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EVALUATING THE EFFECTS OF *COMMIPHORA MOLMOL* (MYRRH) AGAINST OXIDATIVE DNA DAMAGE IN HUMAN LYMPHOCYTES

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Keywords:

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ABSTRACT: Oxidative stress is a condition that might predispose individuals to diseases, including cancer. The 8- hydroxydeoxyguanosine (8-OHdG) is an index that reflects DNA damage caused by oxidative stress in the body. In this study, *Commiphora molmol* (Myrrh) (C. molmol) (Myrrh) that belongs to the family Burseraceae were investigated for its potential favorable properties to blunt DNA damage induced by oxidative stress employing 8-OHdG assay using human lymphocyte cultures. Lymphocytes were separated from blood samples collected from healthy volunteers, cultures, and incubated in aqueous extract of *C. molmol* (Myrrh) at 0, 10, 100, and 1000 µg/ml. Extracts at 10,100 and 1000µg/mL from the oleo-gum resin of *C. molmol* (Myrrh) significantly decreased levels of 8-OHdG. The oleo-gum resin of *C. molmol* (Myrrh) medicinal plants can be used as useful agents to counteract oxidative DNA damage in cultured cells. Collectively, the data revealed that oleo-gum resin of *C. molmol* (Myrrh) possesses favorable antioxidants and therefore lends support to its therapeutic application.

INTRODUCTION: The oxidative stress results from an imbalance between free radicals and antioxidants wherein the production of oxidants overtakes that of antioxidants [1-3].

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Therefore, antioxidants are vital to maintaining the optimal health and well-being of cells and systems [4]. Free radicals are linked to an increased risk for cardiac malfunctions, cancer, and other chronic diseases [5, 6]. Moreover, it damages complex cellular components (such as DNA lipids and proteins) [7].

Previous research has paid considerable attention to the antioxidant properties of plants (vegetables, fruits, medicinal herbs, *etc*.) [8, 9], which are rich in various types of molecules that are able to scavenge free radicals, including terpenoids (including carotenoids), nitrogen compounds (alkaloids, betalains, amines), phenolic compounds (phenolic acids, quinones, flavonoids, lignans, coumarins, tannins) and other endogenous metabolites [10, 11]. Studies show that most of these compounds possess antiatherosclerotic, antimutagenic, antiinflammatory, anticarcinogenic, antibacterial, and antiviral actions [12] and are correlated with lowering the effects of diabetes, cardiovascular disease, and aging-related diseases [5, 6].

DNA is a key objective of ROS, and DNA damage caused by ROS is closely related to several pathological situations, for instance, cancer, cardio-vascular diseases, and diabetes [13]. Medicinal plants include different components of antioxidants, such as polyphenolic and volatile compounds, which protect cells from the adverse effects of ROS [14].

These compounds have an antioxidant effect due to their ability to give electrons to ROS, chelate metal ions, and stimulate antioxidants [15]. The exploration of natural antioxidants has therefore become a popular field of research in recent years, and traditional medicine is regarded as a point of research [16].

C. molmol is a spiny and sturdy shrub with a short trunk, which is about 4m tall. It is a perennial tropical plant mostly found in semi-arid and arid regions of Saudi Arabia, India, and East Africa [17]. The outer bark, which is whitish, bluish or silvery in color and peels in large and small thin paper-like flakes from the greener under-bark. The bark exudates viscid, mostly unscented, and hard-translucent yellowish gum resin. All branches are knotted, and spine-tipped [18]. On the other hand, the Myrrh is mostly grown in Eastern Mediterranean countries, South Arabia, and Africa.

The Myrrh exudes a viscous liquid, which is pale yellow-white in color, from the natural fissures and cracks present in the bark [19]. The viscous liquid is also harvested from the bark by intentionally making fissures and cracks. The exude becomes hard and turns into a reddish-brown mass upon air-drying [20].

This air-dried mass mostly contains white patches, which are about the walnut-size and are the basis of myrrh resin. Summer is considered the ideal season for Myrrh extraction.

The major volatile oils of the shrub include sterols, steroids, and sesquiterpenes, which, together, constitute 2 to 10% [12]. On the other hand, ethanol and water-soluble resins contain 30 to 60% and 25 to 40% of proteins and polysaccharides, respectively [21]. The gum generates a wide variety of sugars upon hydrolysis [22]. The Myrrh's odor is attributed to its furano sesquiterpenes, which are also suggested to possess hypoglycaemic, anti-fungal, antibacterial, and anesthetic properties [23].

Moreover, extracts of *C. molmol* including curzarene and 2 sesquiterpenes, furaneudesma-1-3-diene have been shown to activate opioid receptors in CNS [24]. Additionally, the gum is shown to possess an oxidase enzyme. It has been demonstrated that mixing of oleo-gum-resin with water results in the formation [25].

The myrrh of *C. molmol* has been in use for years for treating wound injuries. A number of studies have proven the health benefits of myrrh as a constituent of medicine [23]. Likewise, Commiphora species are reported to exert anti-ulcer, anti-inflammatory and analgesic, hypolipidemic, and anti-oxidant effects [17, 18, 22, 23, 26, 27]. Commiphora tree is also well-known to contain antischistosomal and antibacterial properties [27, 28].

The 8-OHdG assay has been shown to be useful to screen medicinal plants for their antioxidative properties and to prevent DNA damage induced by different drugs [14, 29].

Accordingly, the present study aims to evaluate the ability of oleo-gum resin of *C. molmol* (Myrrh) to exert beneficial effects in preventing DNA damage induced by oxidative stress. The effect will be evaluated by measuring 8-Hydroxy-2-deoxy-guanosine (8-OH-dG), which is a well-established marker to assess DNA damage.

MATERIALS AND METHODS:

Plant Material: The oleo-gum resin of *C. molmol* (Myrrh) was purchased from a local traditional herb market in Al-Madinah Al-Menawwarh, Kingdom of Saudi Arabia. Plants were identified by Professor SamyZalat, Biology Department, Faculty of Science, Taibah University. 100 mg of the *C. molmol* oleo-gum resin mass material was crushed into a fine powder using sterile mortar and pestle and suspended overnight in DEMSO at room temperature, then warmed until 60 °C with continuous shaking to get a final concentration. A series of concentrations were then prepared from the stock suspension [31].

Subjects: In accordance with the institutional review board of Taibah University, the recruited volunteers were healthy, non-smoking adults (above 18 years of age) recruited from Taibah University. Blood samples were obtained from these volunteers, who were prevented from taking any medication during the course of the experiment. A volume of 0.9 ml heparinized blood was added to 9.1 mL of Peripheral Blood lymphocytes for chromosome analysis, max complete karyotyping media in order to establish lymphocyte cultures. Then, this was incubated at 37 °C temperature in a CO_2 incubator with suitable moisture content [30].

Phytochemical Screening: The occurrence of secondary metabolites, such as saponins, tannins, flavonoids, coumarins, alkaloids, quinone, anthraquinones, and terpenoids, was tested phytochemically. The positive results depend on the color change or precipitation that occurred following the addition of specific reagents.

Blood Cultures: Cultures were made by adding 1 mL of fresh blood samples in a 50 mL culture flask which contains 9 mL of complete karyotyping media (Pb-Max media, Gibco- *in-vitro* gen, Paisley, UK) 31, which contains RPMI-1640, consisting of 3% of phytohemagglutinin, 15% FBS and 1% penicillin-streptomycin.

Then, the cultures were incubated for 72 h in a CO_2 incubator at 37 °C [32]. Various plant extracts of different concentrations (10,100 and 1000 µg/mL) were used, and all concentrations were dissolved in dimethyl sulfoxide (DMSO) 24 h prior to the end of the incubation interval. A vehicle was used for treating the control cultures. The mentioned concentrations were chosen according to earlier studies [31, 33].

Determination of 8-OHdG Assay: The 8-OH-dG is a radical-damaged hydroxyl-guanosine that is usually found during regular metabolism. It increases ROS and RNS generation 8-OH-dG for the estimation of oxidative damage of DNA damage and is regarded as a valuable biomarker.

The 8-OHdG assay was measured according to Khabour *et al.*, 2014 [2]. For this purpose, blood cultures were incubated for 72 h at 37 °C, and washed with RPMI medium, which contains glutamine, Phytohaemagglutinin, and Penicillin-Streptomycin, followed by plant extract and incubation at 37 °C for 6 hours and centrifuged at 1000 xg.

About 200 ul of the filtrate was used for competitive assay of 8-OH-dG by ELISA conferring to the manufactured constructions (Stress Marq's &-OH-dG EIA kit; Biosciences, Canada). 8-OH-dG antibodies and the culture media samples were added in a 96-well plate, which was pre-coated with 8-OH-dG monoclonal antibody. Sample 8-0H-dG competed with the 8-OHdG bond on the plate for 8-OH-dG antibody binding sites.

Then, the plate was protected by plastic films and incubated for 18 h at 4 °C. Wells was emptied and rinsed by washing buffer, and 200 pl of Ellman's reagent, the substrate for 8-OHdG acetyl-cholinesterase was added. The plate was protected with plastic films and left for 90-120 min on a rotating shaker in the dark to obtain optimum development.

Finally, the ELISA plate was read at 405 nm using an automated reader (bio-TEK instrument/USA). Environmental factors that induced the 8-OH-dG in the sample were washed out, while those that reader (ELx 800/universal microplate reader, bio-TEK instrument/USA) [34].

Statistical Analysis: Data were analyzed by Graph Pad Prism software (version 5). The data were presented as mean ± SEM, and ANOVA was performed, followed by the Tukey post-hoc test to ascertain the significance. $P < 0.05$ was considered significant.

RESULTS:

Phytochemical Screening of an Oleo-gum Resin of *C. molmol* (Myrrh): Qualitative screening of phytochemical screening of an oleo-gum resin of *C. molmol* (Myrrh), showed that the plant comprises some secondary metabolites which were tannins, polyphenolic compounds, flavonoids, saponins and triterpenoids, alkaloids and saponins and volatile oils.

The 8-OH-dG Levels: The present study evaluated the effects of an oleo-gum resin of *C. molmol* (Myrrh) on blood lymphocytes by measuring 8-OHdG after 6 H of treatment. The results showed remarkable activity in a dose-dependent manner **Table 1** and **Fig. 1**. A significant decline in 8-OH-dG levels was noticed in samples treated with oleogum resin at different concentrations.

This suggests the favorable effects of resin against oxidative DNA damage (Control: 0.702 ±0.043, 10 µg/ml: 0.509 ± 0.057*, 100 µg/ml: 0.444 ± 0.091*, 1000 µg/ml: 0.416 ± 0.077 **Table 1** and **Fig. 1**.

Hence, *C. molmol* (Myrrh) appears to facilitate oxidative DNA damage in cultured human lymphocytes.

TABLE 1: VALUES OF 8-OH-DG INAN OLEO-GUM RESIN OF *C. MOLMOL* (MYRRH) (MEAN 9± SD)

S. no.	Name of the plant	Part of the plant	8-OH-dG levels (Mean ± SD) with different Plant concentration			
			0 µg/ml	10 µg/ml	100 µg/ml	1000 µg/ml
1	*C. molmol* (Myrrh)	Oleo-gum resin	0.702 ± 0.043	0.509 ± 0.037[a]*	0.444 ± 0.021[a]**	0.416 ± 0.007[a]**

8-OH-dG: 8-hydroydeoxyguanosine. A Compared to control, *p<0.05; **p<0.01

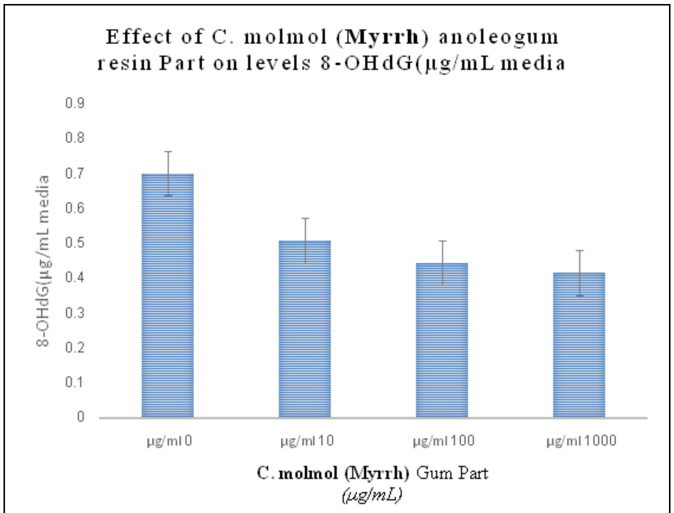

FIG. 1: 8-OHDG LEVELS AFTER TREATMENT WITH DIFFERENT DOSES OF *C. MOLMOL* (MYRRH) GUM PART

Levels of 8- OHdG in control and *C. molmol* (Myrrh) Gum Part groups (0, 10, 100, and 1000 µg/mL) in cultured blood cells. The level of 8-OHdG in *C. molmol* (Myrrh) Gum Part treated groups was significantly lower than that in the control group.

DISCUSSION: The World Health Organization (WHO) evaluates that about 80% of the world's occupants depend predominantly on customary means for health care. Herbs are absolutely the most dominant natural antioxidants and are extremely crucial due to their antioxidant and anti-aging properties. Natural products possess an indefinable variety of chemical structures. The activities of these lead compounds can be improved by de novo synthesis and by chemical management [12]. Currently, numerous medicinal plants proved to be effective in fighting various illnesses, which leads to widespread screening for the presence of medicinally beneficial components. Antioxidants are broadly utilized as components in dietary supplements and used to keep up well-being and avoid oxidative stress-induced illnesses, for example, diabetes, atherosclerosis, aging, cancer, and inflammation. Several antioxidants have been obtained from various plant materials recently [9, 22, 29]. Natural antioxidants are likewise in intense interest for application as food additives and nutraceuticals due to buyer inclinations [35]. Apart from their uses in medicine, these compounds are utilized as food and cosmetic preservatives and for inhibiting the degradation of gasoline and rubber. Antioxidants are also used as essences to protect against food decomposition. Plant polyphenols are specifically significant among natural antioxidants [35].

Nowadays, the exploration for natural compounds abundant in antioxidant, antimicrobial, and anti-cancer properties is heightening a direct result of their significance in controlling numerous chronic disorders for screening of selected example, cancer and cardiovascular infections [36]. It has been surveyed that roughly 66% of anticancer medications endorsed worldwide till 1994 were obtained from plants [37].

The phytochemical screening Oleo-gum resin of *C. molmol* (Myrrh) showed that the plant contains tannins, polyphenolic compounds, flavonoids, saponins and triterpenoids, alkaloids, saponins and volatile oils. These results corroborate with those of [38] who demonstrated the presence of the plant contains tannins, polyphenolic compounds, flavonoids, saponins and triterpenoids, alkaloids and saponins and volatile oils [38]. Phytochemical analysis showed significant flavonoid and total phenolic contents in the oleo-gum resin of *C. molmol* (Myrrh) and in line with their potential free radical scavenging activities. Although the ascorbic acid of this extract was insignificant, indicating that the antioxidant potential is due to its flavonoid and total phenolic contents.

Phenolics are regular common items found in plants and have considerable antioxidant and anti-inflammatory impacts. Different phenolic compounds, for instance, gentisic acid, gallic acid, vanillic acid, protocatechuic acid, phydroxy benzoic acid, ellagic acid, syringic acid, and cinnamic acid derivatives, which incorporate chlorogenic acid, caffeic acid, sinapic acid, ferulic acid and p-coumaric acid, are to a great extent present in plants.

These phenolic compounds are transcendentally accessible in guggul (Myrrh), too, to some extent, adds to its gigantic organic capacity against varied chronic diseases [39]. Anti-oxidants cannot have powerful antiangiogenic activity, between those that have been recognized include vitamin D, vitamin C, vitamin A, vitamin E, rosmarinic acid, 3-hydroxyflavone, 3', 4'- dihydroxyflavone and 2', 3'-dihydroxyflavone [40, 41]. Free radicals are substantial indication that free radicals prompt oxidative damage to biomolecules and show a vital part in aging, cardiovascular diseases, aging, cancer, inflammatory disease and a range of other disorders [3, 4].

Free radical scavenging activity for three concentrations of *C. molmol* (Myrrh) was studied and considered important to help understand the mechanism of action of *C. molmol* oleo-gum resin. It could be due to certain chemical constituents of *C. molmol*, which possess rich oxygen-free radical scavenging and antimutagenic potential.

Antioxidant and defensive properties of *C. molmol* are due to the substance of antioxidant active components such as sesquiterpenes eugenol, and cuminic aldehyde [40]. The presence of terpenes (specifically sesquiterpene) in *C. molmol* may explain the antioxidant mechanisms as described previously, as terpenes may show their pharmacological effect through antioxidant properties. These active compounds consider very potent in contributed to its significant antioxidant [4]. Antioxidants can alter the physiological redox balance that alleviate reactive oxygen species (ROS) that tend to be prevalent in low oxygen tension areas such as that in the tumor [36].

CONCLUSION: Different concentrations of an oleo-gum resin of *C. molmol* (Myrrh) showed the potentially significant capacity to abrogate oxidative DNA damage. It may be due to the presence of several chemical constituents of *C. molmol*, which possess promising antimutagenic and oxygen radical scavenging potential. Antioxidant and defensive properties of *C. molmol* are due to the substance of antioxidant active components such as sesquiterpenes eugenol, and cuminic aldehyde. An oleo-gum resin of *C. molmol* (Myrrh) seems to be interesting for further pharmacological investigations.

HUMAN AND ANIMAL RIGHTS: Volunteers gave written informed consent as required by the institutional ethics committee at Taibah University Study ID: 073 – 1439. IORG0008716 – IRB00010413

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