Evaluation of phytochemical compositition and in vivo antioxidant activity of Gum Arabic aqueous Extract in Gentamicin toxicated rats

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Abstract:

Gum Arabic of *Acacia senegal* is produced in Sudan within Gum Arabic belt which covers about 20% of the country area, where it forms a major source of income to farmers in Kordofan, Darfur and eastern Sudan. Chemically Gum Arabic is a branched chain complex mixture of polysaccharides and glycol proteins either neutral or slightly acidic, found as a mixed calcium, magnesium and potassium salts of polysaccharide acid. In our previous study we confirmed the nephroprotective activity of Gum Arabic aqueous extract. In the present work, we wish to report the in vitro and in vivo antioxidant potentiality of the aqueous extract of Gum Arabic in rat. Antioxidant activity was measured in vitro by measuring the radical scavenging activity (RSA) of Gum Arabic aquous extract, and in vivo by measuring the activity of catalase and superoxide dismutae enzymes.

The radical scavenging activity (RSA) of Gum arabic aqueous extract was found to be 20%. The phytochemical screening of the extracts exhibits the presence of Flavonoids, Saponins and Cumarins. The administration of gum Arabic aqueous extract improved the activities of catalase and superoxide dis mutase in albino rats.

Key words: Gum Arabic, antioxidant, catalase, superoxide dismutase Introduction:

Acacia senegal (Gum Arabic tree) is a shrub or tree usually 2-15 m high, rarely smaller bark on trunk yellowish to grey or grayish-brown, rough or smooth and papery and peeling off crown variable, loose and rounded to dense and flattened.

Gum Arabic is produced in Sudan within Gum Arabic belt which covers about 20% of the country area, where it forms a major source of income to farmers in Kordofan, Darfur and eastern Sudan. Chemically Gum Arabic is a branched chain complex mixture of polysaccharides and glycol proteins either neutral or slightly acidic, found as a mixed calcium, magnesium and potassium salts of polysaccharide acid (Ali et al 2009).

Amino glycoside antibiotics have been widely used for gram-negative bacterial infections. However, their nephrotoxicity and ototoxicity are the major limitations in clinical use.

Amino glycosides throughout the endocytic pathway are taken up into the epithelial cells of the renal proximal tubules and stay there for a long time, which leads to nephrotoxicity. Acidic phospholipids, broadly distributed in the plasma membranes in various tissues, were considered to be the binding site of amino glycosides in brush-border membrane of proximal tubular cells ,Hydroxyl radicals play a role in the pathogenesis of gentamicin nephrotoxicity (M.Pramila Padmini and J. Vijay Kumar 2012)., gentamicin can induce suppression of Na(+)-K(+)-ATPase activity and DNA synthesis in rats proximal tubules leading to renal injury; this injury may be relevant to reactive oxygen metabolites generated by gentamicin. Renal cortical mitochondria is the source of reactive oxygen metabolites, which induces renal injury (M.Pramila Padmini and J. Vijay Kumar 2012).

Materials and Methods:

Animals: albino rats of either sex weighing150-200 gram were used in this study. They were housed in clean polypropylene cages, 10 rats per cage; under controlled laboratory conditions and fed with standard rodent diet and water ad *libitum*. The rodents were allowed to acclimatize to these conditions for one week prior to the commencement of the study.

Preparation of *Acacia senegal* (Gum Arabic) aqueous extract:

Acacia senegal gum was collected from West Sudan (North Kordofan State). The plant material was taxonomically identified and authenticated by taxonomy expert at Herbarium of Medicinal and Aromatic Plants Research Institute (MAPRI), National Center for Research (NCR), Khartoum, Sudan where the voucher specimen has been deposited. Extraction was carried out according to the method described by Handa et. al. (2008). 80 g of the sample was soaked in 500 ml hot distilled water, and left till cooled down with continuous stirring at room temperature. Extract was then filtered and freezed in a deepfreeze. The extract was dried using freezdrier till powdered extract obtained. Yield percentage was calculated.

Samples collection:

After acclimatization, the animals were divided randomly into four groups (n=10), and placed in metabolic cages separately for collecting blood samples. The animals were divided into normal control group (fed with the standarad diet and water ad liptum.), gentamicin group (gentamicin 80mg/kg/day i.p.) and treated group (Gum arabic -250mg/kg/day and 500mg/kg/day, started 4 days prior orally and concurrently with Gentamicin 80 mg/kg i.p. for six days). Blood was collected in the first day (day 0) and then every five days from the orbital plexuses.

Phytochemical analysis:

Phytochemical analysis for the active constituents of Gum Arabic aquous extract was carried out using the methods described by (Martinez et al (2003), Sofowora (1993), Harborne (1984) and Wall et al (1952) with few modifications

In vitro Antioxidant assay:

Determination of DPPH radical scavenging activity:

The free radical scavenging activity was determined by the DPPH assay described by shimada et.al. (1992) with some modification. In 96-wells plate, the test samples were allowed to react with 2.2 Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37'c. The concentration of DPPH was kept as (300µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

In vivo antioxidant assay:

At the end of the experimental period blood was taken for analyses which include measurement of Catalase using commercial kits (Catalase (CAT) assay kit (visible light), A007-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China. spectrophotometric method and Superoxide dismutase (SOD) using commercial kits (SOD) typed assay kit (Hydroxylamine method), A001-2, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) using spectrophotometer.

Statistical analysis:

Data were expressed as mean ± standard error of mean (SEM). Statistical evaluation was done using SPSS (version 16.0). The differences among treated groups were analyzed by one-way ANOVA followed by Tukey's test. ($P < 0.05$) was considered statistically significant.

Results:

The radical scavenging (antioxidant) determination of Gum Arabic extract:
The DPPH radical scavenging was determined in Gum Arabic extract, Table 1, the percentage equal (20%).

Table1. The DPPH radical scavenging determination (antioxidant) of Gum Arabic extract.

No	Sample code	% RSA±SD (DPPH)
1	Gum Arabic	20±0.01
2	Propyl Galate(PG)	
(Reference chemicals)	95±0.01	

Phytochemical screening of Gum Arabic extract:

Phytochemical results, Table 2, revealed that Gum Arabic aqueous extract exhibits the presence of Flavonoids, Saponins and Cumarins.

Table 2.Phytochemical screening of Gum Arabic extract

Key: + Trace ++ Moderate +++ High concentration - No observation

Test	Sample code	% RSA ± SD (DPPH)
Observation	Result	20±0.01
Triterpenes	No observation	-
Flavonoids	No observation	+
Saponins	Foam	+++
Cumarins	UV absorption	+
Tannins	No observation	-
Sterols	No observation	-
Alkaloids	No observation	-
Anthraquenone glycosides	No observation	-
Cyanogenic glycosides	No observation	-

In vivo antioxidant determination:

Serum Catalase activity:

Table 3 shows changes in serum catalase activity of albino rats given gum Arabic extract (250, 500mg/kg B.W) and gentamicin (80mg/kg i.p). At day 0 there was no significant difference observed in the activity of catalase between all groups when compared with the control group (A), at day 10 there was a significant inhibition ($P > 0.05$) in Catalase activity in group (B) which given gentamicin (80 mg/kg B.W-ip) when compared with group (A) the control group, while there was a significant increase in the activity of catalase in the treated group (group C and D which given 250 and 500 mg/kg B.W Gum Arabic extract when compared to Gentamicin group while there was no significant difference when compared to the negative control.

Table3. The activity of Catalse (U/ml) in sera of rats given gum Arabic at different doses with gentamicin

GROUP	DAY 0 Mean ± SE	DAY 5 Mean ± SE	DAY 10 Mean ± SE
A	15.01± 1.06a	14.36 ± 1.07b	14.27 ± 0.47bc
B	16.09 ± 2.32a	13.46± 0.99 b	7.75 ± 1.20a
C	15.43 ± 0.45a	08.79 ± 0.11a	11.90 ± 1.57b
D	15.25 ± 1.51a	15.47± 0.20b	15.97 ± 0.62c

Data are means ± SE
Means in the same column followed by the same letters are not significantly different at ($p > 0.05$).
A= Negative control
B= Positive control (Gentamicin80mg/kg B.W)
C= Gum Arabic 250mg/kg B.W +Gentamicin80mg/kg B.W
D= Gum Arabic 500mg/kg BW +Gentamicin 80 mg/kg B.W

Serum Superoxide Dismutase activity (SOD):

Table 4 demonstrates changes in serum SOD activity of albino rats given gum Arabic extract (250, 500mg/kg B.W) and gentamicin (80mg/kg i.p).

At day 0 and day 5 there was no significant difference in the activity of Catalase between all groups when compared with the control group (A). In day 10 there was a significant decrease ($P < 0.05$) in the activity of SOD in group B which given gentamicin (80mg/kgB.W ip) when compared with group (A) the control group, while there was no significant difference in SOD activity in group C or D which given (250 &500 mg/kg B.W Gum Arabic extracts respectively when compared with group (A) the control which improved towards the normal values.

Table.4 The activity of Superoxide dismutase (U/ml) in sera of rats given gum Arabic at different doses with gentamicin

GROUP	DAY 0 Mean ± SE	DAY 5 Mean ± SE	DAY 10 Mean ± SE
A	90.82 ± 3.22a	92.48 ± 2.45a	92.42 ± 4.82b
B	92.68 ± 1.26a	84.12 ± 2.34a	42.97 ± 9.66a
C	81.98 ± 4.10a	86.97 ± 3.00a	74.80 ± 5.13b
D	88.64A ± 1.75a	87.50 ± 7.47a	64.70 ± 8.37ab

Data are means ± SE.
Means in the same column followed by the same letters are not significantly different at ($p > 0.05$).
A= Negative control
B= Positive control (Gentamicin80mg/kg B.W)
C= Gum Arabic 250mg/kg B.W +Gentamicin80mg/kg B.W
D= Gum Arabic 500mg/kg BW +Gentamicin 80 mg/kg B.W

Discusion:

The proximate analysis of the aqueous extracts of gum Arabic from *Acacia senegal* in the present study exhibited: moisture contents 9.5%, Ash 3.5%, protein 2.45%, Ether extract 1.7%, Fiber 0.5%, Nitrogen free extract 82.35%, Energy 283.8 respectively. Azzouri et al (2015) mentioned that gum Arabic contains: (12.5-16) % moisture, (1.5-2.6) % protein, (0.22-0.39) % nitrogen, 39 42% galactose, 24 27% arabinose, 12 16% rhamnose, 15 16% glucuronic acid. Lelon et al (2010) found that Gum Arabic exudate from Senegal variety of Kenya contain: Moisture (14.9-15) %, Ash (2.94- 3.16) %, Energy (32.96-33.0) %. Yusuf (2011) found that Gum Arabic exudate from Senegal variety of Nigeria contains: Moisture (13.40) %, Ash (3.42) %, protein (2.77)%. Ghashua et al (2013) found that Gum Arabic contains: moisture 13.91%, Ash 3.01%, protein 2.81%, soluble fiber 82.05. The moisture content in this study 9.5% is low than FAO standard levels (13-15) %. Ash content 3.5 agrees with FAO maximum limit (2.0-4.0) % for food and pharmaceutical quality Gum Arabic. Protein content in this study (2.45) agrees with Azzouri et al (2015) who found that Gum arabic contain (1.5-2.6) protein.

The primary phytochemical screening test may be useful in the detection of the bioactive principles and subsequently may lead to drug discovery and development. Further, these tests facilitate their qualitative separation of pharmacological active and chemical compound and also the quantitative assessment (Esraa. 2016).

In the present study, the phytochemical screening tests of *Acacia senegal* aquous extract revealed the presence of Saponins, Cumarin and flavooids, while the other constituent were not found, Shi et al., (2004) showed that a high saponin diet has the ability to manage renal problems. Since phytochemical analysis of the extracts revealed the presence of saponins, it helps to suggest its possible role in renal protection.

Antioxidant activity:

It was known that antioxidant in foods have many health benefits including prevention of various diseases associated with oxidative stress such as cancer, cardiovascular disease, neuro-degeneration and diabetes and others. However, there is a little knowledge of antioxidant activity in a variety of plants.

There is strong evidence that reactive oxygen species including free radicals can lead to lipid peroxidation and oxidative stress which damage biological structures such as proteins, lipids and DNA (Gülçin *et al.* 2003). They result in body ageing and chronic diseases such as heart disease, stroke, certain cancers, neurodegenerative diseases and lung disorders (Yildirim *et al.* 2001). In general, the human body has its own natural antioxidant system to stand against free radicals using certain enzymes. It is believed that an intake of antioxidants reinforces the defense of human antioxidant system. Fruits, vegetables and spices are well known for their natural antioxidants. It has been found that high intake of fruits and vegetables has been associated with lower incidences of chronic diseases such as cancer and heart diseases (Bravo, 1998) .Antioxidant activity of plants or fruits work in several mechanisms including free radical scavenging, hydrogen donation, singlet oxygen quenching, metal ion chelating and acting as a substrate for radicals such as super oxide and hydroxide.

There are several methods to measure total antioxidant activity of a compound or plant extract based on hydrogen atom transfer (HAT) reactions and electron transfer (ET) (Huang et al. 2005). 2, 2- di phenyl- 1-picrylhydrazyl) radical scavenging assay (DPPH) is one of the few stable and commercially available organic nitrogen radicals and has a UV-vis absorption maximum at 515 nm (Miliauskas et *al.* 2003).

In this study the antioxidant activity of Acacia senegal Gum Arabic via DPPH radical scavenging activity was found to be 20%. This result of low antioxidant activity of gum Arabic agree with Ali (2003) while it was in contrast with Almajed et al (2003), Abdalla *et al* 2002 who reported that gum Arabic has a strong antioxidant activity. Amira Kassem and Aminah Abdullah (2015) found that the antioxidant activity of *Acacia senegal* Gum Arabic via DPPH radical scavenging activity of 50% (Acetone extrat , Ethanolic extract , methanolic extract) were found to be (3.00 , 2.39 and 1.70) respectively and they considered that Gum Arabic has a strong antioxidant properties.

In this study the administration of gum Arabic aquous extract improved the activities of catalase and superoxide dis mutase enzymes in albino rats.