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RESEARCH ARTICLE

Protective Role of *Propolis* against Iron Overload Induced genotoxicity and oxidant/antioxidant Status

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Abstract

The current study designed to investigate the protecting role of propolis in DNA damage and oxidaant / antioxidant imbalance caused by iron overload. 24 adult male wister rats divided in to 3 equal groups and handled as following: 1st group(C) as control, 2nd group, IO) group IP iron dextran 100mg/ kg BW each 72 hr. 3rd (IOP) given IP iron dextran 100mg/ kg BW each72 hr. and orally Propolis crude extract (50 mg /kg body weight) daily for two months. Results revealed that administered rats with propolis showed enhancement in oxidant/anti-oxidant indicators, categorized by raise of GSH, and MDA reduction on the contrast with IO group. Propolis adiminstration to IO rats decreased the DNA damage and fragmentation. Conclusion, the present results are established for the first time a new role for the Propolis, in counter and reduce the iron overload harmful effects on DNA mainly via modulation of oxidant status in IO.

Introduction

Iron as an element with high significant biochemical roles in the body, it required for hemoglobin synthesis, cellular proliferation, and oxidation–reduction reactions [1, 2]. In the body, it's necessary for several iron-based enzymes such as, catalase, hydrogenase, cytochromes, and the essential oxygen storage molecule myoglobin [3].So excessive accumulation of iron causes organ dysfunction as a result of reactive oxygen species (ROS) production [4, 5].The increase in dietary iron intake seen to be a risk factor for the incidences of metabolic disease [6].

Iron overload causes oxidative stress because it participates in the formation of free radicals causing cell damage and tissue injury [7]. These free radicals oxidativly produce damage of DNA and RNA [8, 9].Iron overload could be result from hereditary hemochromatosis, blood transfusions in repeated way, any diseases can cause excessive iron deposition in tissue such as bone marrow failure, or myelodysplastic syndrome and beta thalassemia [10, 11]. Iron overload disorders a commen cause of morbidity from liver disease and increase risk of hepatic fibrosis and hepatocellular carcinoma [12].

Propolis is generally known as the “bee glue" which is a generic name that concerning resinous hive product. Propolis, a mucilaginous and balsamic matter, is a honeybee origination took from resinous substantial. It is reaped by bees from buds, blossoms, and exudates of plant life and is acknowledged to form a main spectrum of biological nature [13, 14]. Physically, Propolis considered as a stuck, blackish yellow to brown colored balsam that odors like gum-resin. Propolis used safely for its anti-oxidant [15], anti-inflammatory [16], cardio-protective [17] hepato-protective [18, 15, 19] and Neuro-protective properties [20]. When reviewing previous scientific references in finding natural alternatives that reduce or counter the iron overload deleterious effects mainly on DNA, there were no any signal for propolis. Accordingly, this study is the first study conducted to evaluate propolis efficacy against iron overload

Material and Method

Experiment was performed on 24 healthy adult males waster rats, randomly divided into three groups each one has 8 animals and

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were treated as follows: 1st group consider control (C), 2nd group Iron overload (IO) group, i/p injected with at 72 hours interval [21], 3rd group (IOPr) iron over load (i/p iron dextran 100mg/ kg BW + daily orally propolis (50 mg /kg B.w.) for 60 days. At the end of the experiment blood samples obtained from insthetized animals, then animals were euthetized for tissues samples collection. of the beginning of experiment blood samples collected via retro-orbital sinus by heparinized capillary tube then withdrawn into two seprated tubes, one with anticoagulant (EDTA) for antioxidant (GSH) measurement, another tube with no anticoagulant (gel tube) for serum isolation, were used for measurement of MDA measurement after that animals were euthanized for liver tissue collection for comet assay. Total serum iron measured by colorimetric methods using commercial kits provide by JOURLABS (A Company Of Amshaj Manufacturing plc).

Oxidant – Antioxidant Status

Reduced Glutathione (mg/dl) and Maloneldehyde (µm/mol serum) measured by colorimeteric methods described by Beutler *et al.,* 1963 and Guidet, Shah.1989 [22, 23] respectively.

DNA Damage by Comet Assay

Damage of DNA in liver cells detected by comet assay depends on evaluation of the migrating ability of DNA fragments when they exposed to electric field. The migration of the DNA fragments under electric field formed a tail like shape [24]. Briefly, 200 µg of fresh liver tissues was homogenized in 1.2 ml of ice cold 20 mM EDTA in 1X PBS (Ca2+ and Mg2+ free), and liver cells in the supernatant were isolated after 5 minutes by centrifugation . Comet slides were prepared within one hour following animals sacrifice. In a micro centrifuge tube10µl of cells supernatant with 90µl/ml molten LMA garose (at 37 °C) at a ratio of 1: 10 (v/v) and immediately pipette 50 µl on to Comet Slide. Two slides for each sample were prepared. Slides were kept in flat position at 4 °C in the dark (refrigerator) for 10 min. Slides were immersed in lysing solution (ice) in 4 °C for 30 to 60 minutes. DNA unwinding by immersed slides in freshly prepared alkaline unwinding solution, pH>13.For 20 to 60 minutes at room temperature in the dark, the electrophoresed at C and 21for 30 minutes with mA.

Following electrophoresis, slides dried in cautiously then submerged twice in d H2O for 5 minutes each, then in 70% ethanol for 5 minutes. And dried slides at ≤ 45°C for 10-15 minutes. Drying brings all the cells in a single plane to facilitate observation. After staining slides with SYBER, cells were scored per sample using comet scolar image analyzer soft ware For evaluation of the protective role of propolis against iron overload oxidative damage, the ratio of DNA damage in a 100 cell for each slide, head diameter and area, percentage of DNA in head, tail length and area, percentage of DNA in the tail.

Statistical Analysis

Data obtained from the present experiment were analyzd by analysis and variance (ANOVA), using one-way analysis using SAS (Statistical Analysis System) and Microsoft Office Excel (Microsoft Office Excel for windows; 2010). Least significant differences (LSD) was performed multiple (multiple comparisons), to evaluate significant differences

Results

Iron overload: results in Figure -1 clearly demonstrated that the i/p injection of 100mg.kg iron dextran each 72 hour cosequativly for 2 months caused significant elevation in plasma iron of IO group. Propolis decreased plasma iron to semi normal levels in IOP group.

Figure 1: Protective role of propolis on Serum iron against iron overload for two months, n =6, Means± SE. C = animals control IO = animals with iron dextran 100 mg/kg B.W IP every 72 hours, IOP = animals with iron dextran 100mg/kg B.W IP. Every 72 hours + treated with Propolis 50 mg/kg.BW orally daily

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Oxidant / Anti-oxidant Status

The protective role of propolis on abnormal an oxidant /antioxidant status caused by iron overload shown in Figure-2.

There were significant increase in MDA and decrease in GSH in IO group, while that Propolis decrease MDA and increase GSH in IOP group

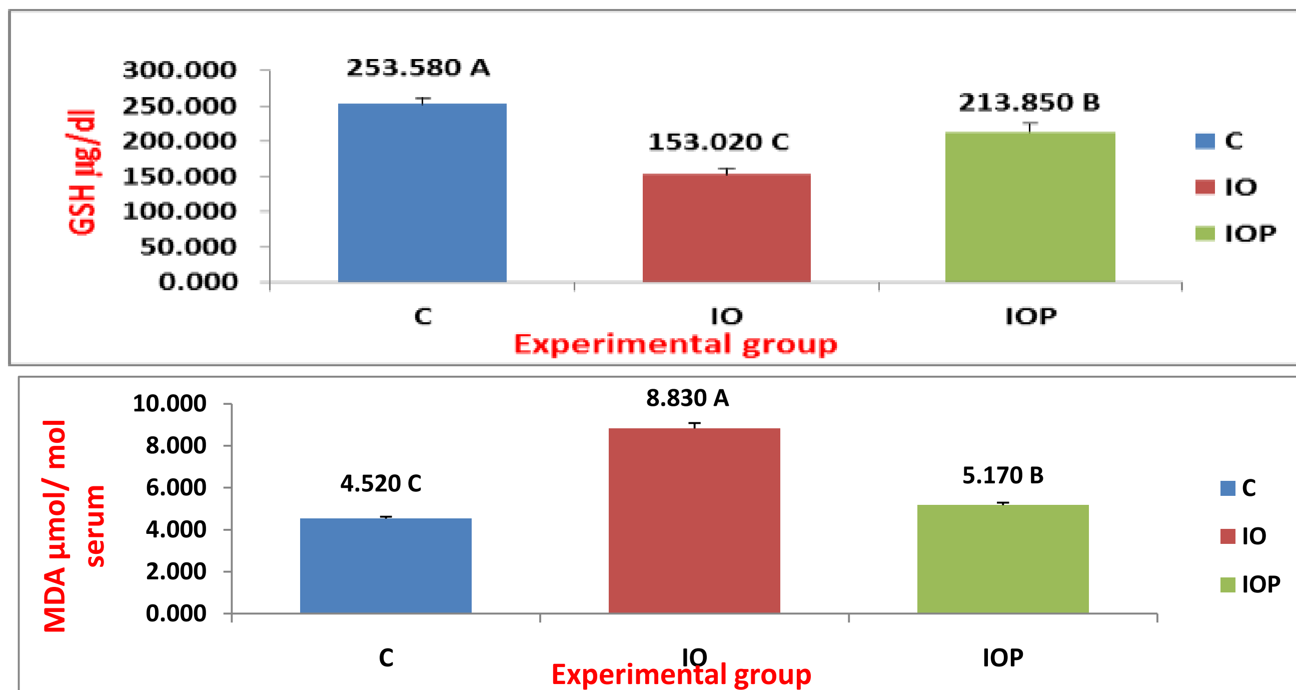

Figure 2: Protective role of propolis on oxidant/anti-oxidant status (glutathione (GSH) mg/dl), malondialdehyde (MDA) µmol/ mol serum) against iron overload for two months, n =6, Means± SE. C = animals control IO = animals with iron dextran 100 mg/kg B.W IP every 72 hours, IOP = animals with iron dextran 100mg/kg B.W IP. Every 72 hours + treated with Propolis 50 mg/kg.BW orally daily

DNA Damage Assay and the Protective Role of propolis against Iron Overload

DNA damage was analyzed by the Comet assay. Comet assay is the test used for investigate protective role of propolis against iron overload induced DNA fragmentation in hepatocytes of rats. Results in Figure 3.Showing the percentage of DNA damage in 100 DNA .The highest ratio of the DNA damage was in IO group (30.71 ± 0.86, 30.28 ± 1.56) Results revealed that administration of propolis caused significant decrease in the ratio of DNA damage (12.71± 0.55).Table 1.Showing the analysis of the comet scholar for the images of hepatocytes DNA gel electrophoresis (Pxl). There were a significant increase in head diamer, head area, tail length, tail area, and percentage of the DNA in the tail in IO group. Results of this test showed the beneficial role of propolis in decreasing all of these measurements.

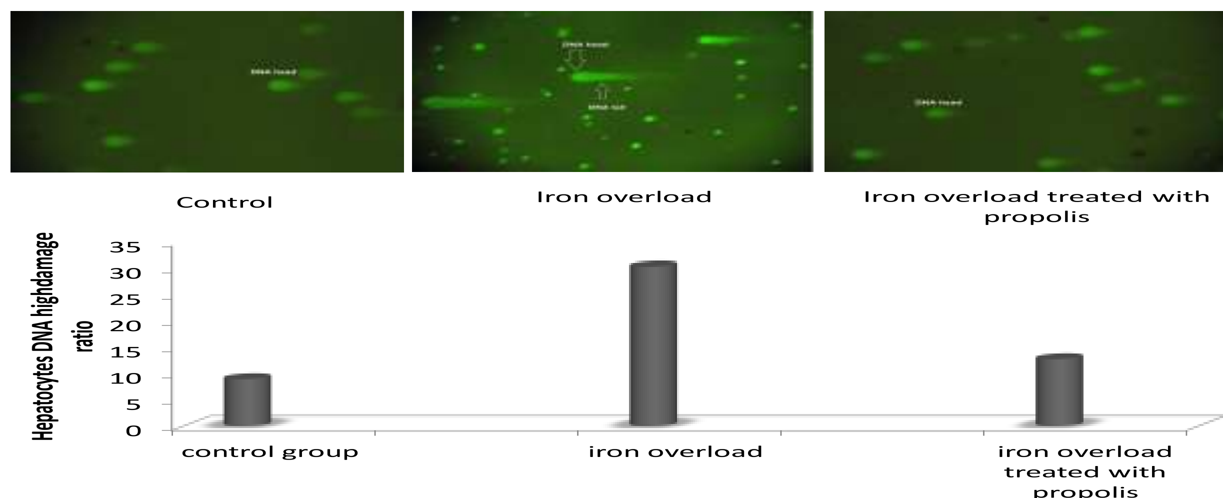

Figure 3: protective role of Propolis on percentage frequency of DNA fragment in hepatocyte (%) against iron overload for two months, n=6, M ± SE C = animals control IO = animals with iron dextran 100 mg/kg B.W IP every 72 hours, IOP= animals with iron dextran 100mg/kg B.W IP. every 72 hours + treated with Propolis 50 mg/kg.BW orally daily LSD =3.06

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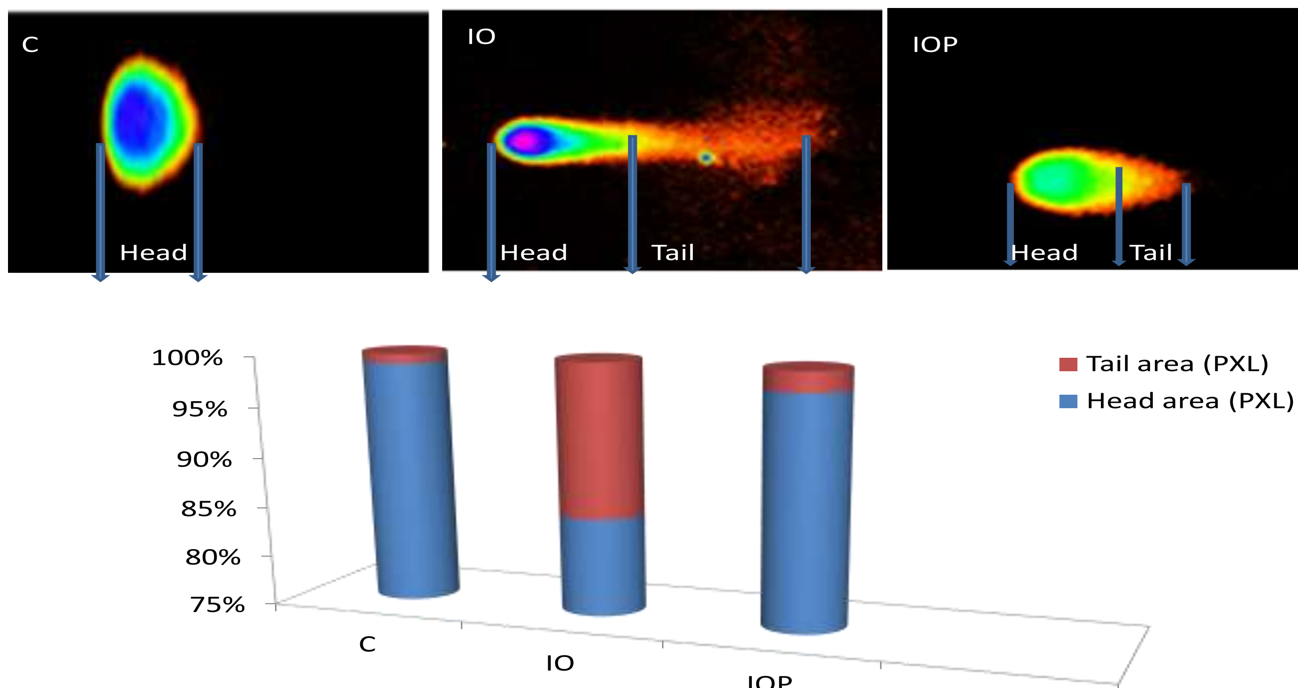

Figure 4: Image of comet scolar analysis shown the protective role of propolis on DNA fragment in liver tissue (px) against iron overload for two months by comet assay of A- control group, B- iron overload group and C- propolis treated group. Showing percentage damage of DNA fragment .SYBR green stain

Table 1: Protective role of propolis on comet scholar assay for DNA fragment in liver tissue (px) against iron overload for two months n =6, Means± SE

Groups / DNA fragmentation	C	IO	IOP	LSD
Head dimer (pxl)	284.43^{C}± 6.15	348.95^{A}± 10.73	331.95^{B}± 7.64	22.232
Head area (pxl)	33524^{C}± 1467.62	57134^{A}± 2509.28	42107^{B}± 1747.91	5193.9
Percent of DNA in head (pxl)	98.96^{A}± 0.66	98.97^{A}± 1.72	98.91^{A}± 0.230	2.602
Tail length (pxl)	2.33^{B}± 0.82	110.12^{A}± 7.73	12.48^{B}± 2.26	10.823
Tail area (pxl)	301.2^{B}± 106.66	10261.8^{A}± 1148.15	838.5^{B}± 206.73	1545.1
Percent of DNA in tail (pxl)	1.04^{B}± 0.65	13.50^{A}± 1.72	1.08^{B}± 0.23	2.602

C = animals control, IOP = animals injected with iron dextran 100mg/kg B.W intraperitonealy every 72 hours + treated with propolis 50mg.kg BW orally daily Iron overload, IO = animals injected with iron dextran 100mg/kg B.W intraperitonealy every 72 hours

Discussion

The present study designed to investigare the protective role of propolis against oxidant/ antioxidant status and DNA damaged induced by iron overload The oxidant in the body related to free radical production and oxidative stress are known to increase in response to environmental stress, including iron overload [25] lipid peroxidation as a consequence of increased free radical generation and increases the MDA level in blood and tissues [26].

Oxidative stress occurs when free radicals production increase or the antioxidant system becomes insufficient, leading to a prooxidant-antioxidant imbalance and an excess amount of pro oxidants. MDA, one of the most important products of lipid peroxidation, can affect ion exchange in the cell membrane and can lead to adverse effects, such as changes in ion permeability and enzyme activity [27]. Heavy metals such as cadmium, Another indicator of cellular damage and lipid peroxidation during the course of MCF may be increased MDA, and decreased GSH .Iron initiates lipid peroxidation by producing highly reactive hydroxyl radicals from hydrogen peroxide via Fenton type reactions or by complexion with oxygen directly to yield reactive perferryl and ferryl ions [28].

Chronic iron intake in rat's increases hepatic and splenic iron contents and these changes

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were associated with elevation in lipid peroxidation and decreased antioxidant defense mechanisms. Excess iron content in the cell is potentially detrimental because it is involved in oxidation reduction reactions, which in turn promote tissue injury by catalyzing lipid peroxidation [29].

Iron initiates lipid peroxidation by producing highly reactive hydroxyl radicals from hydrogen peroxide via Fenton type reactions or by complexing with oxygen directly to yield reactive perferryl and ferryl ions [30]. Anti oxidant mechanism in the body distributed in to enzymatic and non enzymatic, the GSH in a non enzymatic antioxidant with highly distribution in all body tissues particularly red blood cells. The low molecular weight glutathione, which is synthesized in the cell as a tripeptide (glutamic acid), exists in 2 different forms: an oxide and a reductant. It is observed in all cells It has roles in various processes, including antioxidant defense, GSH is also involved in clearing ROS [31].

Measurement of GSH in whole blood has been considering a biomarker for oxidative stress correction [32]. In iron overload rats the decreased blood GSH refer to depletion in red blood cells and hepatic cells in GSH. Iron overload known to cause depletion in sulpfer compound (GSH) in brain [33]. The disturbances in oxidant status of iron overload condition may attribute to abnormal mitochondrial function [34].

In the present study, the increase of antioxidant GSH caused by propolis supplementation may be considered as a protective mechanism against iron overload induced free radical production and lipid peroxidatio .Because of the high chemical complexity of propolis, it is difficult to identify which components are responsible for its biological action. Researchers suggest that propolis might be considered to prevent oxidative stress [35].

Alterations in the oxidant-antioxidant status during the administration of propolis have Also; treatment with Propolis caused reduction in thiobarbituric acid reactive substances (TBARS) level and increased the activities of antioxidant and the level of GSH. These data are in agreement with the results obtained by Jasprica *et al* [36] who reported that Propolis caused reduction in the malondialdehyd (MDA) level and increased the activities of the antioxidant enzymes. The genotoxic effect of iron overload was significantly related to DNA damage as assessed by the comet assay [37]. The comet assay has been used for a variety of applications including studies on toxicology, pollution, aging, exercise, training, and measurement of cell-growth and DNA-repair mechanisms [38]. The comet assay has also been used to study the effects of diet and antioxidant supplementation on oxidative DNA damage [39]. The genotoxic effect of iron overload, using comet assay was underlined by some of the iron indices examined [40].

It is highly likely that the pathologies caused by iron overload involve iron driven oxidation reactions, which the cumulative damage is ultimately translated into organ failure [41]. The most commonly used alkaline comet measures are tail DNA (percentage of DNA in the tail compared to the percentage in the 'head' or un fragmented DNA), tail length (the length of the tail measured from the leading edge of the head). Each of these parameters describes endogenous DNA damage corresponding to DNA strand breakage and/or alkali-labile sites.

In the optimization of the alkaline comet for use with hepatocyte, founded tail DNA to be the most reproducible parameter, therefore, hepatocyte DNA damage has been expressed as tail DNA throughout our studies [42] the honey products and its derivatives can reduce the DNA damage by scavenging of the reactive oxygen species (ROS) when cell were induced with genotoxic agents [43, 45].

Reducing or inhibiting the free radical can decrease the potential of having genotoxic related diseases such as cancer, inflammation disorder and cardiovascular disease [46]. Reducing of free radical was associated with the flavonoid content in the honey and its derivatives [47]. There was a correlation between the total bioactive compound and the protective effect towards DNA damage; higher total phenolic shown strongest protection to the DNA molecules [43].

However, honey and its derivates propolis, royal jelly or bee pollen had beneficial effects when taking in proper doses. It can decrease the free radical effect by chemical, environment, or it also can become a good anti-cancer agent. Surprisingly, honey and its derivatives can also increase the expression of DNA repair genes such as hOGG-1 and NEIL-1 [48]. That are responsible for

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repairmen of single-strand cleavage resulted from oxidative damage by free radicals [49]. Flavonoids that one of main compound of propolis that has shown promising results in protection against various DNA damaging agents, especially since they have antioxidant properties and they can also modulate DNA damage and repair pathways[50].

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