Potential Protective Effect of Local Ethanolic Extract of Propolis against Asthma A Biochemical study on Rats .

Meaad N. Hussein * Ferdous. A. Jabir**
* B. Sc. college of Sciences Al- Qadisyia University.
** Assist. Prof. College of Medicine Al- Qadisyia University.
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الخلاصة

وضعت الدراسة الحالية لدراسة تأثير المستخلص الايثانولي للعكبر المحلي في اخماد الجذور الحرة وكذلك تأثيره على مضادات الاكسدة في الجرذان المصابة بالربو .

تم استعمال ستون جرذ من الذكور من نوع سبراكيو-داولي .قسمت عشوائيا في اربعة مجاميع كل مجموعة تحتوي عل 15 جرذ

المجموعة الاولى اعطيت الماء المقطر لمدة ثلاث اسابيع بعدها حقنت تحت الجلد ب(0.5ml)من السلاين وتم اعتبارها مجموعة مسيطرة .المجموعة الثانيةاعطيت الماء المقطر لمدة ثلاث اسابيع بعدها حقنت تحت الجلد بمادة الاوفالبومين و بواقع ثلاث جرعات في اليوم الاول ،الثالث والخامس .المجموعة الثالثة اعطيت المستخلص الايثانولي لمدة ثلاث اسابيع بعدها حقنت تحت الجلد بمادة الاوفالبومين وبنفس جرعات المجموعة الثانية .المجموعة الاخيرة اعطيت المستخلص الايثانولي لمدة ثلاث اسابيع ثم حقنت تحت الجلد ب(0.5ml)من السلاين بعد 31 يوم من بدء التجربة تم تقدير مستويات المالون ثنائي الالديهايد وفعالية انزيم الكاتليزفي الدم .بينت النتائج وجود زيادة معنوية ($P < 0.05$)في مستويات مالون ثنائي الالديهايد في دم جرذان المجموعة الثانية وحصول انخفاض معنوي($P < 0.05$)في هذا التركيز في دم المجموعة الثالثة .كذلك وضحت هذه الدراسة وجود انخفاض معنوي ($P < 0.05$)في فعالية انزيم الكاتليز في دم المجموعة الثانية ووجود زيادة معنوية ($P < 0.05$)في فعالية هذا الانزيم في دم المجموعة الثالثة من هذه النتائج يتبين ان المستخلص الايثانولي للعكبر المحلي يمتلك امكانية عالية في اخماد الجذور الحرةوكذلك امكانيتة في تزويد وتنشيط مضادات الاكسدة للوقاية من مرض الربو .

Abstract

The present study is designed to investigate the effect of Local Ethanolic Extract of Propolis to inhibition of free radical , and effect in antioxidant defense in asthmatic rats . Sixty healthy adult Sprague-dawley males rats were used in this study, divided into four groups. Every group contained 15 male rats . The first group received distilled water once time daily for three weeks then injected with (subcutaneous) (0.5ml) saline one dose for three time on 1^{st},3^{rd},5^{th}day .The second group was received distilled water for three weeks then injected (s.c.)three doses of 100µg of Egg albumin on 1^{st},3^{rd},5^{th}day . The third group was received local EEP for three weeks at doses 200mg /kg .after that induced asthma by injected (s.c.) three doses of 100µg of egg albumin on 1^{st},3^{rd},5^{th}day . The last group rats received local EEP at doses 200mg /kg for three weeks , then injected (s.c.) with (0.5ml) saline one doses for three time on 1^{st},3^{rd},5^{th}day. After 21 days the experiment finished and the serum samples were collected to determine the MDA content and Catalase activity.

This study showed a significantly ($P < 0.05$) increased in MDA content in asthmatic rats ,but there was significant($P < 0.05$) decrease in MDA in third group who treated EEP before induced asthma ,and there activity of Catalase was significantly ($P < 0.05$) decreased in asthmatic rats but there was increased in rats who received EEP .This result indicate that EEP had potential to inhibited free radical and it can provided and reactivated antioxidant activates .and can protective from asthma by using it as treatment .

Introduction

asthma is chronic inflammatory disease of the airways in which many cell types play a role , in particular mast cell, eosinophils and T lymphocytes .In susceptible individuals ,inflammation causes recurrent episodes of wheezing ,breathlessness ,chest tightness and cough

,particularly at night and or early morning .Inflammation causes an associated increase in airway responsiveness to a variety of stimuli[1]Asthma is not single condition but a heterogeneous collection of clinical phenotypes .It comprises a spectrum of diseases ranging from paroxysms of coughing ,wheezing ,and dyspnoea occurring periodically and with symptom –free periods to severe persistent asthma where symptom are continuously present[2].. Radicals derived from oxygen represent the most important class of radical species generated in living systems[3]

. In the last 20 years ,that free radicals in the form of reactive oxygen species (ROS) have become increasingly recognized as playing a major role in many disease processes [4]. Sources of O_2^- include primarily nicotinamaide adenine dinucleotide phosphate (NADP) oxidase-dependent complex ,the cytosolic xanthine oxidase and the mitochondrial respiratory chain [5]. In the other side of general source of ROS in asthma is derived from environment includes gaseous and particulate air pollution ,like cigarette smoke and oxidant gasses ,such as ozone nitrogen dioxide and sulphur dioxide, diesel exhaust particles. [,6,7]

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Antioxidant it is agents which scavenge the free radicals and prevent the damage caused by them .the can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage [8]. Oxidative stress occurs when this balance is disrupted by extreme production of reactive oxygen species and or by insufficient anti oxidative defenses[9].

Propolis (bee glue) is an adhesive ,dark yellow to brown colored balsam that smells like resin .It is collected from the buds, leaves and similar parts of trees and other plants like pine ,oak, eucalyptus ,poplar, chestnut ,and so on by bee and mixed with their wax and bee enzymes [10]. The studies of chemical composition of ethanolic extract of propolis (EEP) and concluded that propolis contains mainly

2

polyphenolic compounds ,including flavonoids as a major compounds[11]. Propolis is a powerful antioxidant .This effect is due to the high concentration of phenolics and other antioxidant compounds [12]. The anti-inflammatory activity observed in green propolis seems to be due to the presence of prnylated flavonoid and cinamic acid .These compounds have inhibitory activity against cyclooxygenase (COX)and lipooxygenase [13,14].

Material and Methods

Preperation of Ethanol Extract of Propolis (EEP) .

By methods presented by(Al-Mohana, 2004 and Yaghobi et al ,2007) [15,16] prepared the pure local EEP ,taken fifty grams of crude powdered propolis and macerated in 1000 ml of 70% ethanol for 6 days with mixing and shaking by thermomagnatic stirrer (300 rpm at 25c) at long time (4 hour /day).The extract solution was stored overnight at (0-4c)to obtain crystallization of dissolved waxes. The solution was filtered through a whatman filter paper. Then the filtered was dried by using of oven at (35-50c) till complete dryness giving a resin gummy brown products. Complete dryness was examined the weight of pure propolis at three times at about 15 Days.

The yield=weight of EEP/weight of crude propolis*100% .

After that we taken (2)gm of Propolis extract was dissolved in (4)ml of absolute ethanol by using glass stirrer ,when the dissolving occurred the volume complete to 100 ml by adding of distilled water to obtain 2%(w/v) milky solution. The final concentration of ethanol in this milky solution didn’t exceed 5% which had no

effect on *in vivo* and *in vitro* experiment according to what stated by [17].

Animals and Housing

weeks of age , have used in the experiment from the college of veterinary medicine Al-Qadisiya University .Male rats were allowed to acclimatize to the animals house environment in before beginning of experiment .animals were fed on the standard chow and drinking water .Room temperature was maintained at 22+-2 c. The rat were adapted to the new and quiet environment for at least(2)weeks and also thy were exposed to clinical examination produced the beginning of experiment in order to ensure the good healthy status of all the rats.

Experimental Design All adult male Sprague –Dawly rats were randomly divided in to four group . Every group contained of 15 male rats .Animals of all groups were administered as follows:

Group(1) normal*(control negative):*It's given normal feeding, and Administrated orally with distilled water containing 4% ethyl alcohol at a dose of (10ml/ kg .B.W) once time day by using oral drencher for three weeks then injected(subcutaneous) with (0.5ml) saline one doses for three time on 1^{st},3^{rd},5^{th}day .

Group(2)control positive (asthma): These group given orally with distilled water containing 4% ethyl alcohol at a dose of (10ml/ kg .B.W) once time day ,by using oral drencher for three weeks, then injected (s.c.)three doses of 100µg of Egg albumin according of body mass adsorbed on 12mg of aluminum hydroxide gel prepared in 0.5mL of saline on 1^{st},3^{rd},5^{th}day.On10^{th} day of sensation blood was collected [18] .These group don't drencher with EEP.

Group (3)(treatment and induced asthma):These group received local EEP by oral drencher for three weeks at doses 200mg /kg [19] .after that induced asthma by injected (s.c.) three doses of 100µg of egg albumin according of Sixty Mature male Sprague-Dawly rats weighing between 130±10gm and (8-9) body mass adsorbed on 12mg of aluminum hydroxide gel prepared in 0.5mL of saline on 1^{st},3^{rd},5^{th}day . on10^{th} day of sensation blood was collected

Group (4) (treatment): The rats received local EEP at doses 200mg /kg for three weeks by oral drencher, then injected (subcutaneous) with (0.5ml) saline one doses for three time on 1^{st},3^{rd},5^{th}day .On 10^{th} day the blood was collected .

Serum preparation

Blood was collected in non-coagulant test tubes and the serum was separated by using centrifugation at (4000rpm)at 37C for 10minuts[20] . The separated serum of each animal was subdivided nearly in to (3) sample by using of appendroff tubes (500µl) and kept at deep freezer until using for assessment of the biochemical parameters.

Biochemical Tests

1.Determination of serum Malondialdehyde (MDA) concentration.

The principle of this method which is described by (Guidet and shah ;1989)[21] was based on the spectrophotometric measurement of the color occurred during the reaction of MDA with thiobarbituric acid (TBA)

2.Determination of Catalase (CAT) Activity.

Catalase (CAT)activity was determined by the measurement of the decrease in the absorbance due to hydrogen peroxide (H_2O_2) consumptions as described by (Aebi;1974)[22]

Statistical analysis

All the values were expressed mean _+standard error .

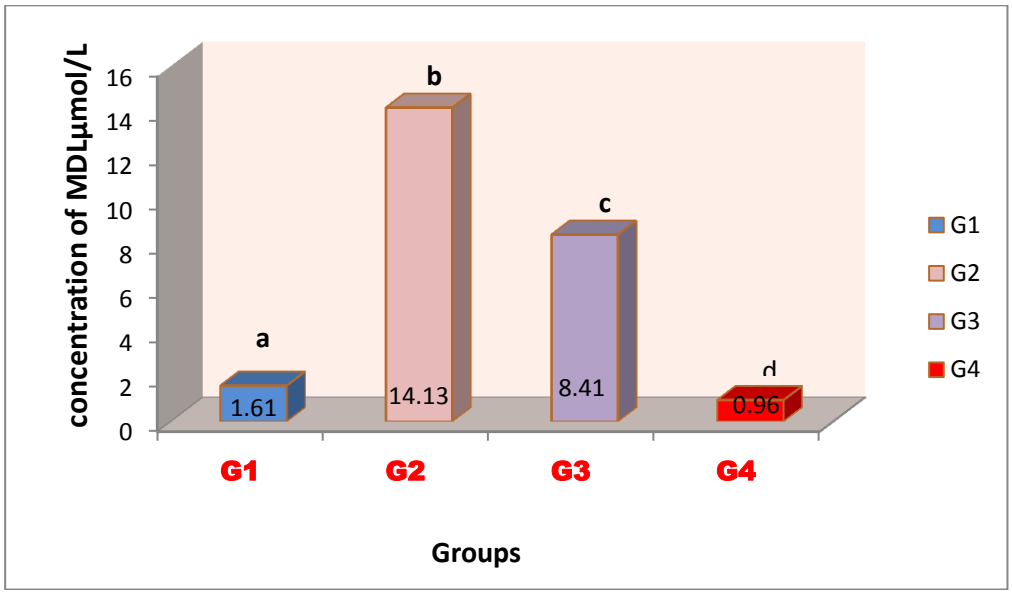
Data were analyzed statistically by using of one way analysis of variance (ANOVA).Analyses were performed ,probability value less than (0.05) was considered statistically significant.

Results

After complete dryness of EEP was viscous and dark brown material ,with a strong ,sweet, leather –like odors .The EEP was transparent but upon dilution with water , It is formed an oil-in water emulsion . The yield of EEP according to different part of Al-Diwaniya province is range between 38%- 42%. The primary chemical tests showed positive results for flavonoids, tannins ,resins ,phenols ,terpenoids , , saponin, and alkaloids .

Effect of EEP on Serum MDA Level.

MDA level in serum of rats induce asthma (group II) was significant($P < 0.05$) increased as compared with control group . The received of EEP to rats before induce asthma (group III) was significant($P < 0.05$) decrease in MDA level as compared with asthmatic group. In addition the level of MDA in serum of rats treated with EEP (without induced asthma)was significant($P < 0.05$) decrease as compared with control group .



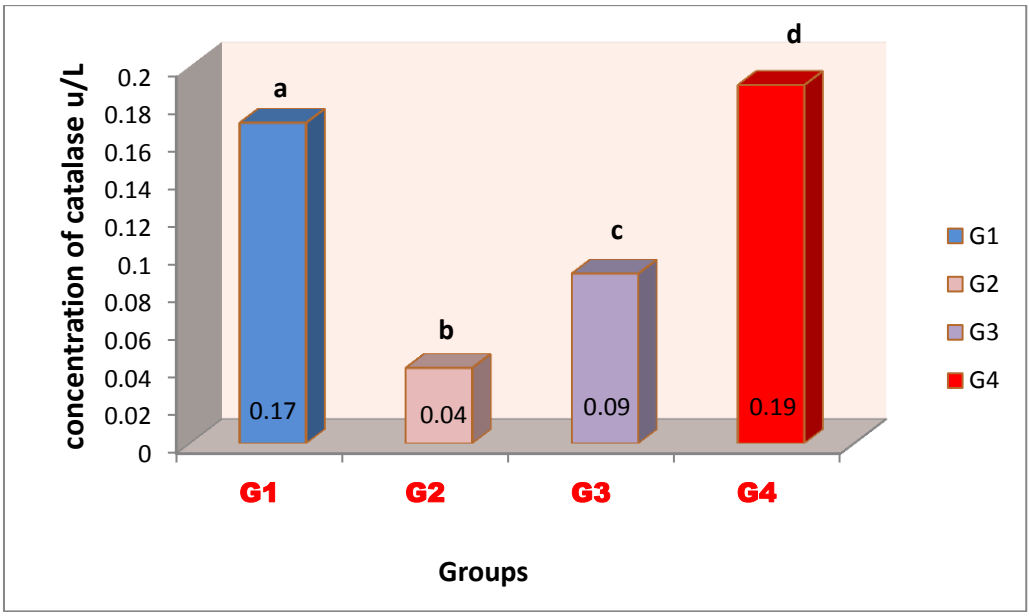
Figure(1): Effect of local EEP on MDA level after induced asthma.G1= Intact control. G2= Asthmatic rats . G3=Treated with EEP and induct asthma .G4= Treated with EEP only

Effect of EEP on Serum Catalase level.

In this study result indicate that animals injected with OVA only (group II) causes a significant($P < 0.05$) declining CAT activity in serum of rats ,compared with control group .

Rats pretreated with EEP orally before injected with OVA (group III) was showed significant($P < 0.05$) increase in CAT activity in the serum of the rats compared with asthmatic group (group II)

The CAT activity in the serum of rats who treated with EEP only (group IV) showed a significant($P < 0.05$) increase as compared with control group



Figure(2): Effect of local EEP on Serum CAT activity after induced asthma.G1= Intact control. G2= Asthmatic rats . G3=Treated with EEP and induce asthma .G4= Treated with EEP only

Discussions

MDA level and EEP

Lipid peroxidation is a free radicals mediated process and acts as potential marker of susceptibility of early and irreversible tissue damage .Lipid peroxidation in vivo destroys biological membrane leading to change in fluidity and permeability [23]The increased level of MDA in group II was may be returned to the effect of OVA albumin and aluminum hydroxide, are well known to potential the damage in the lung tissue

Oxidative damage induced by OVA albumin and aluminum hydroxide resulted in the formation of highly reactive hydroxyl radical ,which stimulated lipid peroxidation leading to distraction and damage to cell membrane [24] The fall in the level of MDA was showed in the group III who treated with EEP , this result indicates antioxidant properties of EEP in the modulation of lung damage due to OVA albumin .

Propolis contains a wide variety of antioxidant compounds mainly phenols, flavonoids and CAPE ,flavonoids in the cell membrane protect the unsaturated fatty acids against oxidant [25] .It was reported that CAPE decreased MDA levels by blocking reactive oxygen species as an antioxidant [26]

In the other hand EEP contain Alkaloids ,that Alkaloids have also been reported to strongly inhibit lipid peroxidation induced in isolated tissues via its antioxidant activity [27].The protection offered by the extract could have been due to the presence of flavonoids and alkaloids .

CTA and EEP

OVA induced asthma results from chronic airway inflammation characteristically associated with the infiltration of macrophages , lymphocytes ,mast cell, neutrophils ,and eosinophils in to the bronchial lumen .[28]

These inflammatory cells have an exception capability to produces ROS, for that the group II have high amount of ROS and that lead to consume the antioxidant enzyme such as decrease CAT activity it became un capability to scavenge the ROS. These result agreement with (Comhair et al) [29,30]

The oral administration of EEP in group III was significantly restored the level of CAT by reactivated the activity of CAT might be via scavenge of free radicals or preventing its formation (Antioxidant activity) .In addition EEP may reduced aggravation of inflammation during asthma by providing antioxidant enzymes protection . These result agreement with (Koo and Park;1997)[31]

Conclusions

The preliminary study investigation of ethanolic extract of propolis showed the presence of flavonoids, tannins ,resins ,phenols ,terpenoids , , saponin, and alkaloids .flavonoids are known to possess anti-inflamatory effects and antioxidant activity which may be responsible for anti-inflammatory and antioxidant activity . Thus the presence of these compound in EEP may further contribute in ova albumin –induced airway inflammatory responses in management of asthma ,therefore ,ours data suggestive of EEP potential in prophylaxis and management of asthma and decreased the oxidative stress .

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