Objective Artificial food colourings and additives (AFCAs) have long been discussed to have adverse effects on cognition and behaviour in children. In this study, our aim was to assess the probable side effects of prenatal exposure to colouring food additives on neurobehaviour and spatial learning process. Methods We administered 'no observable adverse effect levels' (NOAELs) of common used AFCAs as a mixture (erythrosine, Ponceau 4R, Allura Red AC, Sunset yellow FCF, tartrazine, Amaranth, Brilliant Blue, Azorubine and Indigotine) to female rats before and during gestation and tested their effects on spatial working memory and behaviour in their offspring. Effects of AFCAs on spatial working memory were evaluated by Morris water maze, behavioural and locomotor effects by open-field and forced-swim tests. Results Prenatal exposure to commonly used AFCAs had no adverse effects on spatial working memory; however, assessment of interaction of sex and AFCAs on 'latency to locate the visible platform', which was used as a measure of motivation, showed a significant interaction (P < 0.05) on female rats. In addition, AFCAs caused an increase in anxiolytic like effect in the open-field test (P < 0.05) and an increase in mobility time (P < 0.05) in the forced-swim test. We also detected a significant interaction of sex and AFCAs on forced-swim test parameters (P < 0.05). Discussion These findings indicated that prenatal exposure to NOAELs of AFCAs resulted in implicit adverse effects that caused an increase in motility and a decrease in motivation and anxiety in offspring in sex-related manner.