Toxic effect of tannic and related compounds on human plasma proteins*

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ABSTRACT

Objective: To investigate the toxicity of tannic acid related compounds such as gallic acid and polyphenol on the activity of plasma proteins in vitro. Their electrophoretic results show extremely important information, albumin and globulin levels are remarkably changed and characterized by disorder in their fractions avere which occurred frequently.

Methods: All plasma proteins samples of sets A, B and C were treated in sequences with known different concentrations of gallic acid, gallotannin and polypholes, which were separated chromatographically from phenolic extract of fruit peel of punicaceae. These were then treated, A, B and C were subjected to electrophoresis techniques, for identification and quantitation.

Results: The electrophoretic patterns of treated plasma proteins samples, sets A, B and C are arised with remarkable changes in their fraction levels, compared to normal. The results in were also characterized by disorders in their electrophoretic pattern. In this way 5 fractions of treated plasma proteins could be distinguished after sustaining which are albumm and $\alpha 1$, $\alpha 2$ and β and γ globulins.

Conclusion: The biological activity of tannic acid related compounds on plasma proteins in vitro, is important in determining their toxicity, and this toxicity may be depend upon their metabolic processes in the liver. In addition, the electrophoretic techniques used for separation and identifcation of plasma protein is extremely important for future work in the area.

Keywords: Toxic effect, tannic acid and related compounds, plasma proteins, biological activity, electrophoresis techniques.

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Many plant species native to Iraq and the Arab world are known to contain certain chemical compounds, which exert an effect against microorganisms,[1-3] due to their anti-microbial substance known as phonemic compounds.[2] It has been found that tannins are considered as repellents to animal and microbial predators.[4] Tannins or tannic acids are not single homogeneous compounds, but a mixture of phenolic esters such as gallic acids or its related compounds with D-glucose whose exact composition various according to their sources.[5] These types of compounds have been used, as an adjuvant to barium enema for radiological investigations, as it gave sharper definition and better pictures. During the period 1949–1969, there had not been any ill effects. Although some 14–15.000 barium tannic acids enemase had been administered. However, reports of seriously ill dangerous procedures had been observed later.[6] In our area, many people take tannic acids as drug without medical supervision. They are employed in medicine as astringents in the gastrointestinal tract, throat paint, and skin abrasions, in the treatment of burns, as it precipitates the burned proteins, forming a non putrefying protective layer under which new tissues can grow.[4] Usually the tannic acids enter the blood stream after absorption from skin or the gastrointestinal tract of that patient. Therefore accidental or deliberate exposure to toxic levels may be occur frequently. The biological activities of tannic acids or its related phenolic compounds have not been studied widely regarding

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their effect on human plasma proteins in vitro. However, plasma proteins occupy a central position in protein metabolism. They interact with virtually all body tissues or cells and they are intimately related to protein metabolism in the liver. The binding of tannic acids or its related phenolic compounds is of special importance to toxicologists.[7] Thus, this work has largely been directed, to study the effect of these compounds related to tannic acids such as gallic acid, gallottann and polyphenol; because we noted that many people in our area take these chemicals without medical supervision. This work is aiming also to improve electrophoretic methods for separation and identification of treated plasma proteins, with phenolic compounds, by which different migration rates of plasma protein fractions were observed.

Methods. ***Extraction of plant material.*** From punicaceae the peels of healthy fruit were dried at room temperature for at least 6 months, before they were powdered in a mortar and the powder was sieved through, a 100-150 mesh sieve.

Phenolic extract. The powder (50g) was boiled for few minutes in 100 ml ethanol (95%) and left for at least 3 hours. The extract was decanted through fiber glass. The remaining residue was re-extracted twice with ethanol and the combined extracts were concentrated in vacuo to remove ethanol. The heavy viscous residue was obtained as crude phenolic extract.[3]

Paper chromatography. The phenolic extract were chromatographed on whatman number one, paper in 2 dimensions with 6% (v/v) acetic acid (solvent A) and isobutanol: acetic: water (14:1:5) (solvent B) at 25 ±1°C . Phenolics such as gallic acid, gallotannin (β-penta-O-galloyl-D-glucose) and polyphenol (mixture of polyphenols), were revealed by spraying with a freshly prepared reagent of ferric chloride–potassium ferricyanide, Gibbs reagent, saturated potassium iodate reagent and finally a fresh solution of nitrous acid reagent. These gave the phenolic compounds characteristic colors, which helps in their identification.[5]

Isolation of phenolic compounds. The phenolic extracts (10g) obtained as mentioned above, were fractionated chromatographically on sephadex LH-20, column (100x2.5cm). Gallic acid, gallotunnin and polyphenol were obtained homogeneously by fractionation as in **Table 1**. Fraction 2 and 6 were dried at 25°C and 0.01 mmHg, over phosphorous pentoxide and rechromatographed once again over sephadex LH-20. The resultant substances were confirmed by 2 dimensional chromatography as compared with authentic compounds.

Blood collection. At the central clinical laboratory for Ministry of Health, venous bloods were collected from 3 normal healthy individuals, from their antecubital veins, into 3 vacationer tubes containing heparin as an anticoagulant, 10 ml of blood were drawn in each container. Plasma proteins were obtained as a clear yellowish fluid from centrifugation of blood samples according to methods discussed previously.[8]

Sample preparation. Immediately after centrigugation, the 3 samples of plasma proteins were divided into small portions, each one containing exactly one milliliter of plasma proteins. Therefore each sample gave 10 portions, one portion was taken from each sample as a reference and hence the remaining portions were allowed to treat, in sequences, with known different concentrations of tannic acid related compounds such as gallic acid, gallotannin and polyphenol **Table 2**. Three, sets A, B and C, of treated plasma proteins samples were left to stand for 30 minutes at 20°C and subjected to electrophoresis for separation and identification.

Separation of plasma proteins. The sets A, B and C, were subjected to electrophoresis techniques, using cellulose acetate strips, for identification and quantitation.[8,9] The electrophoresis took place at pH 8.6, all sets of samples carry a negative charge to a greater or lesser extant, when the current is turned on. There is a flow or movement of buffer toward the negative electrode (cathode), and this movement passively carries with it, to a small extent, the proteins that tends to migrate toward the anode due to their negative charge. The cellulose acetate stripe was immersed in a trichloro-acetic acid ponceau dye (Allied chemical) solution to fix and stain the treated protein bands, The membrane is made transparent (cleared) by immersing it for approximately one minute in acetic acid mixture placed upon a glass plate and oven-dried at 70°C to 80°C for 15-20 minutes. The clear membranes were placed in plastic envelopes and made ready for inspection for quantitations of the treated plasma protein bands by passing through a recording densitometer which automatically integrates each fraction as a percentage of the total treated plasma proteins in gm/100 ml as well as, the fractions of reference **(Table 3),** according to methods described previously.[10]

Results. This work is concerned only with plant phenolic species, native to Iraq, which contain a high quality of phenolic compounds and which are used in our social lives as drugs without medical supervisions such as the fruit peel of punicaceae in the pomegranate family. This separation and identification of freshly prepared tannic acid related compounds such as gallic acid, gallotannin and polyphenol, is indicated in **Table 1**, and is in agreement with our previous work.[3,5] **Figure 1** shows the electrophoretic patterns of samples of sets A, B and C and also shows their patterns arising with changes in their fraction levels compared to the normal one (such as reference), in this way 5 fractions of treated plasma proteins pattern could be

distinguished after staining, which are: Albumin, alpha–one (α1), and alpha-2 (α2), and beta (β) and gamma (δ)–Globulins.[9] However, according to our results **Table 3** gave more interesting clinical information regarding the effect of tannic acid related compounds on human plasma proteins in vitro: as in the following observations. The first fraction is plasma albumin of a single species of protein and is the most prominent fraction in electrophoregrams (**Figure 1**), it constitutes approximately 60% of the normal plasma proteins and has a capacity to bind many foreign compounds.[9] Our results in **Table 3** show, albumin with the plasma proteins can bind these tannic acid related compounds. The albumin levels of samples of sets A, B and C are markedly increased, from the normal level. Sub-albumin fractions are also indicated in some samples such as 1A, 2A, 6A, 4B and 7B. The 2nd and 3rd fractions, adjacent to albumin are the α1 and α2 Globulin. The are a mizture of many proteins, such as glycoproteins and lipoproteins.[9] In general, non-specific changes in α1 and α2 globulins levels were observed. **Table 3** shows only progressive changes are arising from samples containing high phenolic concentrations such as samples 5B, 6B, 6C, 6C, while samples 5A, 2C, 6B, represent a mixed α1 and α2 globulin fraction. The 4th fraction, as shown in **Figure 1** is b-globulin, which contains the b-lipoproteins, the iron transporting protein (transferrin), fibrinogen, and other lesser known proteins.[9] The treated samples also observed changing in the β-globulin levels. The present results outlined in **Table 3**, indicate β-Globulin levels become relatively less prominent then the normal β–Globulin level as in samples 6B, 7B, 5C, 6C, and 7C while the samples of set A are less effective than others. The 5th fraction, is the δ-Globulin fraction, which contains the immunoglobulins, or circulating proteins of all

Table 1 - Fractionation of gallic acid, gallotannin and polyphenol by column chromatography, using sephadex LH-20.

Fractions	Elution solvent %	Weight (g)	Substance
1	Ethanol (100)	5.56	Unknown
2	Ethanol (100)	1.42	Gallic acid
3	Ethanol (90)	1.70	Mixture of gallic acid and other phenolic substances
4	Ethanol (85)	trace	-
5	Ethanol (80)	trace	-
6	Ethanol (70)	0.68	Gallotannin
7	Ethanol (60)	0.54	Polyphenolic substances

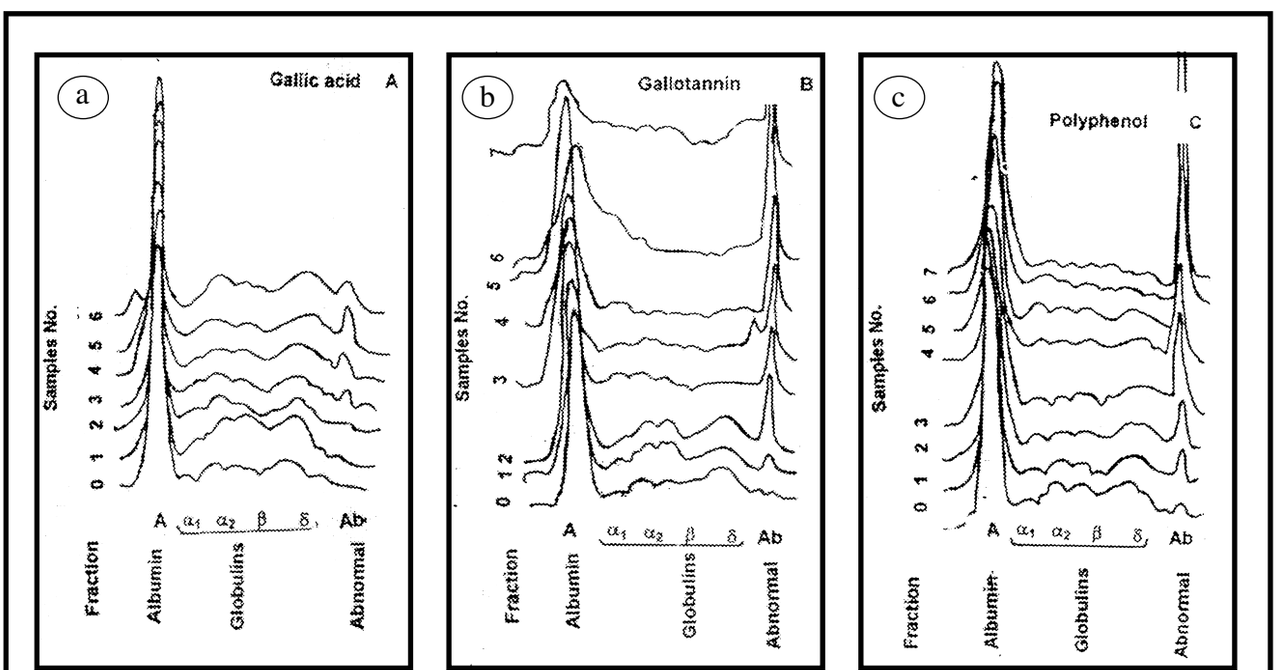

Figure 1 - Electrophoretic patterns of sets of samples **(a)** - gallic acid, **(b) -** gallotannin and **(c) -** polyphenol.

Table 2 - The concentration of tannic acid related compounds in plasma protein samples.

Samples of Set A					Samples of Set B					Samples of Set C				
Vol. plasma proteins (6.6g/ 100ml) ml	**Vol. gallic acid solution (5g/ 100ml) ml**	**Vol. dist. water ml**	**Total vol. of sample ml**	**Gallic acid conce. in g/100ml**	**Vol. Plasma proteins (6.6g/ 100ml) ml**	**Vol. gallotannin solution (5g/100ml) ml**	**Vol. dist. water ml**	**Total vol. of sample ml**	**Gallotannin conce. in g/ 100ml**	**Vol. plasma proteins (6.6g/ 100ml) m1**	**Vol. polyph- enol solution (5g/100ml ml**	**Vol. dist. water ml**	**Total vol. of sample ml**	**Poly- phenol conce. in g/100ml**
1	0	2	3	0	1	0	2	3	0	1	0	2	3	0
1	0.2	1.8	3	$1.11x10^{-2}$	1	0.1	1.9	3	$5.55x10^{-2}$	1	0.1	1.9	3	$5.55x10^{-2}$
1	0.3	1.7	3	$1.67x10^{-1}$	1	0.3	1.7	3	$1.67x10^{-1}$	1	0.3	1.7	3	$1.67x10^{-1}$
1	0.5	1.5	3	$2.78x10^{-1}$	1	0.5	1.5	3	$2.78x10^{-1}$	1	0.5	1.5	3	$2.78x10^{-1}$
1	0.7	1.3	3	$3.89x10^{-1}$	1	0.9	1.1	3	$5.00x10^{-1}$	1	0.7	1.3	3	$3.89x10^{-1}$
1	0.9	1.1	3	$5.00x10^{-1}$	1	1.1	0.9	3	$6.11x10^{-1}$	1	0.9	1.1	3	$5.00x10^{-1}$
1	1.1	0.9	3	$6.11x10^{-1}$	1	1.5	0.5	3	$8.33x10^{-1}$	1	1.1	0.9	3	$6.11x10^{-1}$
-	-	-	-	-	1	2.0	0	3	$11.11x10^{-1}$	1	1.5	0.5	3	$8.33x10^{-1}$
Vol=volume, dist=distilled, conce=concentration														

variaties.[9] The results in **Table 3,** showed that the only samples of set B and set C have a high ability to effect this fraction level and changes were airside and that is in contrast to the samples of set A which shows less influence. The lowest fraction, is an abnormal fraction. The one nearest to δ-Globlulin as shown in **Figure 1.** This result is indicated in **Table 3**, and is extremely important in the present work. Only samples of set B and set C gave unique fractions with distinctive peaks and were in contrast to samples of set A. Very small peaks were indicated such as samples 4A and 5A. Thus this type of fraction may be obtained due to the capability of these phenolics to bind proteins of globulins and migrate further toward the acid composition, our result also indicates the levels of these fractions depend upon the concentrations of phenolic compounds as in set B and set C.

Discussion. Our results indicate, that the electrophoretic patterns of the treated plasma proteins

Table 3 - Fractions of control and treated plasma proteins samples of sets A, B and C obtained by electrophoretic techniques.

Fractions of treated plasma proteins samples of set A (g/100ml)						Fractions of treated plasma proteins samples of set B (g/100ml)						Fractions of treated plasma proteins samples of set C (g/100ml)					
TTPP	**A**	**α1, α2, G**	**βG**	**γG**	**Ab**	**TTPP**	**A**	**α1, α2G**	**βG**	**γG**	**Ab**	**TTPP**	**A**	**α1, α2, G**	**βG**	**γG**	**Ab**
6.6	3.5	0.3, 0.7	0.6	1.5	0	6.6	3.8	0.2, 0.7	0.5	1.4	0	6.6	3.5	0.4, 0.8	0.6	1.4	0
6.5	3.9	0.4, 0.8	0.5	1.5	0	6.5	3.9	0.3, 0.6	0.6	1.0	0.1	6.7	3.5	0.4, 0.8	0.5	1.3	0.2
6.5	3.5	0.4, 0.8	0.5	1.3	0	6.6	3.9	0.3, 0.6	0.4	1.1	0.3	6.6	3.6	0.4, 0.6	0.5	1.2	0.3
6.5	3.7	0.4, 0.8	0.5	1.0	0.1	6.6	3.8	0.5, 0.5	0.3	1.0	0.5	6.7	3.7	0.3, 0.4	0.5	1.2	0.6
6.7	3.6	0.3, 0.7	0.5	1.4	0.2	6.6	4.1	0.4, 0.5	0.6	0.4	0.6	6.6	4.2	0.3, 0.3	0.4	0.6	0.8
6.6	3.6	0.9	0.5	1.4	0.2	6.7	4.6	0.5, 0.1	0.3	0.5	0.7	6.7	4.2	0.5, 0.3	0.4	0.6	0.7
6.6	3.6	1.1	0.4	1.3	0.2	6.6	4.4		0.4	0.2	1.6	6.6	4.4	0.5, 0.4	0.3	0.3	0.7
-	-	-	-	-	-	6.7	2.1	0.6, 0.4	0.4	1.0	2.2	6.7	4.4	0.4, 0.3	0.3	0.2	1.1
TTPP=total treated plasma proteins, A=albumin, α1,α2G=α1 and α2 globulins, β-G=β-globulin, γG=γ-globulin, Ab=abnormal fractions																	

samples (such as sets A, B, C) are markedly changed, albumin and golbulins fractions are gradually changed depending upon the type of phenolic compound and its concentration. Actually these results produced extremely important information regarding their ability to bind proteins and can be characterized by the presence of abnormal fractions, which indicates a particular disorder in their metabolites. At present, the biological activity of tannic acid related compounds on plasma proteins in vitro is important in determining their toxicity. Their biological activity is directly affected by many factors such as chemical factors clinical and biological factors. Thus, the toxicity of tannic acid related compounds depending upon their metabolites (specially in vivo). These phenolic compounds may be converted into more water-soluble metabolites than original compounds in the body, which are readily excreted and hence the potential toxicity is kept to minimum. However in some cases, the reverse occurs and the metabolites produced a more toxic effect than parent compounds. This phenomenon is in agreement with the previous reports, which indicate fatal liver kidney failure.[6] This may be related to the fact that they are very important in the elimination of these tannic acids from the body. In addition, the electrophoretic technique, which was used for separation and identification of plasma proteins, is extremely important for future clinical work and can be characterized from the disorder fractions present.

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