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## Effects of Antioxidant Vitamin Combination on Pregnancy Induced Hyper-Hepatic State

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**Abstract:** The purpose of this present study is to determine the influence of varying combinations of antioxidant Vitamins on the hyper-hepatic state of pregnancy. To achieve this, seventy pregnant Wister albino rats weighing between 250-300 g were procured and grouped into 2 control groups treated with distilled water and vehicle- tween-80, respectively and three cohorts (I, II and III) with four sub-groups each (n = 5). Starting from the 7th day, group I received a varying dose combination of Vitamin A+C, group II Vitamin A+E and group III Vitamin C+E respectively for 11 days. Results of liver function assay revealed that supplementation with Vitamin A+C, A+E and C+E caused a significant reduction ( $p < 0.05$ ) in serum protein and a non-significant ( $p > 0.05$ ) alteration in serum albumin. Except for ALT where Vitamin A+E combination produced no significant alteration, serum AST (Aspartate transaminase) and ALP (Alamine transaminase) were significantly reduced ( $p < 0.05$ ) with antioxidant Vitamin combination therapy when compared with control. Antioxidant vitamin combination may be advantageous in pregnancy induced hyper- hepatic state. However, further study is needed in this respect.

**Key words:** Hyper-hepatic state, supplementation, antioxidant combination, liver function, bi-combination therapy

### INTRODUCTION

It is a known fact that a number of associations between hepatic dysfunction and pregnancy exist (Rahman and Wendon, 2002; Steingrub, 2004). In recent times, the observation that women with pre-eclampsia have decreased plasma and placental concentration of antioxidants especially Vitamin C (Rajmakers *et al.*, 2004; Dehghan and Dehghanan, 2006) led to the proposal that placental under perfusion may mediate a state of oxidative stress (Rumbold *et al.*, 2008). These observations have given rise to increased interest in antioxidants (Kashinakunti *et al.*, 2010). Despite the proposed role of oxidant stress in the pathogenesis of Non-Alcoholic Fatty Liver Disease (NAFLD), antioxidant use have not been investigated sufficiently in NAFLD therapy (Angula, 2002; Chitturi and Farrell, 2001; Reid, 2001). Our previous study on the effects antioxidant Vitamin A, C and E mono-therapy on liver function in pregnancy published in Asian Journal of Medical Sciences (2011),

revealed a mixed effect. We then concluded that the therapeutic benefit of Vitamin A, C and E mono- therapeutic supplementation in pregnancy is not without impact on the functional and cellular integrity of the liver (Iribhogbe *et al.*, 2010).

While increased risk of fetal and maternal morbidity and mortality has been noted in patients with severe hepatic dysfunction in pregnancy (Rahman and Wendon, 2002), antioxidants have been reported for preventing pre-eclampsia (Rumbold *et al.*, 2008) and thus, protect the liver from damage. Also, antioxidant vitamins have been reported to play an important role in the regulation and eventual outcome of human pregnancy (Dakshinamurti and Dakshinamurti, 2001). Available data has attached some importance on the use of several different regimens in combination. This veracity of synergistic effect lead to the postulation that multi-antioxidant vitamin combination therapy may be important in managing pregnancy associated abnormal liver state signified by hyper-hepatic enzymatics. On this

Table 1: Treatment administered to different groups (n = 5 rats per group)

Group	Treatment
Control	Negative Control: Normal feed+Distilled water 1 mL Vehicle: Normal feed+Tween 80 1 mL
Vitamin A+C	1. Normal feed+Vehicle+Dist H <sub>2</sub> O+Vit A 0.6 mg kg <sup>-1</sup> +Vit C 200 mg kg <sup>-1</sup> 2. Normal feed+Vehicle+Dist H <sub>2</sub> O+Vit A 0.7 mg kg <sup>-1</sup> +Vit C 250 mg kg <sup>-1</sup> 3. Normal feed+Vehicle+Dist H <sub>2</sub> O+Vit A 0.8 mg kg <sup>-1</sup> +Vit C 300 mg kg <sup>-1</sup> 4. Normal feed+Vehicle+Dist H <sub>2</sub> O+Vit A 1.0 mg kg <sup>-1</sup> +Vit C 400 mg kg <sup>-1</sup>
Vitamin A+E	1. Normal feed+Vehicle+Dist H <sub>2</sub> O+Vit A 0.6 mg kg <sup>-1</sup> +Vit E 16.4 mg kg <sup>-1</sup> 2. Normal feed+Vehicle+Dist H <sub>2</sub> O+Vit A 0.7 mg kg <sup>-1</sup> +Vit E 18.4 mg kg <sup>-1</sup> 3. Normal feed+Vehicle+Dist H <sub>2</sub> O+Vit A 0.8 mg kg <sup>-1</sup> +Vit E 19.4 mg kg <sup>-1</sup> 4. Normal feed+Vehicle+Dist H <sub>2</sub> O+Vit A 1.0 mg kg <sup>-1</sup> +Vit E 22.4 mg kg <sup>-1</sup>
Vitamin E+C	1. Normal feed+Vehicle+Dist H <sub>2</sub> O+Vit E 16.4 mg+Vit C 200 mg kg <sup>-1</sup> 2. Normal feed+Vehicle+Dist H <sub>2</sub> O+Vit E 18.4 mg+Vit C 250 mg kg <sup>-1</sup> 3. Normal feed+Vehicle+Dist H <sub>2</sub> O+Vit E 19.4 mg kg <sup>-1</sup> +Vit C 300 mg kg <sup>-1</sup> 4. Normal feed+Vehicle+Dist H <sub>2</sub> O+Vit E 22.4 mg kg <sup>-1</sup> +Vit C 400 mg kg <sup>-1</sup>

premise, this study aim to investigate the impact of antioxidant Vitamin A, C and E bi-combinations on elevated liver enzymes in pregnancy.

## MATERIALS AND METHODS

This study was conducted between August and December 2009.

**Experimental animals:** Seventy adult female Wister albino rats weighing (225 - 300 g) were obtained from the Animal House, College of Medicine, Ambrose Alli University, Ekpoma between August and October 2009. They were housed in a stainless steel cage with plastic bottom grid and a wire screen top in physiology Lab II in the Department of Physiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria. They were assigned into five groups; a control group (n = 5), vehicle group (n = 5) and three test groups I, II and III made up of four sub- groups (n = 5). They were fed *ad libitum* with tap water and pelleted feeds purchased from Bendel feeds and flour meal Ewu, Nigeria Limited and allowed to acclimatize for 2 weeks. After which two male Wister albino rats were introduced into each group to allow for mating. The animals were allowed to mate for 6 days after which the male animals were removed from the cage. Pregnancy was confirmed using the palpation method (Agematsu *et al.*, 1983) and vaginal smear microscopy method (Long and Evans, 1922; Daly and Kramer, 1998). From the 7<sup>th</sup> day, administration of the different Vitamin combinations began (Table 1) using orogastric tubes and syringes to minimize the loss of test substance (Ejebe *et al.*, 2009) and lasted a period of 11 days. The administrations were conducted between the h of 8.00 and 10.00 a.m. daily.

**Vitamin preparation and administration:** Vitamin A, C and E were purchased from Clarion Medical Pharmaceuticals Nigeria Limited and Tween 80 vehicle from Sigma Pharmaceuticals Limited. Two hundred milligram of the powdered form of Vitamin C was

dissolved in 10 mL of distilled water and the appropriate dose per kg was prepared for administration. Vitamin A (25,000 IU equivalent to 6 mg retinal and E, 100 mg) was dissolved in 0.2 mL of tween 80 and water in a ratio of 0.2:0.2:9.6. Table 1 shows the doses administrated to the test groups.

**Sample collection:** Twenty-four hours after the last administration of vitamins was carried out, the animals were sacrificed after inhalation of chloroform. Cardiac and jugular vein puncture were used to collect blood samples into tubes containing EDTA as anticoagulant and centrifuged plasma preparation assayed for biomarkers of liver function via standard laboratory procedures.

**Enzymatic assays:** Determination of plasma Albumin (ALB) and Total Protein (TP) for functionality, Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) for cellular integrity and Alkaline Phosphatase (ALP) for conditions linked to the billiary tract were analyzed using standard methods. Albumin (ALB) and Total Protein (TP) level were determined using a Technicon RA-Xt autoanalyzer (Karakileik *et al.*, 2005). Aspartate amino transferase (AST) and Alanine amino transferase (ALT) were determined using the Randox reagent kit and 2, 4-dinitrophenylhydrazine as substrate (Reitman and Frankel, 1957). Alkaline Phosphatase (ALP) was determined using the Randox reagent kit with p-nitrophenylphosphate as substrate (Bassey *et al.*, 1946).

**Data analysis:** The Mean±Standard deviation (X±SD) and one-way ANOVA (LSD) statistical test was performed using SPSS version 17 soft ware. The significance level was set at p<0.05.

## RESULTS

Administration of combinations of Vitamin A+C, A+E and C+E produced a non-steady reduction in serum protein level in pregnant rats when compared with control

Table 2: Effect of vitamin combinations on enzymes of liver function in pregnant rats

Treatments	Protein (g dL <sup>-1</sup> )			Albumin		
	Vit A+C	Vit A+E	Vit C+E	Vit A+C	Vit A+E	Vit C+E
Control	7.18±0.28	7.18±0.28	7.18±0.28	2.92±0.35	2.92±0.35	2.92±0.35
Tween 80	7.00±0.22	7.00±0.22	7.00±0.22	3.08±0.38	3.08±0.38	3.08±0.38
T <sub>1</sub>	7.02±0.67	8.42±0.50*	8.66±0.34*	2.80±0.35	5.24±0.84*	3.28±0.44
T <sub>2</sub>	6.68±0.84	7.54±0.55	7.98±0.49*	3.08±0.44	4.28±0.59*	3.00±0.49
T <sub>3</sub>	7.06±0.42	6.50±0.53	6.80±0.55	2.94±0.35	3.82±0.41*	3.74±0.62*
T <sub>4</sub>	6.38±0.63*	6.44±0.84*	5.38±0.54*	3.10±0.37	3.16±0.31	2.82±0.51

Values are Mean±SD, Vit: Vitamin, T: Treatment, \*p<0.05 compared with control

Table 3: Effect of vitamin combinations on enzymes of liver integrity in pregnant rats

Treatments	AST (IU L <sup>-1</sup> )			ALT		
	Vit A+C	Vit A+E	Vit C+E	Vit A+C	Vit A+E	Vit C+E
Control	266.00±3.67	266.00±3.67	266.00±3.67	88.00±2.00	88.00±2.00	88.00±2.00
Tween80	264.60±7.96	264.60±7.96	264.60±7.96	89.60±1.52	89.60±1.52	89.60±1.52
T <sub>1</sub>	276.60±5.13*	241.20±6.76*	271.60±6.19	92.60±8.44	106.80±6.22*	101.40±3.78*
T <sub>2</sub>	269.60±4.56	223.60±6.27*	269.00±8.51	81.00±6.20	112.00±4.69*	85.40±5.41
T <sub>3</sub>	266.00±5.05	194.80±8.98*	241.00±6.63*	72.20±6.42*	109.40±3.51*	68.60±3.21*
T <sub>4</sub>	253.60±9.02*	207.00±6.86*	240.20±8.93*	60.60±4.16*	103.00±5.10*	62.00±4.90*

Values are Mean±SD, Vit: Vitamin, T: Treatment, \*p<0.05 compared with control

Table 4: Effect of vitamin combinations on enzymes of biliary integrity in pregnant rats

Treatments	ALP (IU L <sup>-1</sup> )		
	Vit A+C	Vit A+E	Vit C+E
Control	88.00±2.24	88.00±2.24	88.00±2.24
Tween80	87.80±5.93	87.80±5.93	87.80±5.93
T <sub>1</sub>	101.60±5.08*	102.00±5.51*	99.80±4.15*
T <sub>2</sub>	92.00±7.31	99.20±4.76*	96.00±5.43*
T <sub>3</sub>	77.60±5.08*	91.80±2.05	79.80±4.97*
T <sub>4</sub>	62.60±5.59*	85.20±7.63	71.20±4.87*

Values are Mean±SD, Vit: Vitamin, T: Treatment, \*p<0.05 compared with control

(7.18±0.28 g dL<sup>-1</sup>). This was significantly (p<0.05) reduced after the 4<sup>th</sup> dosing in the entire vitamin combination groups. Serum albumin levels presented mixed results; dosing with Vitamin A+C combination showed no significant alteration. However, after the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> dosing there was a significant (p>0.05) increase following the administration of Vitamin A+E combination (5.24±0.84, 4.28±0.59 and 3.82±0.41 g dL<sup>-1</sup>) and after the 3<sup>rd</sup> dosing (3.74±0.62 g dL<sup>-1</sup>) with Vitamin C+E combination compared with the control (2.92±0.35 g dL<sup>-1</sup>) (Table 2).

Enzymes of liver integrity (AST and ALT) were also determined. Vitamin A+C, A+E and C+E combination produced a favourable reduction in serum AST levels. Compared with the control (266.00±3.67 IU L<sup>-1</sup>), it was significantly higher (p<0.05) after the 1<sup>st</sup> dosing (276.60±5.13 IU L<sup>-1</sup>), significantly lower (p<0.05) after the 4<sup>th</sup> dosing (253.60±9.02 IU L<sup>-1</sup>) with Vitamin A+C combination, significantly (p<0.05) lower in the entire treatment with Vitamin A+E combination and after the 3<sup>rd</sup> and 4<sup>th</sup> dosing with Vitamin C+E combination. Also, Vitamin A+C and C+E combination produced a

significantly lower serum ALT levels after the 3<sup>rd</sup> and 4<sup>th</sup> dosing while Vitamin A+E presented a significantly higher serum ALT during the entire treatment duration (Table 3).

On enzymes of biliary tract integrity with particular reference to serum ALP, Vitamin A+C and C+E combination produced significantly lower changes (p<0.05) after the 3<sup>rd</sup> and 4<sup>th</sup> treatment when compared with control (88.00±2.24 IU L<sup>-1</sup>). Treatment with Vitamin A+E combination showed a higher serum ALP value which becomes non-significantly different with the control (88.00±2.24 IU L<sup>-1</sup>) after the 3<sup>rd</sup> and 4<sup>th</sup> dosing. (Table 4).

## DISCUSSION

The present study has confirmed that during pregnancy, there are changes in serum protein, albumin, AST, ALP and ALP which are normally associated with an increased risk of liver disease. Numerous publications have reported on the specific causes of abnormal Liver Function Test (LFT) in pregnancy (Knox and Olans, 1996; Castro *et al.*, 1999; Davidson, 1998; Hunt and Sharara, 1999; Sibai *et al.*, 1993). Abnormal LFT can be mild with no long-term consequences, or it can be severe, leading to both maternal and fetal mortality (Riely, 1999). Studies show the diagnostic work-up of abnormal Liver Function Test (LFT) to be challenging, as the conditions peculiar to pregnancy have to be considered in addition to the causes affecting the non-pregnant population (Riely, 1994; Knox, 1998).

In the present investigation, the combination of antioxidant vitamins reduced serum protein in pregnancy.

Also, albumin was also reduced, though this was not significant. Based on these results, combination of antioxidant vitamins during pregnancy keep enzymes of liver function load low as well as ameliorates hyper-proteinemia. Furthermore, antioxidant vitamin combination ameliorate the reported increased AST, ALP and ALT in pregnancy, except for Vitamin A+E combination where ALT was not reduced. In the present investigation, Vitamins A+C, A+E and C+E ameliorated hyper-hepatic states associated with pregnancy. The present study therefore shows that combined vitamin supplementation successfully reverted the hyper-hepatic state of pregnancy in a dose dependent fashion with Vitamin A+C and C+E being more potent. This finding indicates that there is a synergistic effect of antioxidant vitamins on pregnancy induced hyper-hepatic state. Corroboratively, Rumbold *et al.* (2008) reported no increased risk of abnormal liver function in randomized trials following supplementation with Vitamin C and E in pregnancy; this is in consonance with the findings of our study. A study by Ince (2010) reported Vitamin A+E combination to be a potent liver tonic. This finding is also in consonance with present study, except that Vitamin A+E combination did not produce any significant reduction in ALT. In studies with human subjects, Vitamin C supplementation increased plasma lipid standardized  $\alpha$ -tocopherol (Hamilton *et al.*, 2000). Vitamin E and C act as antioxidants independent of each other and protect cells when compared to cells lacking both Vitamin C and E (Madhavi *et al.*, 2009). Plasma  $\alpha$ -tocopherol levels also improved upon supplementation of Vitamin E and C, this improvement in plasma  $\alpha$ -tocopherol levels suggests synergism of Vitamin C with glutathione peroxidase to revitalize Vitamin E (Madhavi *et al.*, 2009). The combination of Vitamin A and E and C and E has been shown to suppress parasitaemia (Umar *et al.*, 2008). The effect of antioxidant in infections with different species of trypanosomes was attributed to the protection of membrane and cellular components against oxidative species by the vitamins (Umar *et al.*, 2008). Several experimental and clinical studies suggest an interaction between micro nutrients as suggested by Smith (1980), Solomons and Russell (1980) and Christian and West (1998). Their study showed the effect of zinc and vitamin A interaction in treatment of Vitamin A deficiency. Other studies revealed that Vitamin A supplementation alone failed to revert Vitamin A deficiency (Rahman *et al.*, 2002). Another study showed that zinc deficiency reduced hepatic cellular RBP (cRBP), which is essential for the intracellular transport of Vitamin A in addition to its well-established role in intercellular transport (Mobarhan *et al.*, 1992). The association between zinc deficiency and Vitamin A metabolism is

further supported by the simultaneous reduction in retinol and Retinol Binding Protein (RBP) in zinc deficient rats (Smith *et al.*, 1974). This suggests that the low plasma retinol concentrations in zinc deficiency might be caused by an impaired ability of the deficient animals to mobilize hepatic retinol (Rahman *et al.*, 2002).

Yakoob *et al.* (2010) has reported widespread maternal vitamin and mineral deficiencies and further recommends it logical to consider supplementation with multiple micronutrient preparations in pregnancy. There is therefore, a need to consider multivitamins and minerals in pregnant state based on the findings of this study and the above literature. A nutrient may affect not only the absorption of other nutrients, but also the transport, tissue uptake, function and metabolism of other nutrients. Hence, concurrent ingestion of several nutrients may result in synergistic, antagonistic, or threshold effects as compared to a single nutrient (Huang *et al.*, 2007). The clinical benefits of such an approach over single-nutrient supplements are unclear (Yakoob *et al.*, 2010) as present study support multiple- vitamin combination therapy in pregnancy.

## CONCLUSION

Conclusively, lack of antioxidant vitamin combination may be one of the explanations for pregnancy complications associated with elevated hepatic status. Further research to explore the present concepts and limitations of maternal multiple-vitamin supplementation during pregnancy cannot be overemphasized.

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## REFERENCES

- Agematsu, Y., H. Ikadai and H. Amao, 1983. Early detection of pregnancy of the rat. *Jikken Dobutsu*, 32: 209-212.
- Angula, P., 2002. Nonalcoholic fatty liver disease. *N. Engl. J. Med.*, 346: 1221-1223.

- Bassey, O.A., O.H. Lowery and M.J. Brock, 1946. A method for the rapid determination of alkaline phosphatase with five cubic millimetres of serum. *J. Biol. Chem.*, 164: 321-329.
- Castro, M.A., M.T. Fassett, T.B. Reynolds, K.J. Shaw and T.M. Goodwin, 1999. Reversible peripartum liver failure. A new perspective on the diagnosis, treatment and cause of acute liver of pregnancy, based on 28 consecutive cases. *Am. J. Obstet. Gynecol.*, 181: 389-395.
- Chitturi, S. and G.C. Farrell, 2001. Etiopathogenesis of nonalcoholic steatohepatitis. *Semin Liver Dis.*, 21: 27-42.
- Christian, P. and K.P. West Jr, 1998. Interactions between zinc and Vitamin A: An update. *Am. J. Clin. Nutr.*, 8: 435S-441S.
- Dakshinamurti, K. and S. Dakshinamurti, 2001. Blood pressure regulation and micronutrients. *Nutr. Res. Rev.*, 14: 3-43.
- Daly, T.J.M. and B. Kramer, 1998. Alterations in rat vaginal histology by exogenous gonadotrophins. *J. Anat.*, 193: 469-472.
- Davidson, K.M., 1998. Intrahepatic cholestasis of pregnancy. *Semin. Perinatol.*, 22: 104-111.
- Dehghan, H.M. and R. Dehghanan, 2006. Plasma level of vitamin C in women with pre-eclampsia in Ardabil, Iran. *Iranian J. Reproductive Med.*, 4: 35-39.
- Ejebe, D.E., I.M. Siminialayi, J.O.T. Emudainohwo, S.I. Ovuakporaye and A.E. Ojeh *et al.*, 2009. An improved technique for oral administration of solutions of test substances to experimental rats using Mediflon/Medicut intravenous cannula. *African J. Biotechnol.*, 8: 960-964.
- Hamilton, I.M.J., W.S. Gilmore, I.F.F. Benzie, C.W. Mulholland and J.J. Strain, 2000. Interactions between Vitamins C and E in human subjects. *Br. J. Nutr.*, 84: 261-267.
- Huang, H., B. Caballero, S. Chang, A.J. Alberg and R.D. Semba *et al.*, 2007. Supplement: Multivitamin/Mineral supplements and chronic disease prevention. *Am. J. Clin. Nutr.*, 85: 265S-268S.
- Hunt, C.M. and A.I. Sharara, 1999. Liver Disease in Pregnancy. *Am. Fam. Physicians*, 59: 829-836.
- Ince, S., 2010. What vitamins help the liver?. eHow, contribution, [http://www.ehow.com/about\\_5092603\\_vitamins-liver.html](http://www.ehow.com/about_5092603_vitamins-liver.html).
- Iribhogbe, O.I., J.E. Emordi, A. Aigbiremolen, O.B. Idonije, E.O. Nwoke and U. Akpamu, 2010. Effects of Vitamin A, C and E on liver function in pregnancy. *Asian J. Med. Sci.*, 3: 8-13.
- Karakilcik, A.Z., A. Hayat, N. Aydilek, M. Zerin and M. Cay, 2005. Effects of vitamin C on liver enzymes and biochemical parameters in rats anesthetized with halothane. *Gen. Physiol. Biophys.*, 24: 47-55.
- Kashinakunti, S.V., H. Sunitha, K. Gurupadappa, D.S. Shankarprasad, G. Suryaprakash and J.B. Ingin, 2010. Lipid peroxidation and antioxidant status in preeclampsia. *Al Ameen J. Med. Sci.*, 3: 38-41.
- Knox, T.A. and L.B. Olans, 1996. Liver disease in pregnancy. *N. Engl. J. Med.*, 335: 569-576.
- Knox, T.A., 1998. Evaluation of abnormal liver function in pregnancy. *Semin. Perinatol.*, 22: 98-103.
- Long, J.A. and H. Evans, 1922. The Oestrous Cycle in the Rat and its Associated Phenomena. In: *Memoirs of the University of California*, Lipman, F. and R. Hedrick (Eds.). Vol. 6, University of California Press, Berkeley, CA., pp: 1-148.
- Madhavi, M., P. Samudram, A.K. Hemanth and L. Victor, 2009. Effect of antioxidant vitamins C and E supplementation on its plasma levels and on lipid profile in pulmonary tuberculosis patients. *Am. J. Infect. Dis.*, 5: 263-272.
- Mobarhan, S., B. Greenberg, R. Mehta, H. Friedman and D. Barch, 1992. Zinc deficiency reduces hepatic cellular retinol-binding protein in rats. *Int. J. Vitam. Nutr. Res.*, 62: 148-154.
- Rahman, T.M. and J. Wendon, 2002. Severe hepatic dysfunction in pregnancy. *QJM*, 95: 343-357.
- Rahman, M.M., M.A. Wahed, J.G. Fuchs, A.H. Baqui and J.O. Alvarez, 2002. Synergistic effect of zinc and vitamin A on the biochemical indexes of Vitamin A nutrition in children. *Am. J. Clin. Nutr.*, 75: 92-98.
- Raijmakers, M.T.M., R. Dechend and L. Poston, 2004. Oxidative stress and preeclampsia; rationale for antioxidant clinical trials. *Hypertension*, 44: 374-380.
- Reid, A.E., 2001. Nonalcoholic steatohepatitis. *Gastroenterology*, 121: 710-723.
- Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.*, 28: 56-63.
- Riely, C.A., 1994. Hepatic disease in pregnancy. *Am. J. Med.*, 96: 18S-22S.
- Riely, C.A., 1999. Liver disease in the pregnant patient. *Am. J. Gastroenterol.*, 94: 1728-1732.
- Rumbold, A., L. Duley, C.A. Crowther and R.R. Haslam, 2008. Antioxidants for preventing pre-eclampsia. *Cochrane Database Syst. Rev.*, (1): CD004227.
- Sibai, B.M., M.K. Ramadan, I. Usta, M. Salamea, B.M. Mercer and S.A. Friedman, 1993. Maternal morbidity and mortality in 442 pregnancies with haemolysis, elevated liver enzymes and low platelets (HELLP syndrome). *Am. J. Obstet. Gynecol.*, 169: 1000-1006.

- Smith, J.E., E.D. Brown and J.C. Smith, 1974. The effect of zinc deficiency on the metabolism of retinol-binding protein in the rat. *J. Lab. Clin. Med.*, 84: 692-697.
- Smith, J.C., 1980. The Vitamin A-zinc connection: A review. *Ann. N. Y. Acad. Sci.*, 355: 62-75.
- Solomons, N.W. and R.M. Russell, 1980. The interaction of vitamin A and zinc: Implications for human nutrition. *Am. J. Clin. Nutr.*, 33: 2031-2040.
- Steingrub, J.S., 2004. Pregnancy-associated severe liver dysfunction. *Critical Care Clinics*, 20: 763-776.
- Umar, I.A., B.L. Rumah, S.L. Bulus, A.A. Kamla and A. Jobin *et al.*, 2008. Effects of intraperitoneal administration of Vitamin C and E or A and E combinations on the severity of *Trypanosoma brucei brucei* infection in rats. *Afr. J. Biochem. Res.*, 2: 88-91.
- Yakoob, Y.M., Y.P. Khan, Z.A. Bhutta, E. Manzotti, D. Lie and S. Fleischman, 2010. Maternal mineral and vitamin supplementation in pregnancy CME. *Exp. Rev. Obst. Gynecol.* Vol. 5.