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Research Article

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Risk Assessment of Paraquat Poisoning and Possible Role of Ferulic Acid as a Therapeutic Usage against Paraquat Toxicity

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ABSTRACT

Objective: To investigate the effectiveness of ferulic acid as naturally product substance for ameliorating the toxicity associated with paraquat administration. Ferulic acid (FA) exhibits a wide range of therapeutic effects against various disorders through its phenolic compound present in its structure. Moreover, nowadays there has been considerable scientific interest in the use of phytochemicals derived from dietary components to combat human diseases.

Method: Paraquat- as fatal herbicide widely used globally and Ferulic acid (FA) as a significant therapeutic Potential against Paraquat toxicity were used for the present study. Three groups of experimental animals were conducted for either Paraquat or ferulic acid (8 rats/group): 1- Control group received orally administration of 1 mL saline. 2- Paraquat (PQ) group received intragastricparaquat (PQ) administration at a dose of 50 mg/kg in 1 mL saline. 3- After one hour of paraquat (PQ) intoxication, rats received oral (intragastric) administration of ferulic acid 150 mg/kg, one time a day for 3 days. Neutrophils count, some inflammatory mediators as TNF-α and IL6, blood gases, C-reactive protein as specific indicator for the presence of inflammation, BUN&creatinine and the oxidative stress biomarker, malondialdehyde (MDA), as well as the antioxidant enzymes such as, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) to assess the antioxidant activity of ferulic acid as a therapeutic Potential agent against Paraquat toxicity.

Results: The number of leukocytes in both blood and BALF(broncho- alveolar lavage fluid) of Paraquat (PQ) intoxicated group showed significant increase in their levels compared with the control group. After ferulic acid (FA) administration, the number of leukocytes in both blood and BALF of the FA treated group was significantly lower than PQ group. Regard to, the inflammatory mediators; TNF-α and IL6 results revealed lower TNF-α and IL-6 concentrations compared to PQ group. Concerning, the effect of Ferulic acid on arterial blood gases; PaCO2 mmHg & PaO2 mmHg, pH value and HCO3 mmol/l, data showed significantly lower concentrations in both PaO2 and PaCO2 of the paraquat intoxicated rats. Additionally, there was insignificant statistical difference in the pH value, while there was a significant decrease in the HCO3 concentration after ferulic acid treatment. Regarding, the oxidative stress biomarker; malondialdehyde (MDA) showed significant increase in its content in lung tissue of paraquat (PQ) intoxicated group. Whereas, data detected significant decrease in all antioxidant enzymes activity of SOD, GPX and CAT in lung tissue of paraquat (PQ) group. While rats treated with ferulic acid showed significant decrease in the malondialdehyde (MDA) content in lung tissue parallel with significant increase in all antioxidant enzymes activity of SOD, GPX and CAT in lung tissue which means it effectives in enhancing the activity of such antioxidant enzymes.

Conclusion: the recorded data of the present study showed that, PQ administration induced inflammatory mediators release and oxidative stress in the exposed rats. Whereas, administration of ferulic acid ameliorated the toxic effects of PQ administration. Consequently, it could be said that ferulic acid has the potential to recover the inflammation and the oxidative stress produced as the result of PQ administration.

Key words: *Paraquat, Ferulic Acid, Blood gases, TNF-α & IL6, Neutrophils count, antioxidant enzymes, C-reactive protein and BUN & creatinine.*

INTRODUCTION

Paraquat poisoning is one of the most common cause fatal herbicide intoxication widely used globally. Also, causing death from suicide by intentional ingestion with reported mortality rate of 60-70% [1]. Additionally, it is

sold under different names by different manufactures as the trade name of paraquat; Gramoxone. Moreover, in the developing countries paraquat (PQ) and organophosphrous (OP) poisoning are the major cause of death and constitute the main clinical problem all over the world [2, 3]. Paraquat is dangerous when absorbed [4]. Therefore, this life-threatening herbicide can expose human population in many countries to various deleterious effects. As indicated by many authors [5-7] the main cause of death in patients intoxicated by PQ is due to lung injury caused by excessive production of oxygen free radicals, also by lipid peroxidation of cellular membrane induced acute lung injure. Additionally, the main mechanism in the tissue injury caused by paraquat intoxication is the inflammatory response [7]. As well as, in the same line. Bowler and Crapo [8] reported that the activated reactive oxygen species (ROS) considered the first cause of Pulmonary toxicity, due to the accumulation of PQ in the alveolar epithelial cells when absorbed into the body [9]. In fact, the easy availability and the lack of awareness of the potential harm towards this chemical substance share in the increasing rate of deaths [2].

Nevertheless, the efficient detoxification therapy for paraquat poisoning has not been found up until now. Thereby, it appears very necessary to search for substance that helps and acts as a good scavenger of oxygen free radicals to avoid the toxicity of such substance and can protect against oxidative stress disorders, as well as, to predict the possible treatment to give a good opportunity for the probability of the survival to those intoxicated patients in order to, save their life Additionally, there are various prognostic potential markers with prognostic significance to evaluation patients with paraquat poisoning, such as arterial blood gases analysis [10]; total and differential leukocytes count. Some antioxidant parameters are of great value such as: catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities, in addition to, malondialdehyde (MDA) production [8]; urea and creatinin [3]; proinflammatory cytokines such as; interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) [11] and C-reactive protein (CRP). According to Ramamoorth and Nallasamy [12] who reported that in the critical illness C-reactive protein(CRP) is considered the specific marker for the presence of inflammation, as it is known as an acute phase protein which increases in the inflammatory diseases. In addition, C-reactive protein is good enough to foretell the prognosis of various and many inflammatory disorders [13]. As well as, it is well know that C-reactive protein is positively correlated with the degree of inflammation during the early stage of illness [14].

Therefore, the evaluation of such potential parameters may be of help to find effective detoxification therapy in cases with PQ poisoning patients. Moreover, Bowler and Crapo [8] reported that pulmonary toxicity is due to the activated reactive oxygen species (ROS), which could cause oxidative stress to the lung tissue. These activated reactive oxygen species (ROS), could cause lung injury due to the accumulation of PQ in the lung tissue through alveolar epithelial cells when absorbed into the body [9].

It is well known that since ancient times and primitive ages, natural plant products have been used in medicine, as they have various health benefit properties, especially anti-inflammatory and antioxidant activities. Also, nowadays people have learned to use a variety of plants as medicines for different purposes. Additionally, as anti-inflammatory therapy include anti-inflammatory drugs that are not free of side effects such as non-steroidal anti-inflammatory drugs corticosteroids and monoclonal antibodies [15].

Therefore, the search for new pharmacologically active agents as anti-inflammatory drugs obtained from natural source such as plant extracts appears very necessary to keep safe treatment for various disorders. The detection of such substances will lead to the discovery of many clinically useful drugs for the treatment of many diseases with less deleterious effects [16-18]. Many drugs of plant origin having antioxidant activity have been reported to have anti-inflammatory activity as mentioned by (Such, 2008) who, reported that drugs of plant origin that having antioxidant activity have been known to have anti-inflammatory activity Additionally, various plant extracts have been known as sources of natural antioxidant and anti-inflammatory drugs, moreover, medicinal plants possess an important source of new chemical substances that have potential therapeutic effects [19]. Ferulic acid is one of the plant extract that have these activities. Therefore, the authors expected that ferulic acid may have significant potential for the development of novel anti-inflammatory and antioxidant drugs and could be used as pharmacological agent in the treatment of some inflammatory disorders in which free radical formation is a pathogenic factor. Ferulic acid (FA) is a phenolic phytochemical naturally occurring substance, usually found in plant cell walls especially in grains such as corn , rice and wheat, as well as derived from dietary components such as fruits and some vegetables, as (tomatoes, carrots and sweet corn) [6, 20] .The beneficial activity of ferulic acid (FA) for human health is derived from its phenolic compound, as it has, strong antioxidant activity. As well as, a phenolic phytochemical compound is a strong membrane antioxidant and known to have positive effects on human health through increasing the free radical scavenger owing to increase the antioxidant protrin expressions such as

glutathione [21]. As, ferulic acid (FA) is an effective scavenger for free radicals, consequently in certain countries, it has been approved as food additive to prevent lipid peroxidation [22] Nowadays, the therapeutic usage of ferulic (FA) is receiving greater attention in the research due to its wide range of therapeutic effects. Most of its therapeutic potentials are derived from its higher antioxidant and anti-inflammatory activities. Moreover, most likely the therapeutic potential of ferulic acid (FA) comes from its staying in the blood stream long time, hence stays in the body long enough to keep the right homeostasis of the cells and to give the opportunity for the antioxidants to act probably in a good manner [22].

Objective of the current study:

Despite of the extensive researches, the limited information about significant therapeutic Potential against Paraquat toxicity is still unknown. Therefore, it seemed necessary to add further information about the risk assessment of paraquat poisoning and to find more accurate method and potential therapy for the development of novel anti-inflammatory and antioxidant drug. Therefore, the present study aimed to investigate the effectiveness of ferulic acid as naturally product substance for ameliorating the toxicity associated with paraquat (PQ) intoxication.

MATERIAL AND METHODS

2.1. Animals:

The experimental animals used in the present study were male adult -old (Eight-weeks) albino rats, obtained from Breeding Unit of the Animal house, Faculty of Science, Tanta University, Tanta, Egypt, with initial body weight ranging from 160-190 gm. All rats were kept under the same environmental conditions. As well as, they were individually housed in a well ventilated animal room at constant temperature of 25± 2°C with a photo cycle of a 12-hour/12-hour light/dark cycle. The animals were fed ad *Libitum* with a standard diet (pellets) and allowed free access of water. All rats were acclimatized to the animal room condition for at least one week before the experiments. Using rats as animal models gives a good opportunity to know the real effect of the given drugs.

2.2. Chemicals:

All chemicals used were of analytical grade. All solutions were prepared in bidistilled water.

2.3. Drugs:

►Paraquat (Sigma Chemical, St. Louis, MO, USA).

►Ferulic acid (Sigma-Aldrich, St. Louis, MO, USA) was diluted in dimethyl sulfoxide (DMSO) to reach the concentration of 150 mg FA/450 µl DMSO

2.4. Doses:

►Intragastric (oral) administration of paraquat (PQ) at a dose of 50 mg/kg in 1 mL saline Zhang et al. (2011).

► Intragastric (Oral) administration of ferulic acid (FA) at a dose of 150 mg/kg, one time a day for 3 days Perluigi et al., 2006.

2.5. Experimental design

Rats were divided into three groups (8-rats/group).1- Control rats group received orally administration of 1 mL saline. 2- Paraqua (PQ) rats group received intragastric administration of paraquat (PQ) at a dose of 50 mg/kg in 1 mL saline according to Zhang et al. [6]. 3- After one hour of paraquat (PQ) intoxication, rats received oral administration of ferulic acid (FA) of 150 mg/kg one time a day for 3 days, the dose of ferulic acid according to Perluigi et al [23].

2.6. Samples collection

After 72 hours, the rats were fasted overnight and were deeply anaesthetized with intramuscular injection of ketamine (90mg/kg) plus xylazine (5mg/kg). Blood samples (5 mL) were collected via the abdominal aorta into plain tubes. Part of the blood was allowed to clot at 4 °C before be
serum yielded was aliquoted and stored at 4 °C for the determination cre
serum. Another part of the whole blood for the determination of blood gases and leucocytes count. After blood collection, lung samples were rapidly dissected out, rinsed with saline, and stored at −80° C for

analyses. one lung used for the determination of anti-oxidant in tissue and the other one for leucocytes count in BALF (bronco-alveolar-lavage- fluid.

Detection of Arterial blood gases (ABG):
Obtained blood was transferred to a 150-µL heparinized microcapillary tube and then processed by analyzing the gases in the blood, through Blood Gas Analysis System. The following parameters were measured; partial CO2 pressure and O2 mmHg; as we well as pH and carbonate (HCO3) values.

Determination of creatinine & urea:
Creatinine and urea were measured in serum by using kits according to manufacturer's protocol.

Determination of C-reactive protein:
C-reactive protein was measured in serum by using an enzyme-linked immunosorbent assay ELISA kits according to manufacturer's protocol.

Cytokine measurement:
Serum TNF- α and IL-6 were measured in serum by using an ELISA kit as per manufacturer's protocol
Determination of cell count in blood and bronco-alveolar lavage fluid (BALF)
BALF was performed on the left lung with 4 mL phosphate- saline solution after cannulation of the left trachea. The collected BALF was centrifuged at 1500 rpm for 10 minutes. Cell pellet was resuspended in 0.3 ml saline solution. The total number of leukocytes was counted in blood and bronchoalveolar lavage fluid using hemocytometer, according to Chen et al. [24].

Detection of antioxidant enzymes and MDA in the lung tissue
The right lung was homogenized in ice-cold potassium phosphate buffer (0.1M, pH 7.4) to produce a 10% (w/v) homogenate using amotor-driven Teflon-glass homogenizer. The samples were then centrifuged at 10,000 ×g for 10min at 4 supernatant aliquots of the resu
dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA), using commercially available assay kits. according to the methods of Beauchamp and Fridovich [25], Beers and Sizer [26], Paglia et al. [27] and Yoshioka et al. [28] respectively.

2.7. Statistical Analysis:
All values are presented as mean ± SEM. Differences between groups were determined with one-way ANOVA followed by Post Hoc test. The minimum level of statistical significance was set at $p<0.05$.

RESULTS

3.1. Effect of ferulic acid (FA) on cells migration in paraquat-intoxicated rats of different groups:
The results of the current study showed signs of damage and deleterious effects after paraquat poisoning, such as reduced oxygen partial pressure, decreased partial pressure of carbon dioxide, the production of some inflammatory mediators such as TNF-α and IL-6 were elevated, in addition to disturbances in antioxidant system.
Collective data of the number of leucocytes count in blood and bronchoalveolar lavage fluid (BALF) were determined after 3 days as affected by oral administration) with ferulic acid.
Data are given in table 1, the mean value of leukocytes count in both blood and BALF showed significant increase in the number of leukocytes count in PQ intoxicated group as compared to control group $P< 0.001$, whereas, after FA administration the number of leukocytes count in both blood and bronchoalveolar lavage fluid (BALF) of the FA treated group was significantly lower than PQ lntoxicated group $P < 0.001$.

3.2. As regards to the effect of ferulic acid (FA) on cytokine production:
Animals treated with FA exhibited significantly lower TNF-α and IL-6 concentrations $p<0.001$. when compared to the PQ group. (Table 1).

3.4. The antioxidant activity of ferulic acid (FA):
The oxidative stress biomarkers; malondialdehyde (MDA) and the antioxidant enzymes such as: catalase (CAT), superoxide dismutase (SOD) as compared to control group $P< 0.001$, on the other hand, the data detected significant

decrease at P< 0.001 level in all activities of antioxidant enzymes, SOD, GPX and CAT in the lung tissue of paraquat (PQ) groups as compared with that of control group. and glutathione peroxidase (GPx) were measured to assess the antioxidant activity of ferulic acid in lung tissue. As illustrated in table2, the data recorded showed significant increase in the malondialdehyde (MDA) contents in lung tissue of paraquat (PQ) intoxicated group Whereas, rats treated with ferulic acid showed significant increase in their levels P< 0.001 which means effectives in enhancing the activity of such antioxidant enzymes (Table 2).

3.5. Regarding the effect of Ferulic acid on arterial blood gases, PaCO2mmHg & PaO2 mmHg, pH value and HCO3mmol/l:

As illustrated in table 3, the data showed significant higher concentration in the PaO2 and PaCO2 of the paraquat intoxicated rats as compared with control group (both P < 0.001). On the other hand, within ferulic acid treated group, significant lower concentration was detected when comparing the paraquat intoxicated group with that of the ferulic acid treated group P < 0.001). Additionally, there was insignificant statistical difference in the pH value, while there was significant decrease in HCO3 concentration after ferulic acid treatment.

3.6. On the basis of the effect of Ferulic acid on serum C-reactive protein (CRP) concentration Table 4:

The statistical analysis revealed, good significant elevation at P < 0.001… level Table 4 when comparing the effect of paraquat in the intoxicated rat's group 947.51 µg/ml with that of control value 649.3. On the other hand, significant decrease was detected after ferulic acid administration at p<0.001 (Table 4).

3.7. Regarding the effect of Ferulic acid on serum creatinine and urea concentrations Table 4

There was significant increase in both creatinine and urea concentrations in PQ-intoxicated rats. Nevertheless, PQ-intoxicated rats that received ferulic acid showed significantly decrease in both serum creatinine and urea concentration at p <0.001. level, (Table 4).

Table 1. Effect of ferulic acid on leukocytes migration and cytokine production in paraquat-intoxicated rats of different groups:

	Control(n=8)	Paraquat(n=8)	Ferulic(n=8)	p
Leukocytes count (x109/ml)Blood	2.33 ± 0.14	8.60a ± 0.28	3.03ab ± 0.28	<0.001*
Leukocytes count (x109/ml)BALF	0.19 ± 0.01	0.48a ± 0.03	0.22b ± 0.05	<0.001*
TNF-α (pg/ml)	12.73 ± 0.56	41.76a ± 2.21	13.18b ± 0.57	<0.001*
IL-6 (pg/ml)	533.15 ± 22.89	1543.21a ± 46.14	550.64b ± 33.20	<0.001*

p values for ANOVA test, Sig. bet. grps was done using Post Hoc Test (LSD)
*: Statistically significant at p ≤ 0.05 a: Significant with control b:Significant with Paraquat

Table 2. Effect of ferulic acid on lipidperoxidation and antioxidant enzymes in paraquat-intoxicated rats of different groups

	Control(n=8)	Paraquat(n=8)	Ferulic(n=8)	p
MDA (µm/ mg protein)	1.78 ± 0.10	3.96a ± 0.40	1.83b ± 0.10	<0.001*
SOD (U/mg protein)	20.82 ± 0.27	10.79a ± 0.45	20.14b ± 1.14	<0.001*
CAT (U/mg protein)	95.89 ± 0.38	42.76a ± 3.21	93.68b ± 1.92	<0.001*
GPX (U/mg protein)	227.43 ± 1.29	110.05a ± 6.39	217.39ab ± 7.24	<0.001*

p values for ANOVA test, Sig. bet. grps was done using Post Hoc Test (LSD)
*: Statistically significant at p ≤ 0.05 a: Significant with control b:Significant with Paraquat

Table 3. Effect of ferulic acid on arterial blood gases, PaCO2 & PaO2, pH value and HCO3 in paraquat-intoxicated rats of different groups

	Control (n=8)	Paraquat (n=8)	Ferulic (n=8)	p
PO2(mmHg)	31.29 ± 0.14	18.98a ± 1.15	31.51b ± 0.28	<0.001*
PCO2(mm Hg)	38.26 ± 0.70	20.29a ± 1.18	37.28b ± 1.08	<0.001*
HCO3(mmol/l)	20.27 ± 0.19	20.96a ± 0.34	20.23b ± 0.24	<0.001*
pH value	7.24 ± 0.05	7.30 ± 0.09	7.26 ± 0.07	0.265

p values for ANOVA test, Sig. bet. grps was done using Post Hoc Test (LSD)
*: Statistically significant at p ≤ 0.05 a: Significant with control b: Significant with Paraquat

Table 4. Effect of ferulic acid on C- reactive protein, creatinine and urea in paraquat-intoxicated rats of different groups

	Control (n=8)	Paraquat (n=8)	Ferulic (n=8)	p
CRP (µg/ml)	604.61 ± 3.62	1066.90a ± 31.11	627.26ab ± 19.60	<0.001*
Creatinine (mg/dl)	0.54 ± 0.05	1.18a ± 0.15	0.58b ± 0.07	<0.001*
Urea (mg/dl)	37.79 ± 0.70	71.14a ± 4.36	38.03b ± 0.72	<0.001*

p values for ANOVA test, Sig. bet. groups was done using Post Hoc Test (LSD)
*: Statistically significant at $p \leq 0.05$ a: Significant with control b: Significant with Paraquat

DISCUSSION

Death from Paraquat poisoning constitutes the major clinical problem all over the world (2; 3)

Therefore, this herbicide can expose human population in many countries to various deleterious effects. Continuous low level exposure to such substance may increase the susceptibility of the host to various noxious effects. Under limited information about the drug toxicity of paraquat (PQ), especially the efficient detoxification therapy for paraquat poisoning has not been found up until now. Thereby, it seemed necessary to add farther information about the biochemical changes associated with paraquat toxicity, as well as search for new pharmacologically active agents as anti-inflammatory drug that obtained from natural source as plant extract products, to keep safe the treatment of such disorders. Additionally, the initial and main mechanism in the tissue injury caused by paraquat intoxication is the inflammatory response [7].

Moreover, as anti-inflammatory drugs are not free of side effects, this will lead to the discovery of many clinically useful drugs with less deleterious effects. It is well known that, since ancient times natural plant products have been used in medicine, as they have various health benefit properties, especially anti-inflammatory and antioxidant activities.

Ferulic acid may have significant potential for the development of novel anti-inflammatory and antioxidant drug and could be used as pharmacological agent in the treatment of some inflammatory disorders in which free radical formation is a pathogenic factor. As well as, Ferulic acid (FA) is a naturally occurring substance usually found in plant cell especially in grains such as corn and rice, also derived from dietary components such as fruits mainly the orange and some vegetables as tomatoes, carrots and sweet corn. Therefore, the present study aimed to investigate the effectiveness of ferulic acid for ameliorating the toxicity associated with paraquat (PQ) intoxication.

There are various prognostic potential markers to evaluation patients with paraquat poisoning, such as arterial blood gases analysis [10]; total and differential leukocytes count [24]. Some antioxidant parameters are of great value such as: catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities, in addition to malondialdehyde (MDA) production [8]; urea and creatinin; [3]; pro-inflamatory cytokines such as; interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) and C-reactive protein (CRP), because, C-reactive protein is good enough to foretell prognosis of various and many inflammatory disorders. [13]. Additionally, C-reactive protein is positively correlated with the degree of inflammation during the early stage of illness Moreover, the initial and main mechanism in the tissue injury caused by paraquat intoxication is the inflammatory response [7].Therefore, the evaluation of such potential parameters may be of help to find effective detoxification remedy in cases with paraquat poisoning patients. Moreover, Bowler and Crapo [8] reported that Pulmonary toxicity is due to the activated reactive oxygen species (ROS), which could cause oxidative stress to the lung tissue due to the accumulation of PQ in the lung tissue through alveolar epithelial cells when absorbed into the body Dinis-Oliveira et al. [9]. Therefore, it appears very necessary to evaluate the anti-oxidant enzymes to avoid tissue injury caused by paraquatpoisoning ,

Cell migration in paraquat-intoxicated rats and the impact of ferulic acid:

In the current study, the number of leucocytes in both blood and BALF showed significantly increase in their numbers after 3 days of intragastricparaquat (PQ) administration. The migration of leukocytes in blood and BALF caused by paraquat (PQ) correlates with acute lung injury which comes in the same line with Pfafflin and Schleicher [29]. On the other hand, the data recorded showed significant decrease after ferulic acid administration in the number of leucocytes in both blood and BALF. Therefore ferulic acid could reduce acute lung injury by inhibiting leukocytes migration in blood and BALF in rats exposed to paraquat (PQ). Therefore, the dramatic reduction in leukocytes migration could be given as a marker of anti- inflammatory activity of ferulic acid.

Cytokines production in paraquat-intoxicated rats and the possible effects of ferulic acid:

The results of the current study showed signs of damage afterparaquat poisoning, such as production of some inflammatory mediators as TNF-α and IL-6. With regard to the data concerning cytokine production in paraquat intoxicated rats, the results indicated that the intragastricparaquat (PQ) administration produced a significant increase in the mean concentrations of both TNF-α and IL6. On contrally, rats treated with ferulic acid exhibited significantly lower TNF-α and IL-6.

One of the essential features of PQ poisoning is accompanied with strong inflammatory response, TNF-α and IL-6. In addition, TNF-α is one of the molecules that involved in the various stages of inflammation, Lin et al. [30]. In PQ intoxicated rats, there was up-regulation in IL-6 in both BALF and blood which was consistent to early inflammation in injury lung. Therefore, IL-6 and TNF-α could be taken as key marker to treat early damage in lung caused by paraquat (PQ) poisoning.

As regards the effect of ferulic acid on oxygen partial pressure and carbon dioxide partial pressure after intragastricparaquat (PQ) administration:

The results of the current study showed detrimental effects after paraquat poisoning, such as reduced oxygen partial pressure which could be explained as the result of progression of lung injury and the restriction of lung volume, as well as, the decreased partial pressure of carbon dioxide may be attributed to tachypnea. This assumption is supported by the work of Lee et al. [31]

Moreover, various organ systems have been known to injure by paraquat intoxication including lung and kidney. Additionally, in acute lung injury the most cases of death is due to paraquat toxicity [32]. On the other hand, intragastric administration of ferulic acid produced a significant higher PaCO2, as well as higher PaO2 compared with the rats in the PQ group, suggesting that ferulic acid may have a role in the regulation of PaCO2 and PaO2. . Moreover, Bowler and Crapo [8] reported that Pulmonary toxicity is due to the activated reactive oxygen species (ROS), which could cause oxidative stress to the lung tissue. Additionally, the activated reactive oxygen species (ROS), could cause oxidative stress to the lung tissues due to the accumulation of PQ in the lung tissue through alveolar epithelial cells when absorbed into the body [9]. Therefore, it appears very necessary to evaluate the anti-oxidant enzymes to avoid tissue injury caused by paraquattoxicity,

As regard to malondialdehyde (MDA) content and the antioxidant enzymes activities such as: catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the lung tissue after paraquat poisoning and the role played by ferulic acid administration:

The oxidative stress biomarker, malondialdehyde (MDA) and the antioxidant enzymes such as: catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured to assess the antioxidant activity of ferulic acid as a therapeutic Potential agent against Paraquat toxicity.

Free radicals play a major role in the persistence of inflammation. Moreover, according to Krishnamoorthy and Honan [33] during the process of inflammation, the phagocytes secrete chemically reactive oxygen species (ROS) and radicals, thereby, the insistence of inflammation maintained as long as free radicals are found in the surrounding environment of the host. These radicals cause the damage to the surrounding host tissue [34].

In addition, authers suggested that, the antioxidants which can scavenge reactive oxygen species (ROS) are expected to improve the inflammatory disorders. Mahdi [19] reported that many drugs of plant origin which having antioxidant activity have anti-inflammatory activity [35]. For this reason, various plant extracts have attracted interest as sources of natural antioxidant and anti-inflammatory drugs.

The results of the present study showed higher MDA content in lung tissue of paraquat group than that of both control and FA groups. This could be attributed to the attack of the free-radicals on the plasma membranes. Moreover, according to Mansour [36] malondialdehyde (MDA) levels, indirectly reflect the extent of cellular damage by free radicals and are widely used as an index of free radical mediated lipid peroxidation. Another possible explanation is through the release of reactive oxygen species (ROS) from activated neutrophils and macrophages, these overproduction lead to tissue injury, as well as lipid peroxidation of membranes. Furthermore, in the present study the significant increase in the MDA content suggest the reinforce lipid peroxidation due to the failure of antioxidant defense mechanisms leading to tissue damage.

This assumption is in agreement with Amresh et al. [37]. Additionally, the reactive oxygen species induce inflammation by stimulating release of cytokines which in turn causes induction of additional neutrophils and macrophages [38], consequently, ROS may be considered as the main factor that provoking inflammatory process.

Therefore, the neutralization of these radicals by antioxidants and radical scavengers are very important to reduce inflammation process.

On the other hand, CAT, SOD and GPx activities showed decline in their levels after paraquat administration. The present findings revealed that the treatment with ferulic acid improved the disturbance of the antioxidant system caused by paraquat poisoning. Thereby, these results suggested that the decrease of MDA production after ferulic acid treatment may be due to the increase of antioxidant enzymes; CAT, SOD and GPx activities. In addition, the anti-inflammatory and antioxidant mechanisms of ferulic acid may be related to the inhibition of the free radicals and therby the increase in the activities of antioxidant enzymes; CAT, SOD and GPx which posses the ability of free radicals scavenging mechanism and by that means lead finally to anti-inflammatory and antioxidant activities of ferulic acid.

In summary, the current results suggested that the anti-inflammatory mechanism of ferulic acid may be related to the inhibition of locally-produced pro- inflammatory mediators and reduce the leucocytes infiltration mainly neutrophils. This inhibition is associated with the increase in the activities of the antioxidant enzymes; CAT, SOD and GPx.

Additionally, the beneficial activity of ferulic acid (FA) for human health is derived from its phenolic compound, as it has strong antioxidant activity. As well as, a phenolic phytochemical compound is a strong membrane antioxidant and known to have positive effects on human health through increasing the free radical scavenger owing to increase the antioxidant protrin expressions such as glutathione [21] Moreover, The ameliorating effect of the antioxidant activities following ferulic acid supplementation has previously been reported in animal [39- 41].

Effect of ferulic acid following the paraquatposining on serum C-reactive protein (C-RP):

The C- reactive protein (CRP) was estimated as specific indicator for the presence of inflammation. In the current study Serum C- reactive protein (CRP) concentration in the group intoxicated with paraquat showed elevation in its level, this could be explained in the view that, C- reactive protein (CRP) is considered as specific measure for the presence of inflammation, because it is an acute phase protein which increases in the inflammatory disorders.

This is consistent with [12] who reported increase of C- reactive protein (CRP) levels in cases of inflammatory disorders. In addition, ferulic acid administration showed significantly decrease in the CRP concentration which means, it has the ameliorating effect through the improvement of the disturbance in the antioxidant system and hence improve the inflammatory effect induced by paraquat poisoning.

Effect of ferulic acid following the paraquat poisoning on serum creatinine and urea concentration:

After 72 hours of paraquatintragastric administration, there was significant increase in BUN and creatinine. These data suggest that PQ exerts its deleterious effect by causing abnormal renal function, thereby the increase in both BUN and creatinine may lead to renal failure therefore, the malfunction of which is involved in kidney pathogenesis.

This explanation comes in the same line as reported by Koo et al. [42], Roberts et al. [43] that renal failure in paraquat toxicity, resulting in increasing BUN and creatinine, as well as proteinuria, glycosuria, microscopic hematuria, phosphaturia and aminoaciduria. Moreover, Pavan [44] indicated that acute kidney injury, and renal dysfunction develop rapidly following paraquat poisoning. On the other hand, PQ-intoxicated rats received ferulic acid showed significantly decrease in both serum BUN and creatinine which means that, ferulic acid may ameliorate the deleterious effect of paraquat, as these parameters are considered as a good markers for the pathogenesis of kidney function. Furthermore, according to Jo et al. [45] acute kidney injury is the common cause of death in patients with paraquat intoxication.

The main pathogenesis of paraquat intoxication includes redox cycling and intracellular oxidative stress generation; however, inflammatory reaction plays a vital role in paraquat toxicity, thereby authors suggested that suppressing the inflammation may be of help to reduce acute kidney injury. Additionally, toxicokinetics Studies of paraquat have showed that, it is eliminated mainly through the kidney within 12 to 24 hours of ingestion, more than 90% of paraquat is excreted unchanged by the kidney if the renal function remains normal. [46]. Moreover, the data of this study suggested that ferulic acid involvement had a protective effect on renal function.

CONCLUSION

The model of the present study showed that PQ administration induced inflammatory mediators release and oxidative stress in the exposed rats. Administration of ferulic acid ameliorated the toxic effects produced as the

result of PQ administration. Therefore, it could be said that ferulic acid has the potential to recover inflammation and oxidative stress. Moreover, the beneficial activity of ferulic acid (FA) is derived from its phenolic compound. Ferulic acid (FA) has a strong antioxidant activity, as well as it is an effective scavenger for free radicals and prevent lipid peroxidation of the membranes due to its phenolic hydroxyl group in its structure. Therefore, ferulic acid may have significant potential for the development of novel anti-inflammatory and antioxidant drugs, hopped to find in the near future an accurate therapy, to get the probability of survival to the host.

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