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Combined administration of curcumin and gallic acid inhibits gallic acid-induced suppression of steroidogenesis, sperm output, antioxidant defenses and inflammatory responsive genes

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ABSTRACT

In this study, we investigated the effects of administration of gallic acid (Gal) with or without curcumin (Cur) on the sperm output, steroid level and antioxidant defenses in rat testis *in vivo* and the expression of inflammatory responsive genes *in vitro*. Male Wistar rats were divided randomly into four groups and given oral Gal (100 mg/kg/day) and Cur (100 mg/kg/day) alone or in combination for four weeks. The sperm quality was impaired following Gal treatment, while Cur prevented this and also improved the sperm count as well as the efficiency of sperm production (DSP/gm testis). The inhibitory effects of Gal on plasma testosterone level, glutathione levels, activities of glutathione peroxidase, catalase, superoxide dismutase and steroidogenic enzymes, 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-HSD in the rat testis was blocked by Cur. Interestingly, the level of testosterone and the activities of the steroidogenic enzymes were significantly increased after treatment with Cur alone. Malondialdehyde concentration was unchanged following Gal treatment, while a significant decrease in malondialdehyde level was observed following treatment with Cur alone or in combination with Gal. We further analyzed the effects of Cur and Gal (25–100 μM) on the 93RS2 Sertoli cell-lines and observed that Cur blocked the Gal-induced suppression of inflammatory mediators such as TNF-α and IL-6, while Gal blocked the suppressive effect of Cur on IL-1α expression. Furthermore, the stimulatory or inhibitory effects of Gal on the expressions Tgf-β1 and CD-14 was concentration-dependent and could be blocked by Cur. When cultures of primary Sertoli cells were exposed to both Cur and Gal for 24 h, p-JNK/SAPK expression remain stable, whereas Gal-induced p-p65 (NF-κB) expression and IκBα degradation was seen to be blocked by Cur but not Gal-induced expression of pERK1/2. Overall, Cur has stimulatory reproductive effects and could protect the testis from the toxic effects of Gal by mechanisms that could not be explained by its effects on the expressions of inflammatory cytokines but by its anti-oxidant properties.

1. Introduction

Gallic acid (3,4,5-trihydroxybenzoic acid) and curcumin (diferuloylmethane) are both natural plant phenols (Fig. 1) with well-known antioxidant and anti-inflammatory properties [1–3]. Curcumin is the principal curcuminoid found in turmeric, and is generally considered as its most active constituent [4]. It is commonly used in India and in most tropical and subtropical regions of the world as a spice and medical agent [4]. Gallic acid is widely consumed in the form of gallnuts, grapes, tea leaves, oak bark, strawberries, pineapples, bananas, lemons, both in its free state and as part of the tannin molecule [5,6]. Apart its antioxidative and anti-inflammatory properties, Gal is also well known for its antiallergic, antimutagenic, anticarcinogenic, antiviral, and anti-bacterial abilities [6]. It has also been found to induce apoptosis in some tumor cell lines [7,8] and plays an important role in preventing malignant transformation and cancer development *in vivo* [9]. Furthermore, many studies—including *in vitro* and animal studies have suggested that Cur may have antitumor, antiarthritic, anti-amyloid, and anti-ischemic properties [10]. The most important feature of Cur is that it has no toxic effects despite being a therapeutic agent with multiple beneficial functions [11]. It acts as a scavenger of free radicals and is considered to be an effective antioxidant against oxidative tissue damage [12].

Some phytochemicals, including, Gal also has the potential to function as pro-oxidant [1] in addition to acting as antioxidant [13].

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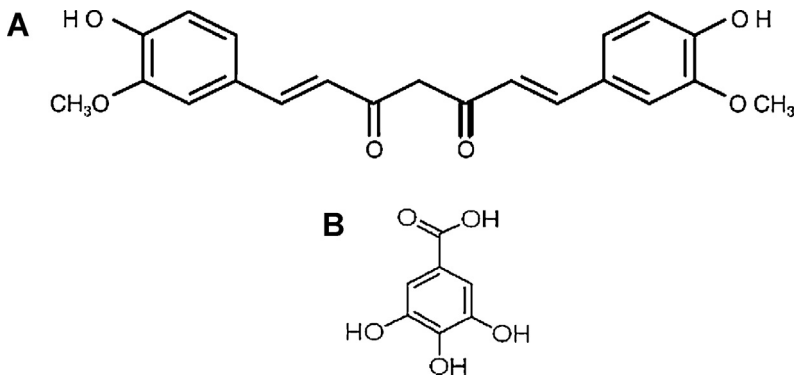

Fig. 1. Chemical structures of Curcumin (A) and Gallic acid (B).

This potential of Gal as pro-oxidant might explain the increased cytotoxicity in hepatocyte cell line HL-7702 [6], human blood lymphocytes [1], liver, kidney and blood of rats [14] and in mouse models testicular cell lines such as the spermatogonia, spermatocytes, Sertoli and Leydig cells [1,15]. These effects may be explained by a concentration-dependent manner; that is high doses of a phytochemical may produce a deleterious effects, whereas low doses may be beneficial to human health including chemoprevention of cancers and cardiovascular diseases [16].

There are also contrasting results on the male fertility effects of some plant-derived polyphenols depending on whether the experimental design was *in vivo* or *in vitro*. For instance, tannic acid and Cur inhibits mammalian sperm motility *in vitro* [1,17–19], gossypol inhibits spermatogenesis and sperm motility *in vivo* [1], the flavonoid, chrysin, improves testosterone levels and sperm quality of rats [20,21]. Similarly, anecdotal evidence claims that some plants enhance fertility by improving spermatogenesis [22]. However, there is yet no *in vivo* data on the reproductive effects of Cur and Gal. Furthermore, our current knowledge about the reproductive effects of Cur is mainly from researches based on disease models. For example, Cur displayed a protective function on testicular tissues under various pathological conditions [3,23–26]. However, its molecular effect on normal testicular tissues or cells has not been systematically evaluated.

The capacity of polyphenolic compounds to function as prooxidants may be dependent on their consumption sources [16]. Gallic acid is abundant in many fruits and plants and are added to foods to prevent oxygen-induced lipid peroxidation [1] and Cur is the principal component of the curry spice turmeric (*Curcuma longa)* used in many parts of the world [4]. Nevertheless, the combined effects of these compounds have not been evaluated *in vivo*, although both have been in use as food additive for many years. Therefore, this study was performed to investigate the effect of a 30-day treatment (100 mg/kg/day) of Gal and Cur alone or together on the antioxidant defense system in rat testis, steroidogenesis and sperm quality of rat *in vivo*. Because, Sertoli cells are important in regulating inflammatory processes that control testicular functions [27], they were incubated *in vitro* with different concentrations of Cur (25–100 μM) and Gal (25–100 μM) alone or in combination and the expressions of inflammatory responsive genes were evaluated as endpoint markers of inflammatory processes.

2. Materials and methods

2.1. Chemicals and reagents

Curcumin and Gallic acid were purchased from Sigma-Aldrich, Chemie, GmbH, Taufkirchen, Germany). All other used reagents were of analytical grade and purchased from Sigma unless otherwise stated.

2.2. Animal treatment for in vivo experiment

Twenty male Wistar rats (average weight of 128.25 g) were obtained from an animal vendor at the University of Ibadan, Nigeria and were allowed to acclimatize for 1 week prior to the start of study. The animals were housed in metal cages under a well ventilated condition, maintained under a 12-h light/dark cycle and provided with standard commercial pelleted feed and water *ad libitum*. After acclimatization, the animals were assigned randomly into four groups of five animals, including a control group and three experimental groups. The three treatment groups of rats received 100 mg/kg/day of Cur or Gal, or the combination of Cur and Gal (100 mg/kg/day + 100 mg/kg/day). Control rats were administered equivalent volumes of corn oil only. Cur and Gal were diluted with the vehicle (corn oil) and administered to all animals (0.3 mL/kg) by gavage four times a week for 30 days. Experimental protocols were in accordance with the principles and procedures of the National Institute of Health Guidelines for Animal Care and Use of Laboratory Animals. At the end of 30 days, the animals were fasted overnight, weighed and killed by cervical dislocation. Body weight was recorded prior to sacrifice. Blood samples were collected for hormonal analysis. The paired testes, epididymides, prostate and seminal vesicles (intact) were dissected out quickly and washed in 1.15% KCl (ice cold) and pat-dried and the wet weight taken. One side testis was fixed in Bouin's fluid for histopathological examination.

The dose of Gal (100 mg/kg) was based on its no-observed-adverse-effect level (NOAEL) in rats [14]. The dose of Cur was chosen based on previous studies which reported protective function on testicular tissues under various pathological conditions [28,29].

2.3. Assessment of testis oxidative stress

Testis tissues were homogenized in ice-cold 0.1 M Tris–HCl buffer (pH 7.4) to produce 10% homogenate. The homogenate was centrifuged at 5000 rpm and 4 °C for 15 min and the supernatant was separated to measure the biochemical parameters described below. Levels of malondialdehyde (MDA) and glutathione (GSH) and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) were measured. Protein concentration of the tissue homogenate was determined by the Lowry method [30], using bovine serum albumin as the standard.

2.3.1. Assay of antioxidant systems

The MDA content, a measure of lipid peroxidation, was measured as thiobarbituric acid reactive substances in testicular tissues according to the method described previously [31]. End products of