IN VITRO TOXICITY EVALUATION OF BISPHENOL A ON HUMAN ERYTHROCYTE AND ITS AMELIORATION BY QUERCETIN

Sanman Samova, Hetal Doctor and R.J. Verma*

***Department of Zoology, BMTC and HG, School of Sciences, Gujarat University, Ahmedabad-380009, India.**

***Corresponding Author Email: ramtejverma2000@yahoo.com**

ABSTRACT

Bisphenol A is well-known xenoestrogen, used in plastics and food can liners manufacture. Food is considered as main source of exposure. Experimental studies revealed that BPA causes negative health effect. Quercetin is natural antioxidant present in various fruits and vegetables. The present study was undertaken to analyze the direct effect of BPA on erythrocyte and its amelioration by quercetin. Intravenous blood samples were collected, and erythrocyte suspension was prepared. Study was divided in two phases. In phase I: Effect of BPA on erythrocyte suspension was analyzed, BPA was treated to erythrocyte with different doses (0-150 µg/ml). The highest concentration of BPA (150 µg/ml) showing hemolysis was selected for ameliorative study. In phase II: ameliorative study different concentration of quercetin (0-30 µg/ml) + BPA (150 µg/ml) -treated tubes containing 2 ml of erythrocyte suspension. In the present study we conclude that there was dose dependent reduction in BPA-induced hemolysis has been observed due to quercetin. Reduction in BPA induced hemolysis due to quercetin might be due to antioxidant capacity of quercetin.

KEY WORDS

Bisphenol A, Quercetin, Hemolysis, Amelioration

INTRODUCTION

Bisphenol A (BPA) is a chemical produced in large quantities for use primarily in the production of polycarbonate plastics and epoxy resins (Ballesteros-Gómez *et al*., 2011; Hoekstra *et al*., 2013). Bisphenol A is used in plastics and food can liners manufacture (Erler and Novak. 2010). Hence, it becomes an integral part of the food chain. Food is considered as main source of exposure of BPA as a consequence of BPA migration from food containers. (Biles *et al*., 1997; Ballesteros-Gómez *et al.,* 2009; Schecter *et al*., 2010).

Due to the broad spectrum of use of BPA to manufacture products used in many applications, it has been speculated that human exposures to BPA may be widespread and it has been postulated that these exposures may reach high levels (Tsai, 2006; Ranjit *et al*.,

2010; Vandenberg *et al*., 2013; Rochester *et al.,* 2013). Bisphenol A shows negative health effect in experimental studies (Peretz *et al*., 2014).

Quercetin is one of the most effective antioxidants of the flavonoids (Harborne *et al*., 2013). Dietary flavonoids can exert a positive effect regardless of their poor absorption. Some study suggests that quercetin do not necessarily need to be absorbed to exert an effect (Manach *et al*., 2004; Halliwell *et al*., 2005). Quercetin is natural antioxidant present in various fruits and vegetables. Diet containing quercetin will be beneficial against oxidative stress causing agents.

Various studies suggest that quercetin could be a substantially promising organoprotective agent against toxic effects and perhaps against other toxic metal

chemicals or drugs (Hamza *et al*., 2015; Kumar *et al*., 2016; Kocahan *et al*., 2017).

The present study was undertaken to analyze the direct effect of BPA on erythrocyte and its amelioration by quercetin.

Hypotheses Proposed:

- BPA may not be causing significant, concentration- and time-dependent effect on erythrocyte: *in vitro*.
- Quercetin may not be causing significant, concentration- and time-dependent amelioration on BPA induced toxicity on erythrocyte: *in vitro*.

MATERIALS AND METHODS

Collection of Blood and sample preparation

Intravenous blood samples were collected from healthy adult volunteers (23-25 years) in EDTA vials. Samples were diluted with phosphate buffered saline and centrifuged at 1000×g for 10 min. Erythrocyte pellets were washed thrice and diluted with saline to get cell density of 2×10^4 cells/ml.

Phase I: Effect of BPA on erythrocytes:

To study the effect of BPA on erythrocyte suspension, following sets of tubes were prepared;

1. Control tubes containing 2 ml of erythrocyte suspension and 2 ml of PBS.
2. 100% hemolysis tubes containing 2 ml of distilled water and 2 ml of erythrocyte suspension.
3. BPA-treated tubes containing 2 ml of erythrocyte suspension and 0 to 200 µg/ml BPA.

The total volume of each tube was made to 4 ml with addition of PBS.

The incubation medium containing erythrocyte suspension were mixed gently and incubated at 37ºC for 2 h with intermittent shaking. Thereafter the tubes were centrifuged at 1000×g for 10 min. The colour density of supernatant was measured spectrophotometrically at 540 nm.

Phase II: Ameliorative effect of quercetin on BPA-induced toxicity on erythrocytes

Erythrocyte suspensions were prepared as explained earlier. High dose of BPA was selected on the bases of set A. Following sets of tubes were prepared for the ameliorative study:

1. Control tubes containing 2 ml of erythrocyte suspension and 2 ml of PBS.
2. 100% hemolysis tubes containing 2 ml of distilled water and 2 ml of erythrocyte suspension.
3. Antidote control containing 2 ml of erythrocyte suspension and 200 µg/ml quercetin.
4. Different concentration of quercetin (0-30 µg/ml) + BPA (150 µg/ml) -treated tubes containing 2 ml of erythrocyte suspension.

The total volume of each tube was made up to 4 mL with addition of PBS and proceed as mentioned earlier.

RESULT

Bisphenol A cytotoxicity was examined in human erythrocytes. In control tubes the supernatant remained clear and erythrocyte settled in the bottom of the tubes were normal. In BPA -treated tubes reddish colour appeared in supernatant which indicates hemolysis. BPA-treated tubes number of cells settled in the bottom of the tubes reduced. Bisphenol A treated erythrocyte showed swelling. Addition of BPA (25-200µg/ml) to suspension of erythrocyte caused significant ($p<0.05$) increase in hemolysis (Table.1). This increase was concentration-dependent ($r=0.991$). Hemolysis was maximum on addition of 150 µg/ml of BPA. Hemolysis may be because of influx of BPA into the cells causing alteration in erythrocyte membrane, swelling and eventual cell lysis (Fig.1).

Maximum toxic effect on erythrocyte was seen in 150 µg/ml BPA. Addition of 30 µg/mL of quercetin to erythrocyte suspension did not cause any significant increase in the rate of hemolysis (Table.2). However, concurrent addition of quercetin (0-200 µg/ml) in erythrocyte suspension significantly reduced bisphenol A (150 µg/ml) -induced hemolysis. The reduction caused by quercetin was significant and dose-dependent ($r=0.959$). Maximal retardation in hemolysis was observed with 30 µg/ml concentration of quercetin. The results also revealed that quercetin is protective to prevent BPA -induced hemolysis (Fig.2).

Table 1: Bisphenol A-induced hemolysis in human erythrocyte in vitro

| Groups	Bisphenol A conc. (μg /ml)	Hemolysis (%)
Control		
1	Control	2.20 ± 0.42
2	Vehicle control	2.40± 0.27
Bisphenol A- treated		
3	25	17.60 ±1.04[a]
4	50	23.40 ±1.88 [a]
5	75	59.40 ±1.68 [a]
6	100	68.60 ±2.46 [a]
7	125	80.60 ±2.36 [a]
8	150	96.20 ±1.03 [a]

Results are expressed as mean ± SEM; n = 10

Significant at the level [a]$p < 0.05$ as compared to vehicle control

Table 2: Retardation in bisphenol A-induced hemolysis by quercetin

Groups	Concentration (μg /ml)		Hemolysis (%)	Retardation (%)
	BPA	**Quercetin**		
Control				
Control	0	0	2.20 ±0.82	-
Vehicle control	0	0	3.60 ±1.04	-
Antidote Control	0	150	3.40 ±1.03	-
Bisphenol A- treated				
BPA	150	0	96.20 ±1.03[a]	-
Bisphenol A+ quercetin – treated				
1	150	50	54.20 ±2.56 [b]	45.80±2.55 [b]
2	150	100	40.80 ±1.78 [b]	59.20±1.78 [b]
3	150	150	24.40 ±2.36 [b]	75.60±2.36 [b]
4	150	200	6.60 ±1.20 [b]	93.40±1.20 [b]

Results are expressed as mean ± SEM ; n = 10

Level of significance: [a]$p < 0.05$ as compared to vehicle control; [b]$p < 0.05$ as compared to BPA-treated
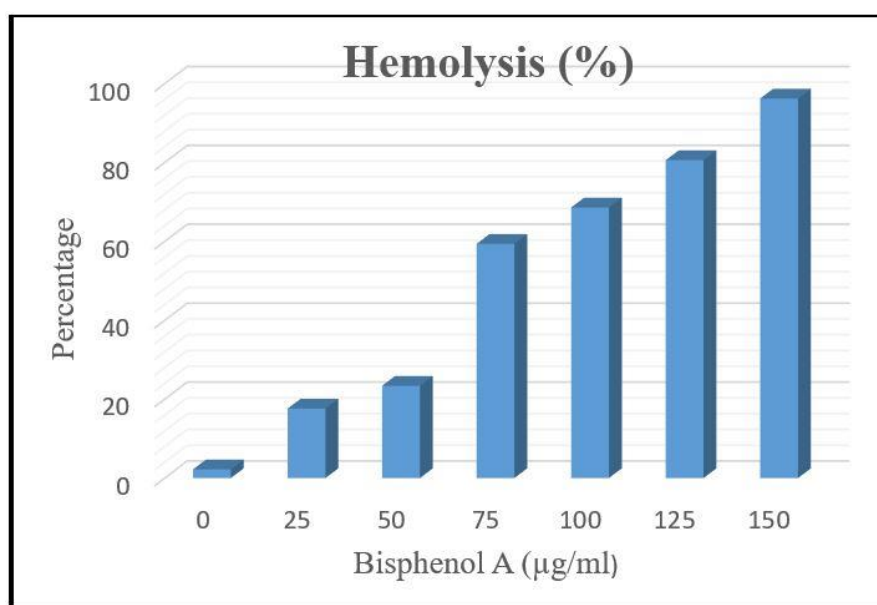
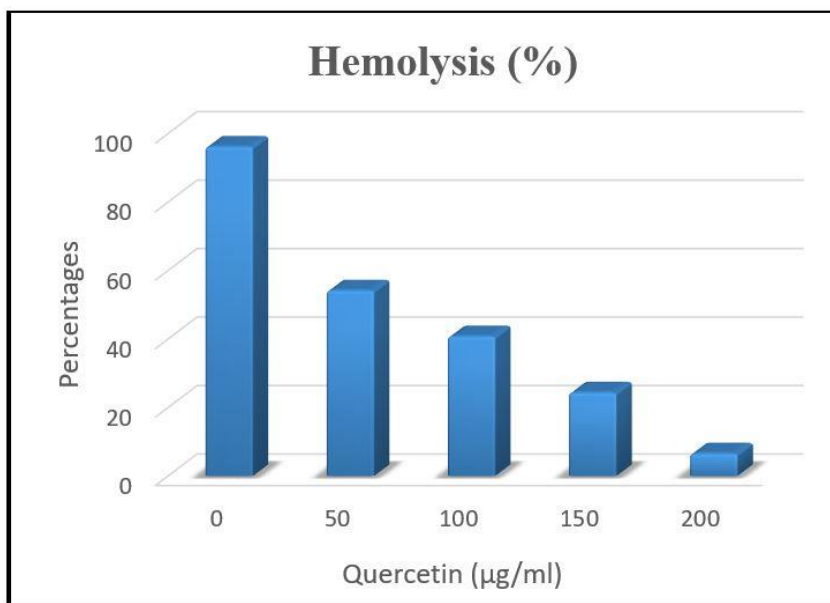**Fig. 1: Bisphenol A- induced hemolysis: *in vitro***

Fig.2: Quercetin induced amelioration in BPA- induced reduction in hemolysis of erythrocyte*: in vitro*

DISCUSSION

In this study we have assessed the effect of BPA on membrane of erythrocyte, which is the first barrier that must be overcome to penetrate the cell. Erythrocyte membrane contains 60% phospholipids of the total lipid components. Studies have shown that bisphenol A, a phenolic compound may alter erythrocyte membrane (Miller and Deinzer. 1980; Maćczak *et al*., 2015).

In vitro study of bisphenol A on erythrocyte shows its toxic effect. Dose-dependent hemolysis was observed in human erythrocyte. Hemolysis may be because of influx of BPA into the cells causing alteration in erythrocyte membrane, swelling and eventual cell lysis (Fujisawa *et al*., 1978; Verma and Sangai, 2009). However, the exact mechanism of action is not clearly understood. BPA disturbs phospholipids metabolism, structure and function by generating hydroxyl radical in human erythrocytes. (Taniguchi *et al*., 1981; Shalel *et al*., 2002). Thus, hemolysis might be due to oxidative damage and alteration of phospholipid membrane. Maćczak *et al*. (2017) have noticed that BPA and it analogs induced oxidative stress and caused lipid peroxidation in erythrocytes, which could have contributed to deterioration of the structure of erythrocyte membrane.

Experimental studies suggested that a decrease in the number of erythrocytes might have been due to disruption of erythropoiesis and/or increase in the destruction of red blood cells (Schaer *et al*., 2013). Study by Ahmed *et al*. (2015) revealed that albino adult male rats treated with BPA had reduced blood count and packed cell volume, which resulted in development of anemia. Iida *et al*. (2003) revealed that exposure to bisphenol A brought about morphologic changes in the cultured Sertoli cells, such as membrane blebs, cell rounding, cytoskeletal collapse, and chromatin condensation.

Quercetin is natural antioxidant having beneficial effect on human health. In the present study dose dependent reduction in BPA-induced hemolysis has been observed due to quercetin addition. Quercetin is rapidly and avidly taken up by human erythrocyte via a passive diffusion mechanism, driven by flavonoid binding to hemoglobin and resulting in almost quantitative accumulation of the flavonoid ((Fiorani *et al*., 2003; Serrano Casasola *et al*., 2016). Also, it may be useful in diminishing oxidative damage to erythrocyte.

Various studies have shown that quercetin having antioxidative effect in experimental studies (Łuczaj and Skrzydlewska. 2005; Pourmorad *et al*., 2006; Boots *et al*.,2006). In spite of the free radical scavenging activities, quercetin is also involved in the indirect induction of detoxifying agent which might be involved in detoxification of bisphenol A and its toxicity (Satoh *et al*., 1999; Verma *et al*., 2009; Fan *et al*., 2012).

Reduction in BPA induced hemolysis due to quercetin might be due to antioxidant capacity of quercetin or compatative binding of BPA-Quercetin. Our previous *in*

silico study revelaed that quercetin can bind to BPA and reduce its effect (Samova *et al*., 2018).

CONCLUSION

Present *in vitro* study revealed that Exposure of bisphenol A causes hemolysis in erythrocyte. The effect was dose-dependent. Moreover, quercetin is potent enough to ameliorate BPA –induced hemolysis.

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*Corresponding Author:
R.J. Verma*
Email: __ramtejverma2000@yahoo.com__