rad. Membranes were blocked for 1 h in blocking buffer (10ml TBS) and incubate on shaking for 1 hour. Incubate overnight at 4°C with the primary antibodies diluted in blocking buffer (1:1000). After 4x washing with 0.1%TBST, the membranes were incubated with appropriate secondary antibodies for 1 h at room temperature. Proteins were visualized using an enhanced chemiluminescence system. The density ratio of each band compared to its corresponding GAPDH band was determined. All antibodies were purchased from Abcam.

CHAPTER 4

RESULTS and DISCUSSION

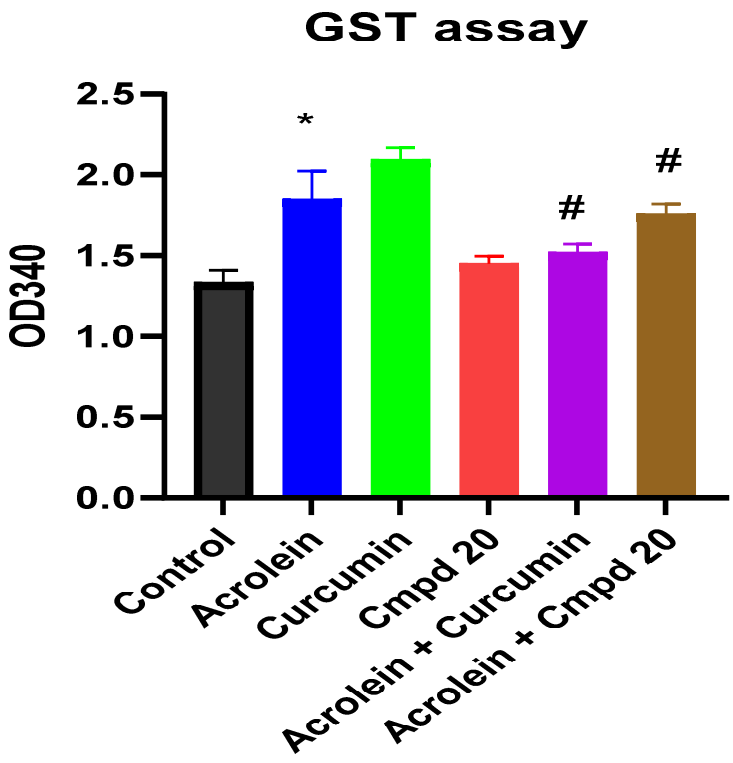

Figure 8: Glutathione-S-Transferase (GST) Assay

Based on Figure 8, Increased activity of GST is linked to an enhanced detoxification system. The GST enzymes catalyzed the conjugation of toxic substance to GSH before expelling it out. The GST activity increased significantly in Acrolein, Curcumin and Acrolein + Compound20, whereas there was slight increase in Compound20 and Acrolein + Curcumin compared to Control using a T test statistical analysis. $P<0.05$, and $n=5$

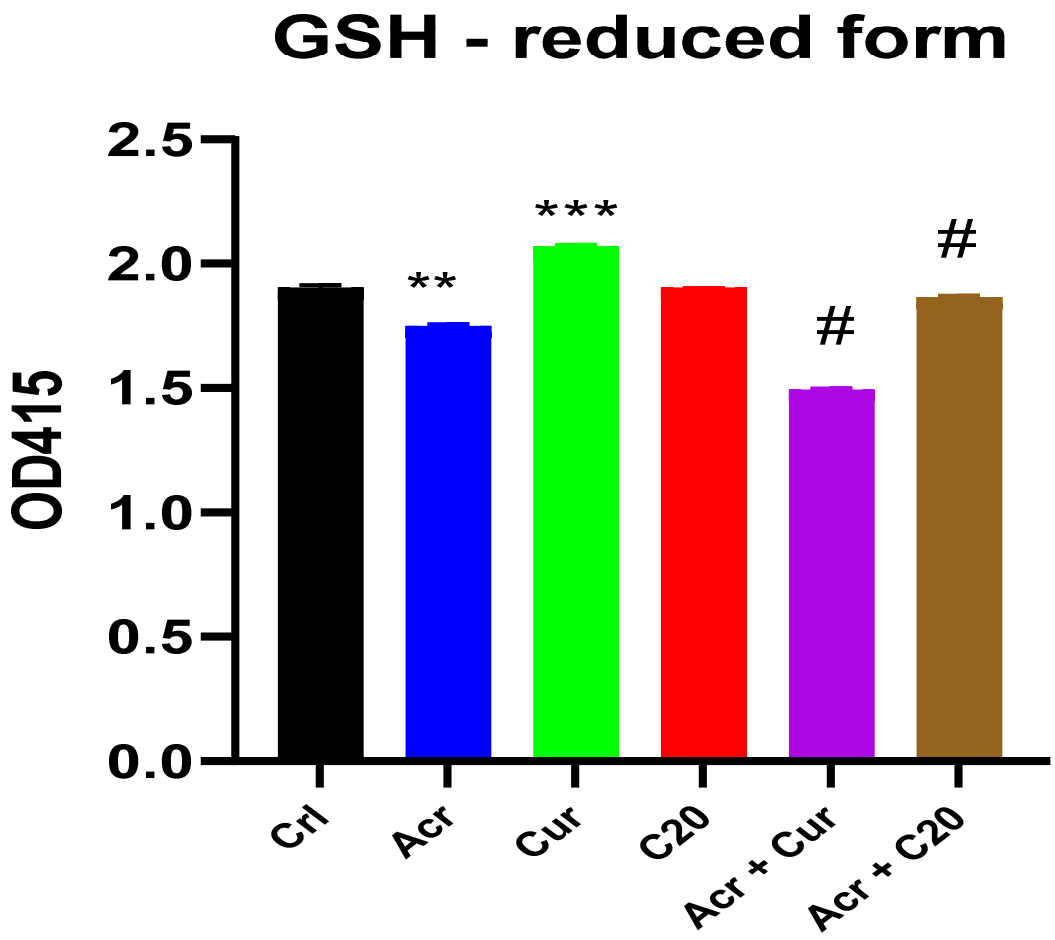

Figure 9: Reduced Glutathione (GSH) Assay

There is a significant decrease in GSH content in Acrolein whereas, there was a significant increase in GSH in Curcumin and slight increase in Acrolein + Compound20 groups compared to control after treatment period. Interestingly, acrolein depleted GSH content in Acrolein + Curcumin treatment group. Treatment with Compound20 showed no significant effect on GSH content compared to control (Figure 9). $P < 0.05$, and n=5

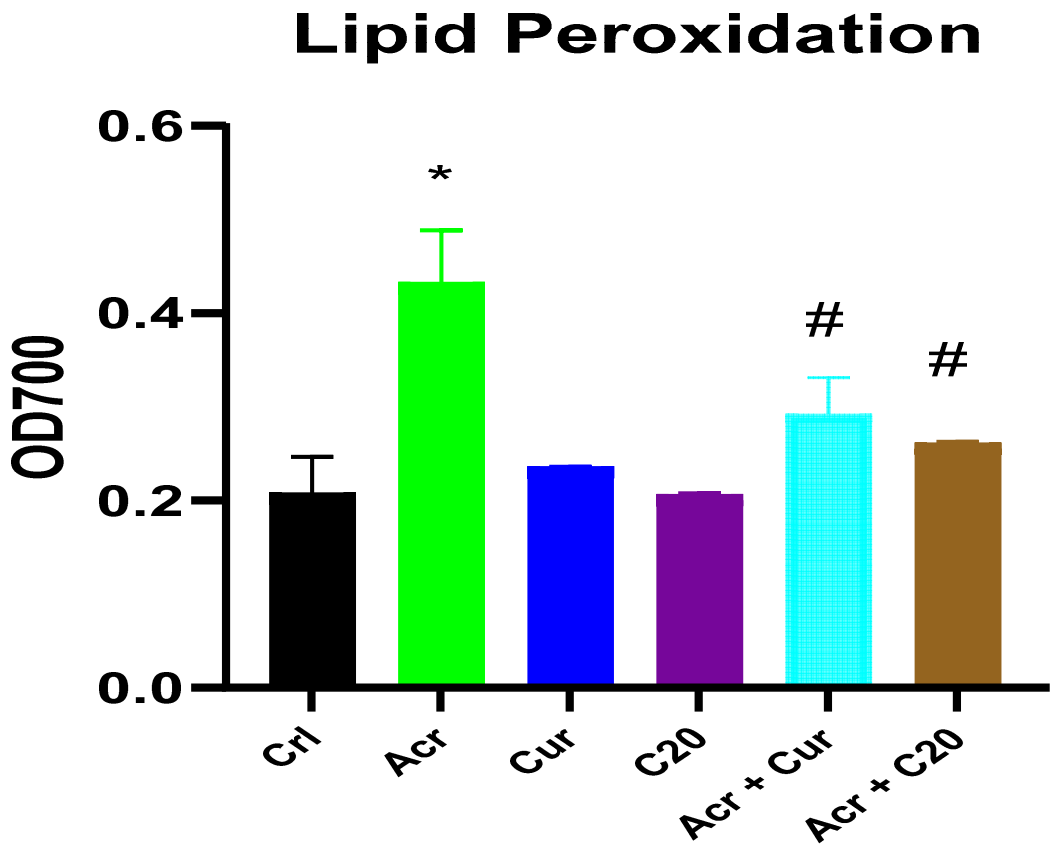

Figure 10: Lipid Peroxidation Assay

Effect of curcumin and compound20 on lipid peroxidation exposure of rats to acrolein had no significant difference in MDA levels in the small intestine as compared with controls ($P<0.05$). A significant increase in MDA levels in the small intestine were observed in rats treated with acrolein compared to the control as seen in Figure 10. This suggests that the MDA level in the small intestine tends to correlate with the supplemented curcumin and compound20 concentrations, n=5.

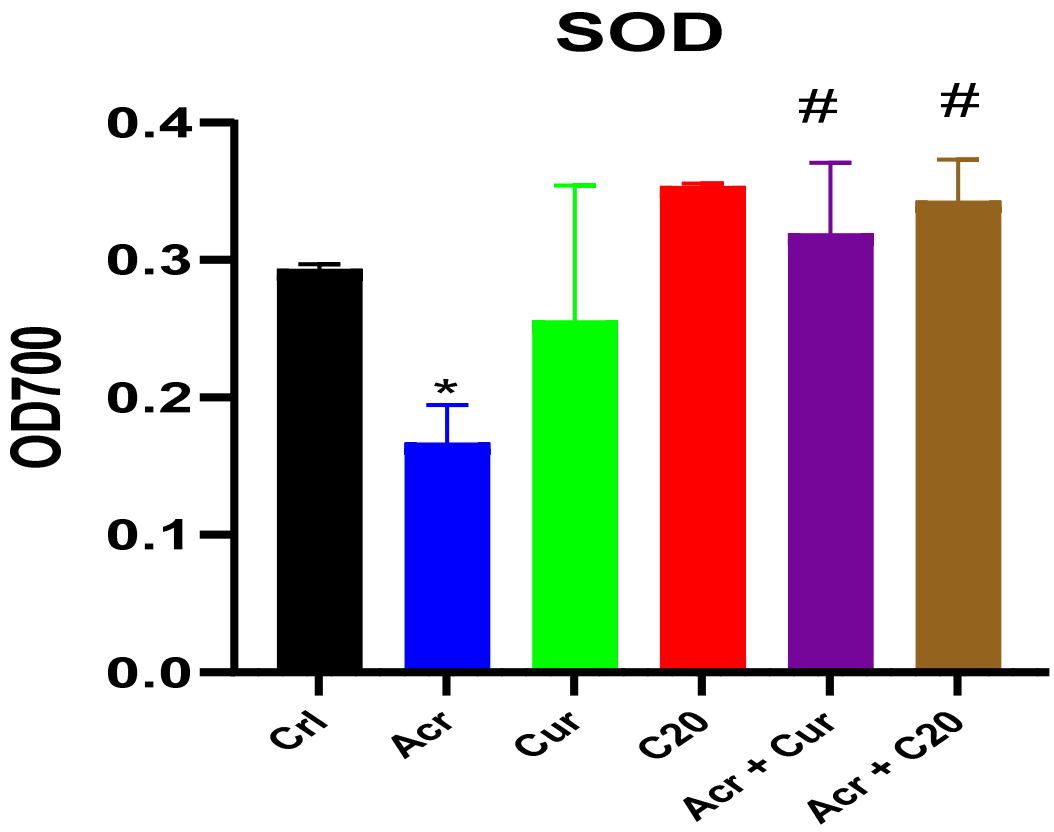

Figure 11: Superoxide dismutase Assay

The Figure 11 above shows effect of acrolein on small intestine antioxidant enzymes. The antioxidant enzymes SOD are the members of endogenous antioxidants which plays an important role in protection of the biological systems against oxidative damages. The activities of SOD in the small intestine tissue are demonstrated above. The administration of acrolein to rats significantly reduced the small intestine activities of SOD as compared to the control ($P < 0.05$). However, a significant increase in the activities of SOD, upon pretreatment with curcumin and compound20 compared to the control. compared to the control. Compared to the control, the compound20 diet increased mean SOD activity in the small intestine ($P<0.05$), n=5.

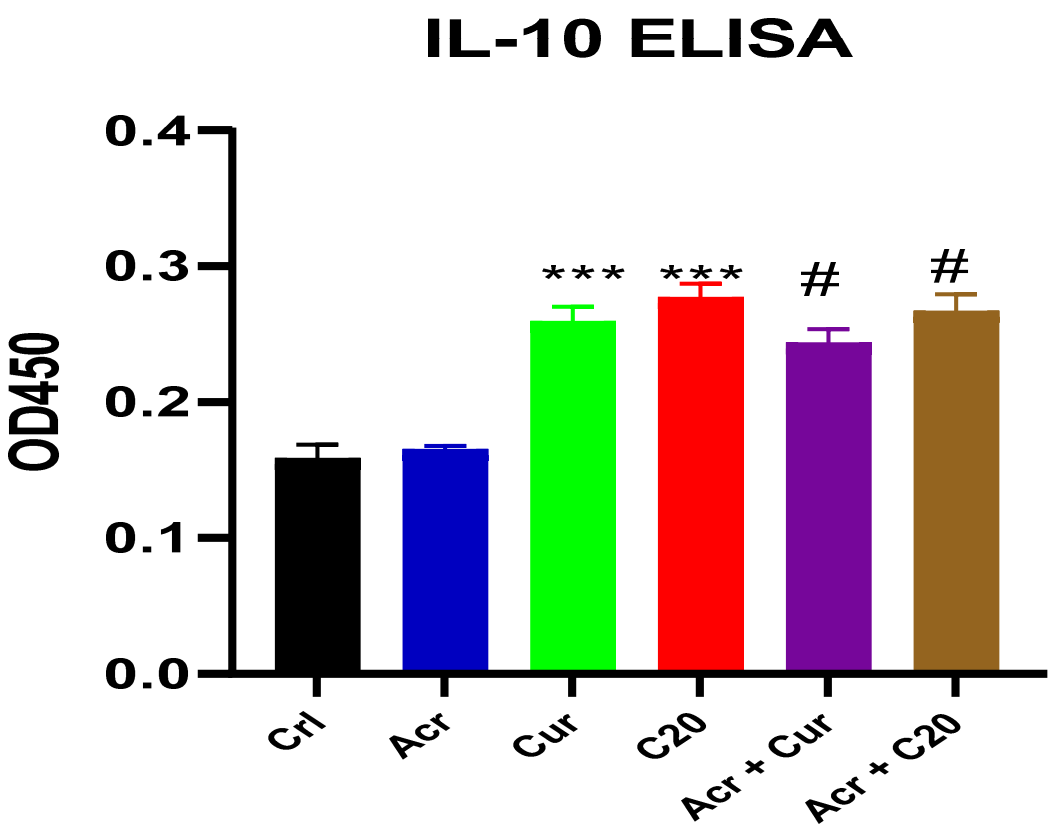

Figure 12: IL-10 ELISA

The Figure 12 above shows no significant difference of IL-10 ELISA in Acrolein compared to Control. Meanwhile, Curcumin, Compound20, Acrolein + Curcumin, and Acrolein + Compound20 shows significant increase compared to Control. This significant decrease suggests that curcumin and compound20 leads to a reduction in the inflammation of the small intestine. $P < 0.05$ and $n=5$

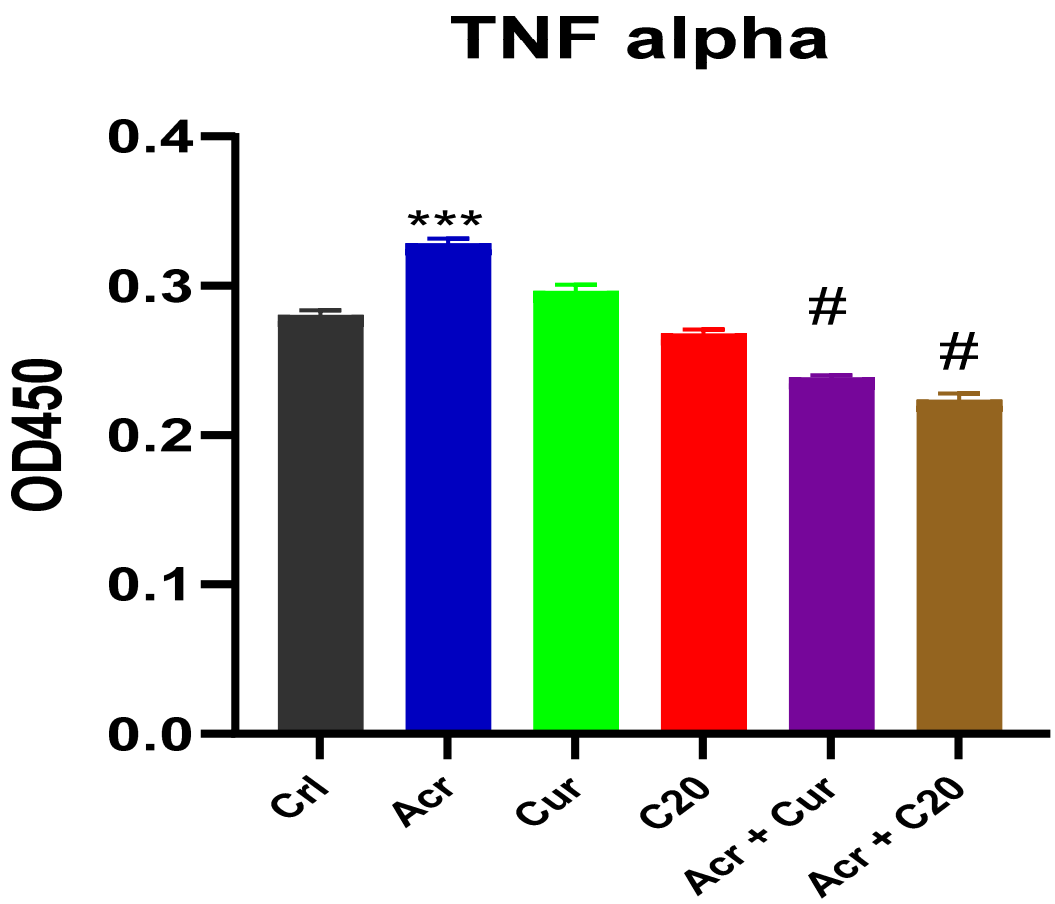

Figure 13: TNF alpha Assay

The Figure 13 above shows significant increase of TNF alpha in Acrolein, Curcumin compared to Control. Meanwhile, Acrolein + Curcumin, and Acrolein + Compound20 shows significant decrease compared to Control. Whereas no change observes with Compound 20 only treatment. $P < 0.05$ and $n=5$

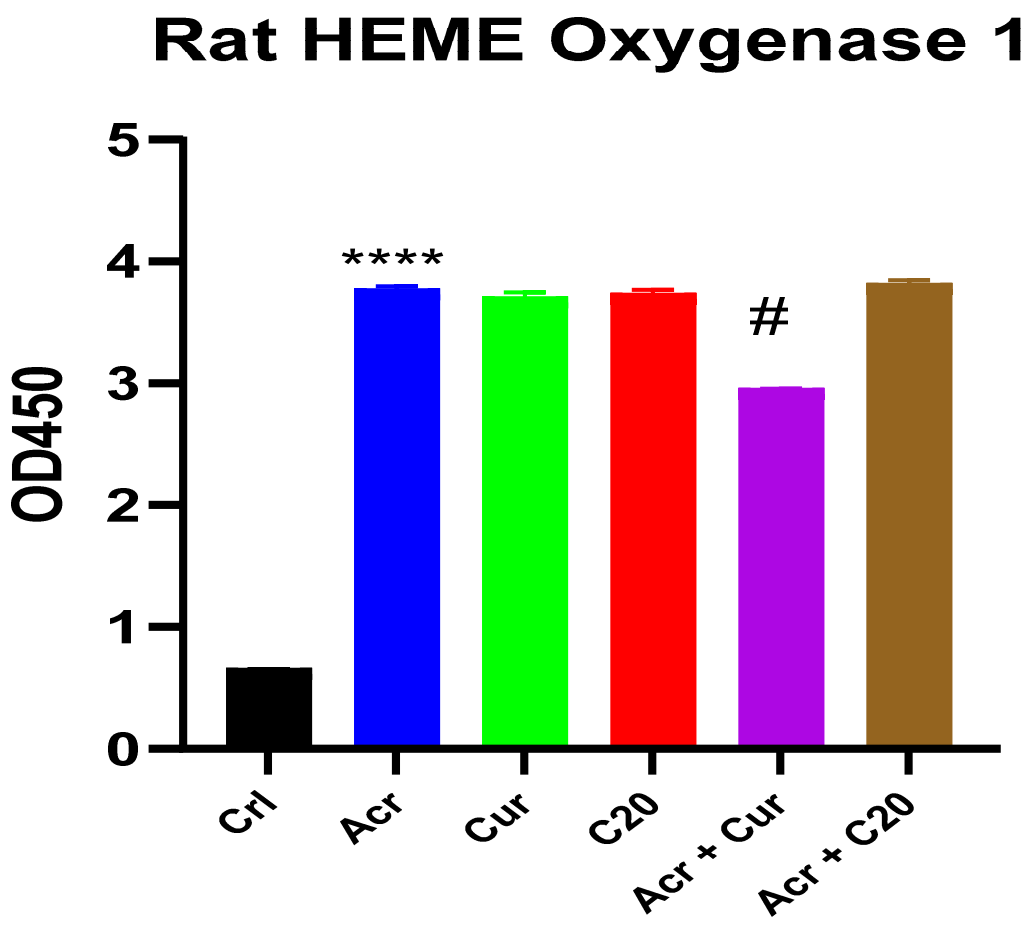

Figure 14: Rat HEME Oxygenase 1

The Figure 14 above shows significant increase of Rat HEME Oxygenase 1 in Acrolein compared to control. No significant difference observed for Curcumin, Compound20, and Acrolein + Compound20 compared to acrolein. Significant increase noted with Acrolein + Curcumin compared to acrolein. ($P<0.05$), n=5.

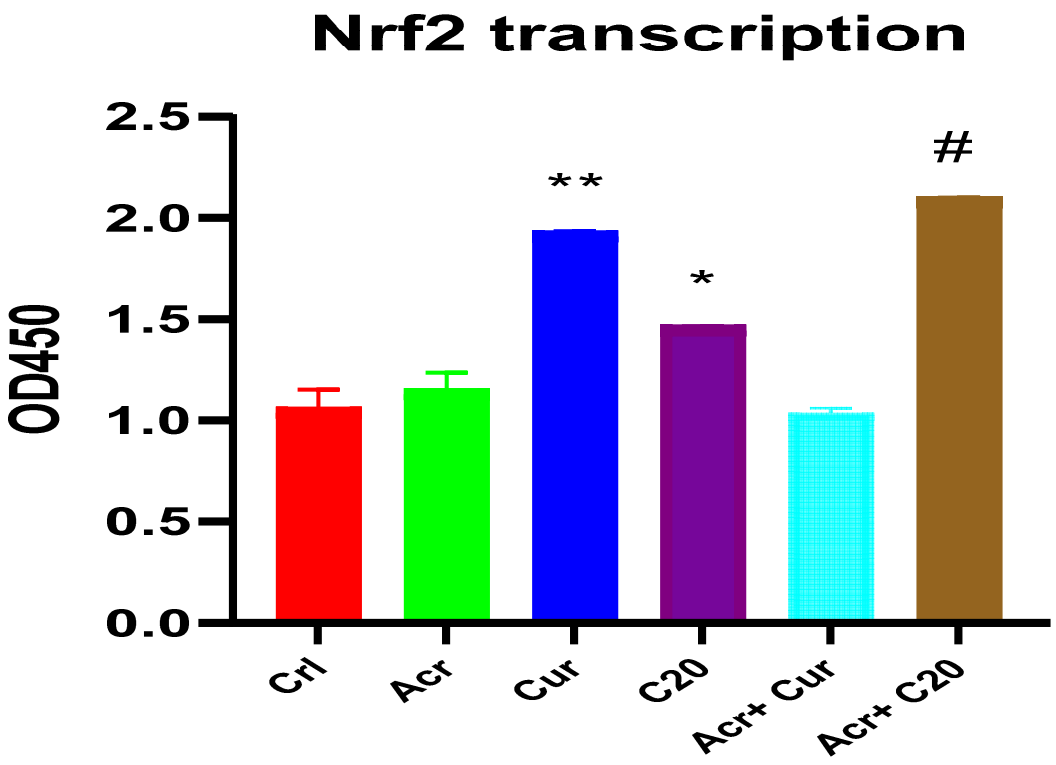

Figure 15: Nrf2 Transcription Factor assay

The Figure 15 above shows significant increase of Nrf2 in Curcumin, Compound20 and Acrolein + Compound20 compared to Control. Meanwhile, Acrolein and Acrolein + Curcumin shows no significant difference compared to Control. $P < 0.05$ and n=5

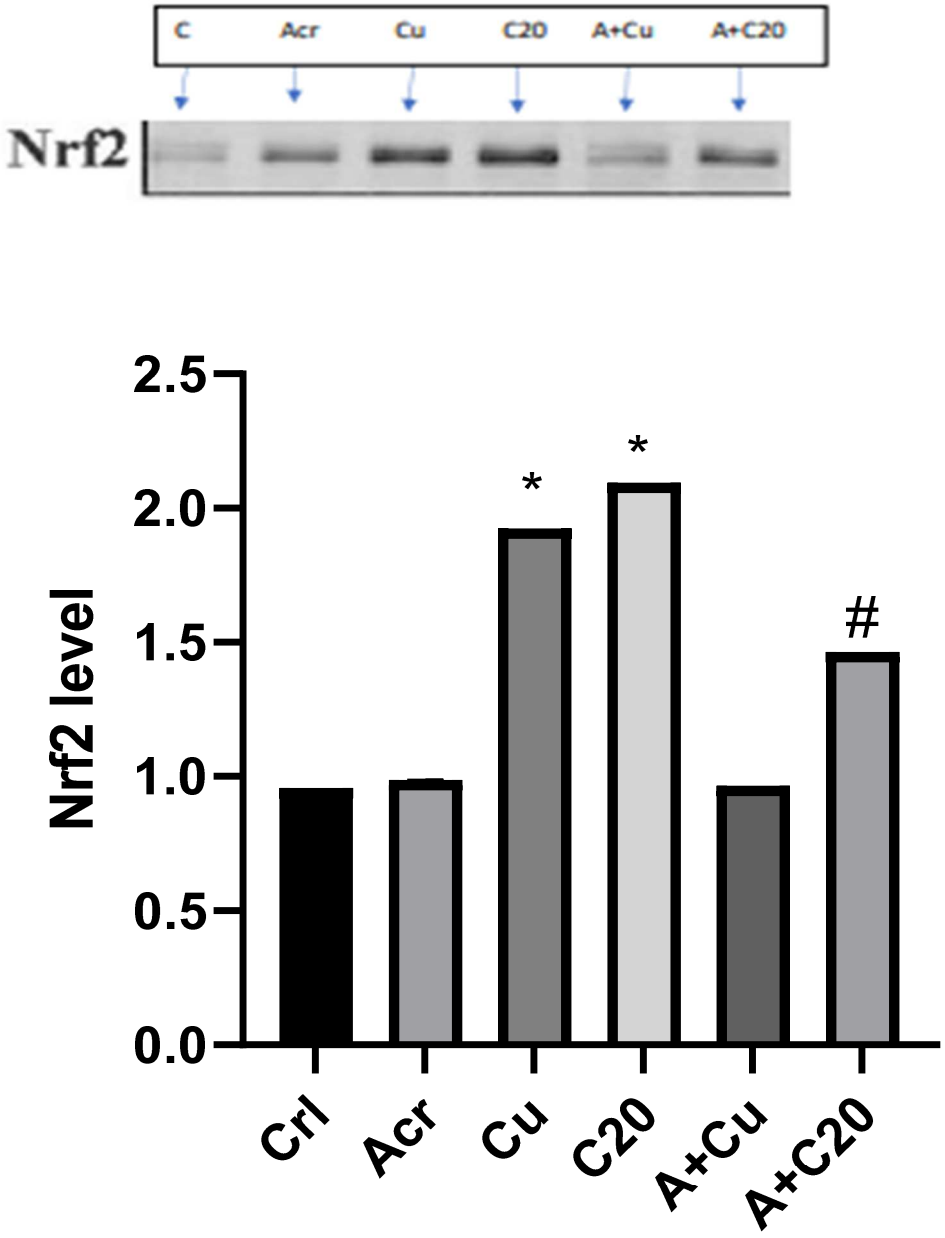

Figure 16: Western blot – Nrf2

Western blot activities of Nrf2 transcription factor on the small intestine of rats. Acrolein only treatment, does not upregulate phase II detoxifying enzymes or antioxidant proteins in the small intestine as seen in this figure.

- Statistical Analysis

All data are expressed as mean ± Standard Error mean (S.E.M.) statistical comparisons were made relative to the appropriate control group by t test. The 0.05 level was selected as point of minimal statistical significance in every comparison.

The evaluation of the impact of acrolein treatment at normal doses and the ameliorative effect of curcumin and compound20 were monitored by determining the levels of lipid peroxidation, SOD, GST and GSH in the rat small intestine. Increased activity of GST is linked to an enhanced detoxification system. The GST enzymes catalyzed the conjugation of toxic substance to GSH before expelling it out. The GST activity increased significantly in Acrolein, Curcumin and Acrolein + Compound20, whereas there was slight increase in Compound20 and Acrolein + Curcumin compared to Control using a T test statistical analysis ($P< 0.05$) as shown in Figure 9. Oxidative stress can be evaluated by the assessment of the end products of oxidative damage like MDA which is indicated by the lipid peroxidation of cell membrane and the content of GSH, which scavenges free radicals and detoxifies different xenobiotics. The level of MDA in the small intestine tissue was shown in Figure 10. The small intestine tissue level of MDA was significantly increased in Acrolein-treated group compared to the control ($P< 0.05$). The treatment with acrolein + curcumin and Acrolein + Compound20 groups resulted in no effect in MDA value compared to control group. Curcumin and Compound20 alone at doses 100 mg/Kg and 10mg/kg respectively, did not increase MDA level compared to control group. The content of GSH in small intestine was shown in Figure 9. In Acrolein-treated group, small intestine content of GSH was significantly reduced compared to the control

($P< 0.05$). However, pretreatment with curcumin and Acrolein + Compound20 groups significantly increased the content of GSH compared to control ($P< 0.05$). The result of this study indicated that treatment of rats with Compound20 alone at dose 10 mg/kg did not change the content of GSH compared to control group. The level of GSH in the small intestine of rat was slightly decreased with acrolein + curcumin as compared to that of the control group.

The antioxidant enzymes SOD is a member of endogenous antioxidants that play important role in the protection of the biological systems against oxidative damages. The activities of SOD in the small intestine tissue were shown in Figure 11. The administration of Acrolein to rats significantly reduced the small intestine activities of SOD as compared to the control ($P< 0.05$). However, a significant increase in the activities of SOD was observed with the treatment with Compound20 alone compared to the control group ($P< 0.05$). The data also showed that treatment with Curcumin alone did not decrease the small intestine activities of SOD compared to the control.

Cytokines are small essential, secreted proteins that are released by various types of cells in the body. They have very specific effects on cellular signaling and communication through binding onto their receptors on the cell surfaces such as IL-10 and tumor necrosis factor (TNF). This significant decrease shown in figure 12, suggests that curcumin and compound20 only and as well as when exposed to acrolein leads to a reduction in the inflammation in the small intestine tissue. This satisfies the hypothesis that curcumin reduces inflammation in the small intestine. The acrolein dosing level of 2mg/kg/day

produced a gross significant increase in small intestine tissue in rats treated with acrolein only.

The figure 13 shows that acrolein only treatment group, has the TNF-α level in small intestine tissue was significantly increased as compared to that in the control group. The TNF-α level in the Compound20 group was close to that observed in the control group. The treatment groups of Acrolein + Curcumin and Acrolein +Compound20 shows slight significant decrease compared to the control. The results of the statistical analysis showed that the difference in TNF-α levels between the control and lutein groups was statistically significant ($p < 0.005$) because acrolein cause inflammation but curcumin and compound20 attenuate the inflammatory effect of TNF alpha.

Heme Oxygenase 1(HO-1) is a redox-sensitive inducible protein that provides efficient cytoprotection (cell protection) against oxidative stress. Curcumin and its synthetic such as Compound 20, possess anti-inflammatory properties, has been reported to induce potently HO-1 expression in vascular endothelial cells, Scapagnini et al. (2002). In this study, as observed in figure 14, all treatment groups of the small intestine tissue, shows significant upregulation of HO-1 as compared to the control. Curcumin and Compound20, induces HO-1 expression in the epithelial cells thus, leading to increased resistance to oxidative stress-mediated damage of the small intestine tissue, Scapagnini et al. (2002).

The Nuclear erythroid 2-related factor (Nrf2) defends cells against oxidative stress by the transcriptional regulation of cytoprotectional genes, in which the expression in all types of epidermal cells were observed at a very high level, Gęgotek et al. (2015). In the figure

16, shows significant increase of Nrf2 in Curcumin, Compound20 and Acrolein + Compound20 compared to Control ($P<0.05$). Meanwhile, Acrolein and Acrolein + Curcumin shows no significant difference compared to Control. The Nrf2 level in the cytoplasm is regulated by the formation of Nrf2- Keap1 complex, Gęgotek et al. (2015).

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The hypothesis was satisfied as shown that curcumin and compound20 (antioxidant), prevent the oxidation of ROS in the small intestine following the exposure of toxins such as acrolein.

Cytokines are small essential, secreted proteins that are released by various types of cells in the body. They have very specific effects on cellular signaling and communication through binding onto their receptors on the cell surfaces such as IL-10 and tumor necrosis factor (TNF). This significant decrease shown in figure 12, suggests that curcumin and compound20 only and as well as when exposed to acrolein leads to a reduction in the inflammation in the small intestine tissue. This satisfies the hypothesis that curcumin

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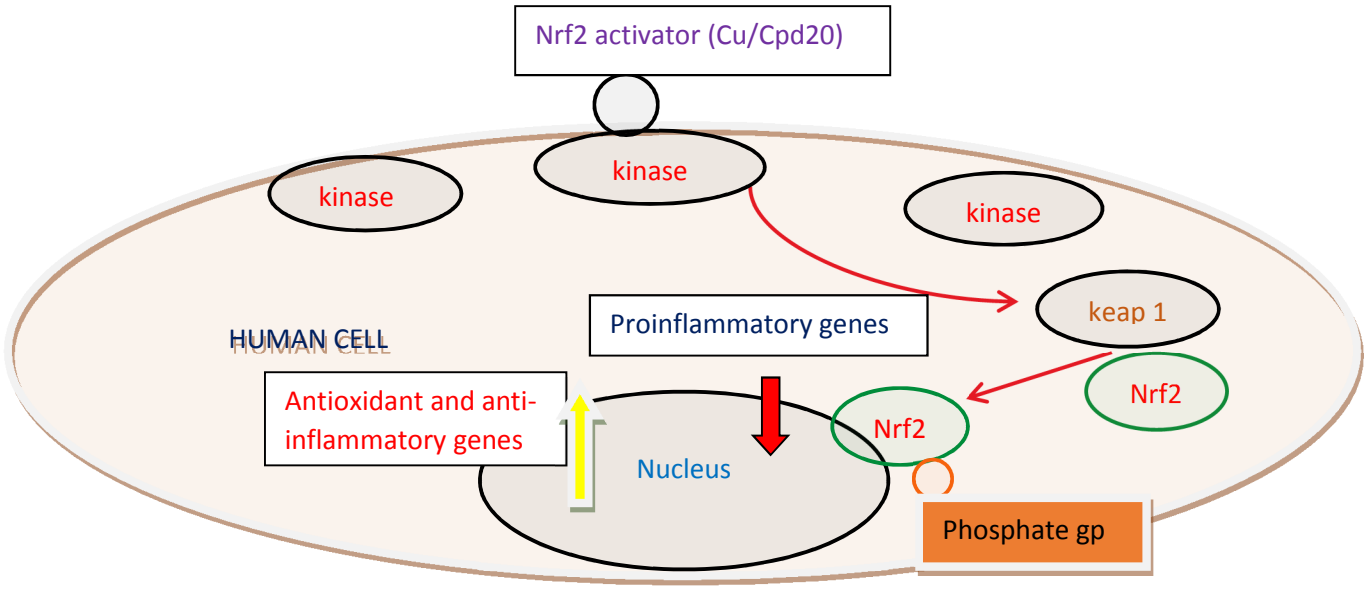

Figure 17: Mechanism of action of Curcumin and Compound 20

Nrf2 transcription factor is a critical transcription factor that regulates the expression of over 500 genes in the cell under normal or stressed conditions. Helps the body balance. Nrf2 helps regulate work of antioxidant proteins that help protect against antioxidant cell damage. Free radicals occur naturally in our body and antioxidants also have antioxidants that fight these free radicals. But, when there's an overload of these free radicals due to exposure to toxins, pollutants, inflammation, the body can suffer from oxidative stress.

However, Nrf2 activation was found to promote the body to produce more antioxidants which will protect the body from the effects of free radicals. Nrf2 also upregulate genes known as survival genes such as antioxidants, anti-inflammatory and antifibrosis genes (Qiang Ma, 2013). Nrf2 is sometimes referred to as Master regulator.

In this study, Cu and Compound20 are shown to activate Nrf2. The figure above, shows curcumin and compound20 activates the Kinase which then carries to the Kinase which is been held inside the cell by Keap 1. The activated NRF2 has phosphate functional group added attached to it which changes its structure that allows it to enter the nucleus, which then activates over 500 genes. Some will go up and some goes down. In this study, Nrf2 upregulates antioxidant and anti-inflammatory genes, thus prevent oxidative stress and down regulate proinflammatory genes.

CHAPTER 5

SUMMARY

In conclusion, this study supported my hypothesis. Curcumin and Compound20 oral consumption did in fact reduce the inflammatory and oxidative effects of acrolein on the small intestine of rats. Though the effects of compound20 are more significant than curcumin. When ten-days curcumin and compound20 consumption results were analyzed, it did show significant changes in decreasing the oxidative and inflammatory effects of the small intestine when exposed to acrolein with the most effects seen in the group treated with compound20 when compared with the control group. This study did show a larger effect size suggesting that long term consumption of curcumin and compound20 especially, may have some positive effect in preventing or reducing oxidative stress and inflammation of the small intestine. Thus, it was observed that curcumin consumption has more of a long-term effect than of an acute, short-term effect due to its hydrophobic nature unlike its synthetic such as compound20.

While overwhelming evidence has been presented in the existing literature on the effectiveness of curcumin in reversing acrolein-induced inflammation, the subject has not been exhaustively researched. Notably, most past studies on the protective effects of curcumin against acrolein-induced inflammation focus on different parts of the body such as lungs, airways, liver, and hippocampal cells. No study has been conducted on the

reversal effect of curcumin on inflammation induced by acrolein, specifically in the ileum of rats.

Research study limitations

While curcumin may have protective properties, particularly in rats, its reduced bioavailability limits its proper usage as a treatment modality for reversing inflammation and antioxidant of the endothelial tissue of the gastrointestinal tract. As such, more research is needed to address this challenge.

Also, more research and information are needed on the curcumin synthetic(compound20) used in this study.

Only one treatment concentrations for curcumin and compound20 were administered, therefore limited the confirmation of determining an optimal dose.

Future research study

More studies are needed to measure the long-term administration of curcumin and most especially compound20 and their effects on the small intestine inflammatory and oxidative stress levels. If this crossover study is repeated, more animals of both sexes should be used as the different hormones can influence the metabolism of the stressor such as acrolein in the presence of either curcumin or compound20. Also, various concentrations doses of curcumin and compound20 should be administered to determine the optimal concentration that will provide the most difference(benefit).

A study must be conducted to address this gap in knowledge and provide more evidence on the effectiveness of curcumin and compound20. Additionally, very few scholars have addressed the question of increasing the bioavailability of curcumin. More future study

will be to measure the Keap 1, Nrf2 inside the nucleus and also find out if other pathways can be activated other than Nrf2 transcription factor.

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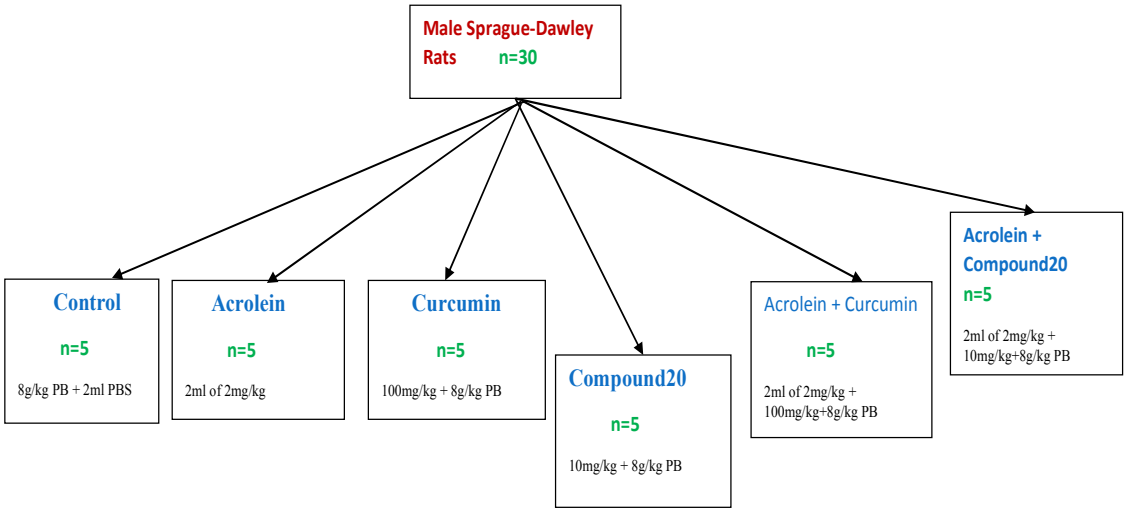
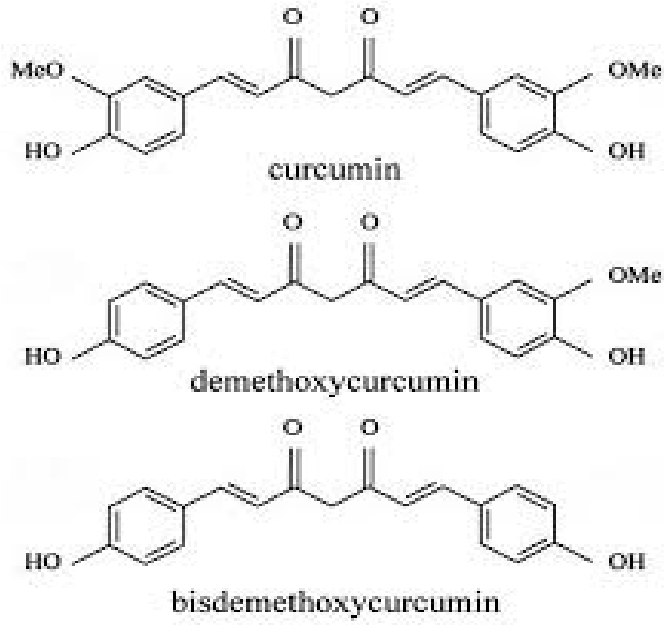
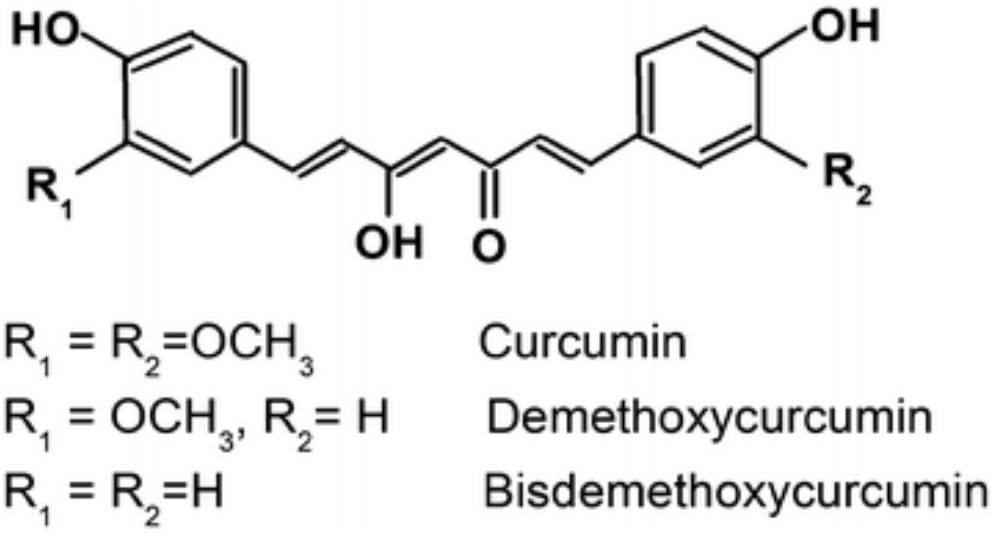
APPENDIX A



Experimental design diagram

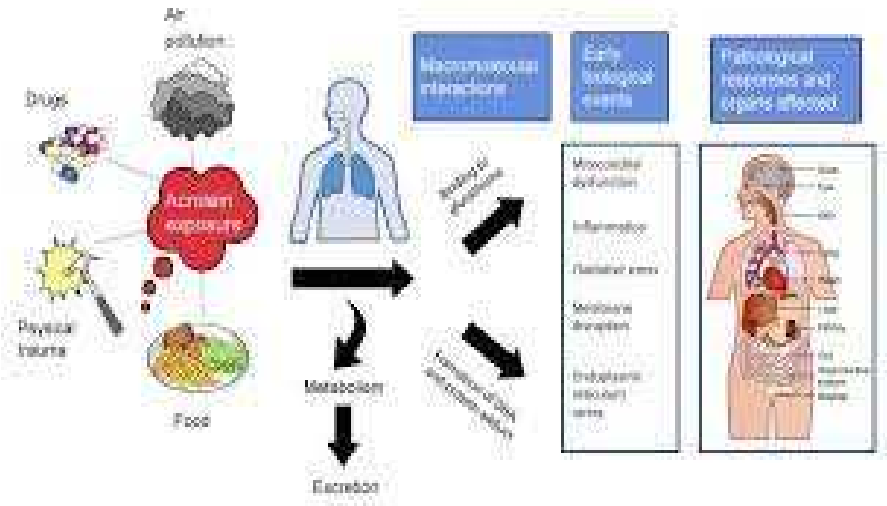


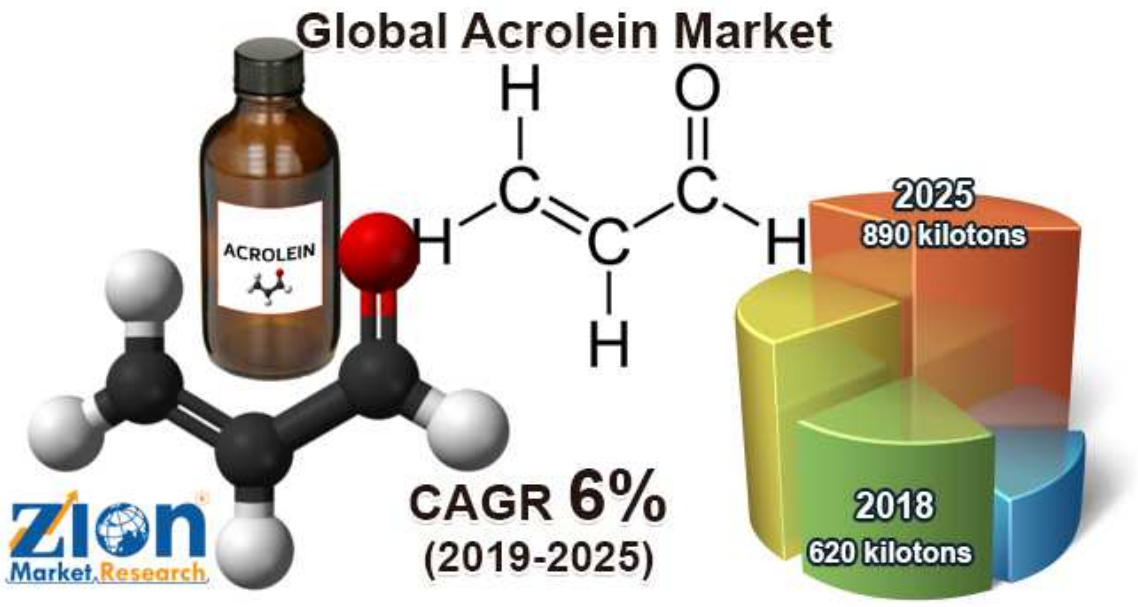


Images got from google.

All pictures in both Appendixes were taken from google search.

APPENDIX B





All pictures in both Appendixes were taken from google search